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Separation and purification of water-soluble iridoid glucosides by high speed counter-current chromatography combined with macroporous resin column separation

Hui-Lan Yue [a], Xiao-Hui Zhao [a,b,*], Qi-Lan Wang [a], Yan-Duo Tao [a,**]

[a] Key Laboratory of Tibetan Medicine Research, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, China
[b] Graduate University of the Chinese Academy of Sciences, Beijing 100049, China

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ABSTRACT

Four iridoid glucosides, shanzhiside methyl ester, phloyoside II, chlorotuberside, and penstemonoside, were isolated and purified from an herbal medicinal plant for the first time by high-speed counter-current chromatography (HSCCC) using a two-phase solvent system composed of ethyl acetate–*n*-butanol–water (5:14:12, v/v/v). A total of 37 mg of shanzhiside methyl ester, 29 mg of phloyoside II, 27 mg of chlorotuberside, and 21 mg of penstemonoside with the purity of 99.2%, 98.5%, 97.3%, and 99.3%, respectively, were obtained in one-step separation within 4 h from 150 mg of crude extract. To the best of our knowledge, this is the first report of separation and purification of iridoid glucosides from natural sources by HSCCC. The chemical structures of all the four compounds were identified by ESI-MS, ^{1}H NMR, and ^{13}C NMR.

1. Introduction

The genus *Lamiophlomis* (family Lamiacea), distributed mainly in Qinghai-Tibet Plateau in northwest of China, comprises only one species, *Lamiophlomis rotata* (Benth.) Kudo. For thousands years, *L. rotata* (Benth.) Kudo has been used as one of the traditional drugs in Tibetans, Mongolians and Na-Xi nations with the effects of alleviating pain, detumescence, hemostasis, promoting blood circulation, subduing swelling, and removing blood stasis [1]. Previous phytochemical studies demonstrated that *L. rotata* (Benth.) Kudo contains various constituents, including iridoid glucosides, phenylethanoid glycosides (PhGs), flavonoids, and certain miscellaneous substances [2–5]. Pharmacological investigations revealed that iridoid glucosides are the main hemostatic components of *L. rotata* (Benth.) Kudo [6,7]. In addition, the iridoid glucosides, shanzhiside methyl ester, phloyoside II, chlorotuberside, and penstemonoside, were shown to have diverse biological activities including anti-nociceptive [8], anti-inflammatory [8], anti-influenza virus [9], *in vitro* antispasmodic [10], and *in vitro* and *in vivo* immunomodulatory activities [11].

In view of their wide pharmacological activities, large quantities of pure compounds are urgently needed as chemical reference standards and for further pharmacological studies. The preparative separation and purification of iridoid glucosides from many plants by conventional methods including silica gel and gel filtration chromatography are time consuming, require large amounts of organic solvents, and typically require numerous chromatographic steps resulting lower recovery and higher cost. Therefore, effective methods for the isolation and purification of iridoid glucosides from natural sources become necessary.

High-speed counter-current chromatography (HSCCC), a support-free liquid–liquid partition chromatographic technique based on partitioning of compounds between two immiscible liquid phases without support matrix, eliminates irreversible adsorption in the solid support and has been widely used in preparative separation of natural products. Macroporous resin (MR), as the adsorption material for column chromatography, has been widely applied in combination of HSCCC for the preseparation due to its low-cost, high-efficiency, easy-recycling, and simple scaling-up performance.

To the best of our knowledge, only a few reports on the use of HSCCC for separation and purification of iridoid glucosides from natural sources have been successfully published [12]. The present paper describes the successful preparative separation and purification of four iridoid glucosides, shanzhiside methyl ester, phloyoside II, chlorotuberside, and penstemonoside, from an herbal medicinal plant for the first time by HSCCC. The chemical structures of four iridoid glucosides were show in Fig. 1.

* Corresponding author at: Key Laboratory of Tibetan Medicine Research, Northwest Institute of Plateau Biology, Chinese Academy of Sciences, Xining 810001, China. Tel.: +86 971 6117264; fax: +86 971 6117264.
** Corresponding author. Tel.: +86 971 6117264; fax: +86 971 6117264.
E-mail addresses: xhzhao@nwipb.ac.cn (X.-H. Zhao), taoyanduo@163.com (Y.-D. Tao).

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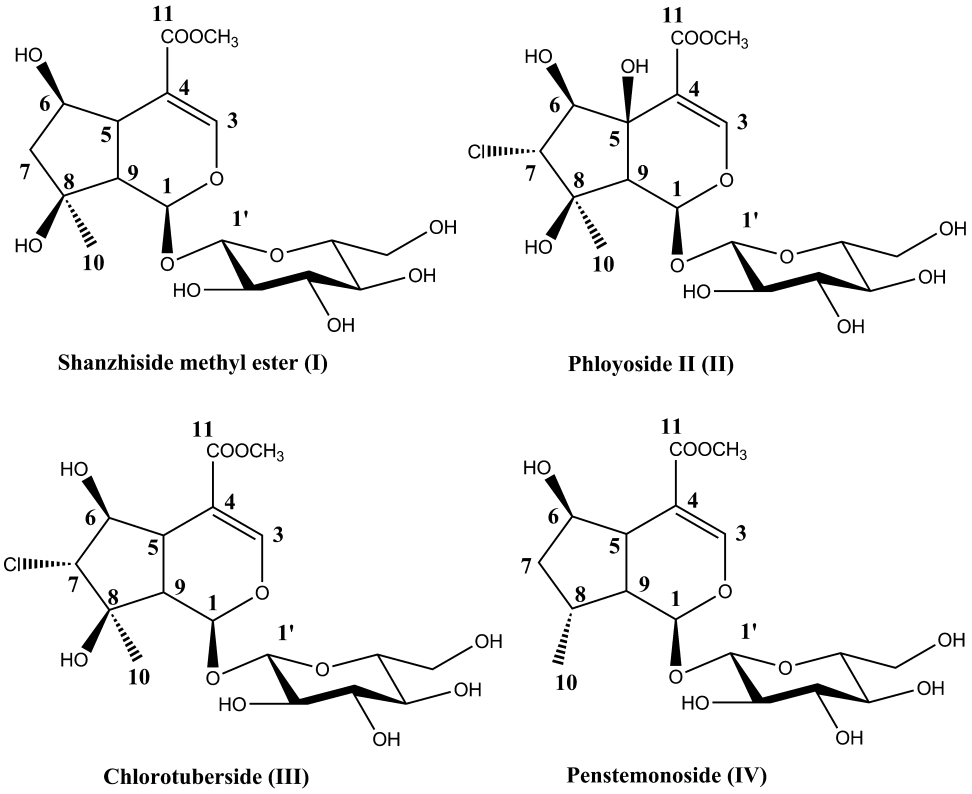

Fig. 1. The chemical structures of shanzhiside methyl ester (I), phloyoside II (II), chlorotuberside (III), and penstemonoside (IV).

2. Experimental

2.1. Reagents and materials

L. rotata (Benth.) Kudo was collected from Yushu, Qinghai, China in September 2011, and identified by Professor Lijuan Mei of the Northwest Institute of Plateau Biology, Chinese Academy of Sciences. The voucher specimen (Mei 201109) was deposited in the Herbarium of the Northwest Institute of Plateau Biology, Xining, Qinghai Province.

All organic solvents used for sample preparation and HSCCC were of analytical grade and purchased from the Tianjin Chemical Factory (Tianjin, China). Methanol used for HPLC analysis was of chromatographic grade, and was purchased from Yuwang Chemical Ltd. (Shandong, China). The water used was purified using a PAT-125 (Chengdu Ultra Technology Co., Ltd., Chengdu, Sichuan, China) laboratory ultra pure water system with a 0.4 μm filter.

2.2. Apparatus

The HSCCC instrument used in the present study was a TBE-300B high-speed counter-current chromatography instrument (Shanghai Tauto Biotech Co., Ltd., Shanghai, China) with a three preparative polytetrafluoroethylene (PTFE) coils (tube diameter: 2.6 mm, total volume: 280 mL), and a 20 mL sample loop. The revolution radius or the distance between the holder axis and the central axis of the centrifuge (R) was 5 cm. The β-values of the multilayer coil ranged from 0.5 at the 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. The HSCCC system was equipped with a model TBP-5002 constant-flow pump (Shanghai Tauto Biotech Co., Ltd., Shanghai, China), a model UV500 detector (Shanghai Tauto Biotech Co., Ltd., Shanghai, China) operating at 230 nm, and a model of N2010 workstation (Zhejiang University Star Information Technology Co., Ltd., Hangzhou, China). The separation temperature was controlled by a DC-0506 constant temperature circulating instrument (Shanghai Shunyuhengping Science Instruments Co. Ltd., Shanghai, China).

The HPLC equipment was an Agilent 1200 system, equipped with a G1354A solvent delivery unit, a G1315B UV–vis photodiode array detector, a G1316A column thermostat, a G1313A autosampler, an Eclipse XDB – C_{18} 4 μm, 4.6 mm × 250 mm analytical column, and an Agilent HPLC workstation (Agilent Technologies Co. Ltd., USA).

The mass spectrometer was an Agilent 1100 series LC/MSD (Agilent Technologies Co. Ltd., USA). Nitrogen was used as the drying gas at a flow rate of 8.0 L/min and as the nebulizing gas at a pressure of 40.0 psi. The nebulizer temperature was set at 325 °C.

The nuclear magnetic resonance (NMR) spectrometer was a Mercury-600BB NMR (Varian Co. Ltd., Palo Alto, CA, USA) with tetramethylsilane (TMS) as the internal standard.

2.3. Extraction of the medicinal plant

About 4 kg of dried *L. rotata* (Benth.) Kudo was powdered and extracted three times using 65% ethanol (25 L) under reflux, each time for 2 h. After concentration under vacuum, the residues were suspended in distilled water and extracted with light petroleum (b.p. 60–90 °C, 9 L), ethyl acetate (9 L), and *n*-butanol (10 L), respectively. The *n*-butanol solutions were evaporated to dryness under vacuum at 65 °C to generate 400 g of *n*-butanol extract.

2.4. Macroporous resin column chromatography

In order to enrich the targeted components, the extract of *n*-butanol (400 g) was dissolved in deionized water, loaded into a macroporous resin column (140 cm × 12 cm, containing 10 kg D101

macroporous resin), and eluted with various proportions of a mixture of water–ethanol (100:0, 95:5, 90:10, 70:30, 60:40, 40:60, and 20:80 v/v; about 15 L for each gradient). The flow rate was controlled at about 50 mL/min. The eluted fractions were collected individually and then analyzed by HPLC (HPLC spectrogram), and the water–ethanol (90:10) fraction was concentrated to produce 5 g of crude sample for subsequent HSCCC isolation and purification. All these fractions were stored at 4 °C in darkness.

2.5. Measurement of partition coefficient

The partition coefficients were determined by HPLC as follows. A suitable amount of crude sample was added into a series of pre-equilibrated two-phase solvent systems, and the solution was then fully shaken to reach the partition equilibrium. Subsequently, the same volumes of upper and lower phase were each evaporated to dryness. The residues were diluted into 2 mL methanol and then analyzed by HPLC. The K value was defined as the peak area of the component in the upper phase divided by the peak area of the component in the lower phase.

2.6. Preparation of the two-phase solvent system and sample solution

In the present study, the two-phase solvent system composed of ethyl acetate–n-butanol–water (5:14:12, v/v/v) 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 shortly before use. The sample solution for HSCCC separation was prepared by dissolving 150 mg of the dried powder of the crude extract after cleaning up by D101 macroporous resin in the 10 mL of the lower phase of the two-phase solvent system.

2.7. HSCCC separation procedure

First, the multilayer coil column was entirely filled with the upper phase (stationary phase). Then the apparatus was rotated at 950 rpm, while the lower phase (mobile phase) was pumped into the column at a flow rate of 1.5 mL/min. After hydrodynamic equilibrium was reached, as indicated by the emergence of the mobile phase, 10 mL of sample solution containing 150 mg of the crude extract was injected into the column through the injection valve. The eluant from the tail end of the column was continuously monitored with a UV detector at 230 nm, and the chromatogram was recorded. The temperature of the apparatus was set to 40 °C. The peak fractions were collected manually according to the elution profile. Evaporation under reduced pressure was then carried out, and the residues were dissolved in methanol for subsequent purity analysis by HPLC. The purity was obtained by HPLC peak area calculation.

2.8. HPLC analysis and identification of HSCCC peak fractions

The crude sample and each peak fraction obtained by HSCCC were analyzed by HPLC. HPLC analysis was performed on a reversed-phase Agilent Eclipse XDB-C18 (4 μm, 4.6 mm × 250 mm) analytical column with gradient elution, at a column temperature of 30 °C. Methanol–water in the following percentage and corresponding time was used as the mobile phase: (methanol: 0–30 min, 36–45%). The flow-rate and detection wavelength were set at 1.0 mL/min and 230 nm, respectively. The crude sample and peak fractions separated by HSCCC were analyzed by HPLC under the optimum analytical conditions, and the chromatograms were presented in Fig. 2. Identification of HSCCC peak fraction was performed by ESI-MS and NMR.

3. Results and discussion

3.1. Selection of solvent system and other HSCCC conditions

Successful HSCCC separation depends upon the selection of a suitable two-phase solvent system, which requires the following considerations [13,14]: (1) the settling time of the solvent system should be short (<30 s); (2) the partition coefficient (K) of the target compound should fall within a suitable range (*i.e.* usually between 0.2 and 5); (3) the separation factor ($\alpha = K_1/K_2$, $K_1 > K_2$) between any two compounds all should be greater than 1.5.

In this experiment, different two-phase solvent systems such as: ethyl acetate–n-butanol–water and n-hexane–ethyl acetate–methanol–water, were tested according to the polarity of the target compounds. The K-values of the four target compounds corresponded to peak fraction I, II, III, and IV in different solvent systems were determined by HPLC and the results were shown in Table 1. The two-phase solvent systems with n-hexane–ethyl acetate–methanol–water (5:15:10:10, 10:12:9:12 and 3:15:12: 8, v/v/v/v) were tested first; small K values and poor retention of target compounds in the upper phase resulted. Then, two-phase solvent systems comprised of ethyl acetate–n-butanol–water (12:6:12, 10:12:12, 8:12:12, 8:12:15, 8:12:14, 6:13:12, 5.5:13.5:12, and 5:14:12, v/v/v) were further investigated. When a ratio of 12:6:12, 10:12:12, 8:12:12, 8:12:15, 8:12:14, or 6:13:12 (v/v/v) was used for the separation, the K value of compound I was small and might lead to poor retention of target compound in the upper phase. When a ratio of 5.5:13.5:12 (v/v/v) was tested, the K values of the target compounds were all suitable. However, the separation factors between compounds I, II, III, and IV were too small and not suitable for the separation of the four iridoid glucosides from the crude sample. Fortunately, when a ratio of 5:14:12 (v/v/v) was tested, the K values of the four target compounds were between 0.2 and 5, and the separation factors were large enough for the four iridoid glucosides to exhibit large resolutions relative to one another.

In addition to screening a suitable two-phase solvent system, other factors such as separation temperature and flow rate of the mobile phase were also investigated. Firstly, effects of separation temperature on the retention of the stationary phase were tested. The results demonstrated that the retention of the stationary phase was improved with an increase in separation temperature, but high temperature would often trap a lot of air in the apparatus and decrease the separating efficiency of the apparatus to some degree. Therefore, the separation temperature was set at 40 °C in this experiment. Then, different flow rates of the mobile phase (1.0, 1.5, and 2.0 mL/min) were tested in order to determine their effect on separation time, stationary phase retention, and targeted compound purity (Table 2). Results indicated that the low flow rate of the mobile phase could improve the peak resolution and increase stationary phase retention, but more time and more amount of mobile phase would be required, and the chromatogram peak was extended. Considering the elucidated separation time and the purity of the targeted compounds, a flow rate of 1.5 mL/min was used. Additionally, the revolution speed could also impact stationary phase retention, the lower revolution speed would reduce the volume of the stationary phase retained in the column leading to lower peak resolution and purities of targeted compounds, and the higher revolution speed might produce emulsification [15]. In the

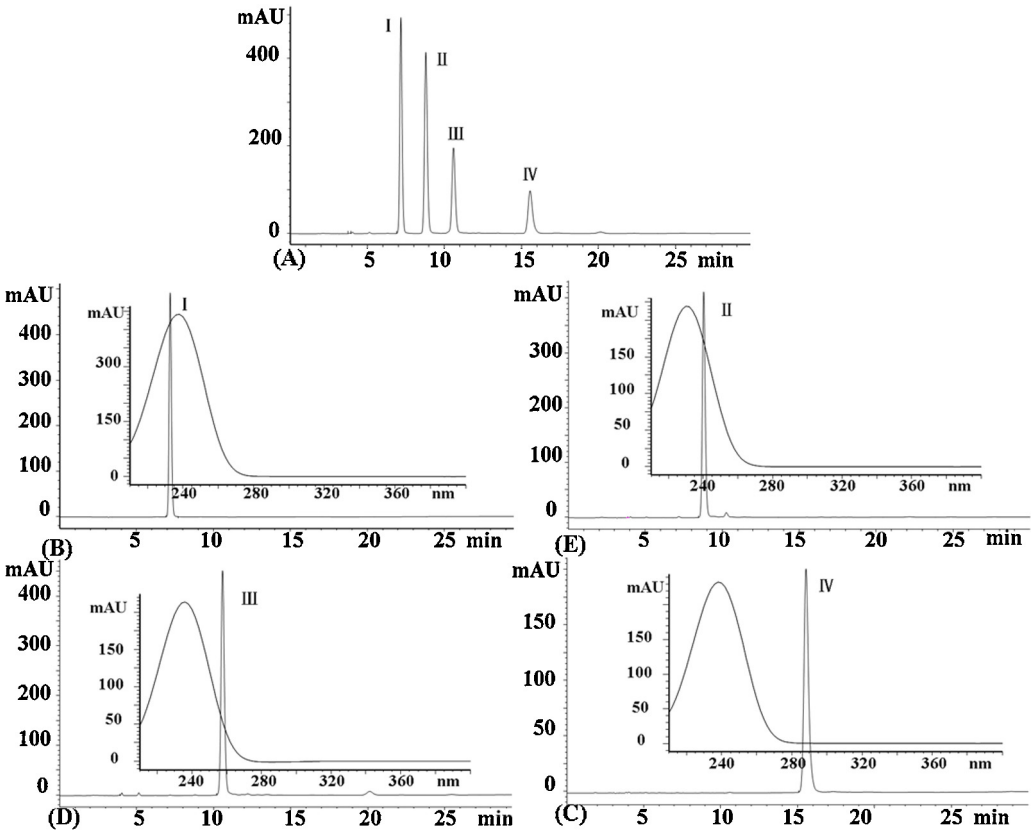

Fig. 2. HPLC chromatograms of the crude extract from *L. rotata* (Benth.) Kudo (A) after clean up by macroporous resin chromatography and the four targeted compounds (peak fractions I, II, III, and IV B–E) purified by HSCCC. Conditions: column: Eclipse XDB-C_{18} column (250 mm × 4.6 mm i.d., 4 μm); mobile phase: methanol–water (methanol: 0–30 min, 36–45%); flow-rate: 1.0 mL/min; column temperature: 30 °C; and detection wavelength: 230 nm.

present study, the emulsification has not been produced at 950 rpm. Thus, the 950 rpm/min speed was mainly considered in our isolation procedure.

Under the optimized conditions, four fractions (I, II, III, and IV) were obtained in one step separation within 4 h, which were shanzhiside methyl ester (peak I, collected during 96–107 min, 37 mg), phloyoside II (peak II, collected during 112–125 min, 29 mg), chlorotuberside (peak III, 27 mg), and penstemonoside (peak IV, 21 mg). The HPLC analysis of each HSCCC fraction revealed that the purities of these four compounds were 99.2%, 98.5%, 97.3%, and 99.3%, respectively. The HSCCC chromatogram was shown in Fig. 3.

Table 1
The *K* values of the target compounds in different solvent systems.

Solvent systems	Ratio (v/v)	Settling time (s)	*K* values			
			I	II	III	IV
n-Hexane–ethyl acetate–methanol–water	5:15:10:10	20	−∞	−∞	−∞	−∞
n-Hexane–ethyl acetate–methanol–water	3:15:12:8	24	−∞	−∞	−∞	−∞
n-Hexane–ethyl acetate–methanol–water	10:12:9:12	18	−∞	−∞	−∞	−∞
Ethyl acetate–*n*-butanol–water	12:6:12	16	0.073	0.129	0.245	0.263
Ethyl acetate–*n*-butanol–water	10:12:12	11	0.112	0.233	0.375	0.448
Ethyl acetate–*n*-butanol–water	8:12:12	13	0.174	0.270	0.462	0.592
Ethyl acetate–*n*-butanol–water	8:12:15	13	0.156	0.248	0.403	0.609
Ethyl acetate–*n*-butanol–water	8:12:14	13	0.157	0.259	0.465	0.653
Ethyl acetate–*n*-butanol–water	6:13:12	12	0.191	0.281	0.485	0.682
Ethyl acetate–*n*-butanol–water	5.5:13.5:12	19	0.203	0.323	0.527	0.782
Ethyl acetate–*n*-butanol–water	**5:14:12**	**19**	**0.235**	**0.385**	**0.632**	**1.030**

Table 2
Comparison of separation time, stationary phase retention, and purities of four compounds under different flow rates.

Flow-rate (mL/min)	Separation-time (min)	Retention (%)	Purity (%)			
			I	II	III	IV
1.0	300	63.1	99.3	98.7	98.0	99.5
1.5	**210**	**60.0**	**99.2**	**98.5**	**97.3**	**99.3**
2.0	160	53.7	98.1	96.3	94.7	98.9

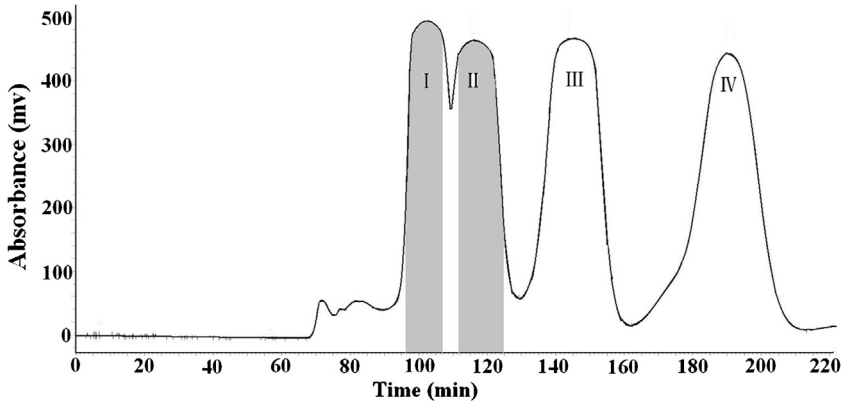

Fig. 3. HSCCC chromatogram of the crude extract from *L. rotata* (Benth.) Kudo after clean up by macroporous resin column chromatography. Two-phase solvent system: ethyl acetate–*n*-butanol–water at a ratio of 5:14:12 (v/v/v); stationary phase: upper phase; mobile phase: lower phase; flow-rate: 1.5 mL/min; revolution speed: 950 rpm; detection wavelength: 230 nm; sample size: 150 mg of crude sample dissolved in 10 mL of the lower phase; and separation temperature: 40 °C.

3.2. The structure identification of the HSCCC peak fractions

According to ESI-MS (Fig. 4), ^{1}H NMR, and ^{13}C NMR data, the chemical structures of the peak fractions separated by HSCCC were identified. In comparison with reference data, peak I, peak II, peak III and peak IV were effectively identified as shanzhiside methyl ester, phloyoside II, chlorotuberside, and penstemonoside, respectively. Results for each peak fraction were as follows.

Peak fraction I: Shanzhiside methyl ester, yellow needles, $[a]_D^{25}$ −121.7° (MeOH, c = 2.5); ESI-MS m/z: 405 $[M-H]^-$; ^{1}H NMR (CD_3OD, 600 MHz) δ = 7.40 (d, 1H, J = 0.7 Hz, H-3), 5.56 (d, 1H, J = 2.6 Hz, H-1), 4.62 (d, 1H, J = 7.9 Hz, Glu H-1), 4.02 (m, 1H, H-6), 3.88 (dd, 1H, J = 2.0 Hz, J = 11.9 Hz, Glu H-6), 3.72 (s, 3H, COOMe), 3.64 (m, 1H, J = 6.2 Hz, J = 11.9 Hz, Glu H-6), 3.35 (t, 1H, J = 9.0 Hz, Glu H-3), 3.29 (m, 1H, Glu H-5), 3.24 (t, 1H, J = 8.9 Hz, Glu H-4), 3.15 (t, 1H, J = 8.0 Hz, Glu H-2), 2.98 (dd, 1H, J = 3.1 Hz, J = 10.0 Hz, H-5), 2.60 (dd, 1H, J = 2.4 Hz, J = 10.3 Hz, H-9), 2.00 (dd, 1H, J = 6.4 Hz, J = 13.3 Hz, H-7), 1.81 (dd, 1H, J = 6.0 Hz, J = 13.2 Hz, H-7), 1.25 (s, 3H, H-10); ^{13}C NMR (CD_3OD, 600 MHz) δ = 169.7 (C-11), 152.8 (C-3), 111.4 (C-4), 99.8 (C-1′), 94.8 (C-1), 79.0 (C-8), 78.4 (C-3′), 78.0 (C-6), 77.5 (C-5′), 74.7 (C-2′), 71.6 (C-4′), 62.9 (C-6′), 51.9 (OMe), 51.8 (C-7), 49.1 (C-9), 41.5 (C-5), 24.7 (C-10). Comparing with the reported data, the ^{1}H NMR and ^{13}C NMR data are in agreement with that of shanzhiside methyl ester in the literature [16].

Peak fraction II: Phloyoside II, yellow needles, $[a]_D^{25}$ −122.4° (MeOH, c = 1.6); ESI-MS m/z: 455 $[M-H]^-$; ^{1}H NMR (CD_3OD, 600 MHz) δ = 7.49 (s, 1H, H-3), 5.84 (s, 1H, H-1), 4.59 (d, 1H, J = 7.9 Hz, Glu H-1), 4.03 (d, 1H, J = 9.7 Hz, H-7), 3.88 (d, 1H, J = 2.0 Hz, J = 12.0 Hz, Glu H-6), 3.72 (s, 3H, COOMe), 3.71 (m, 1H, H-6), 3.65 (dd, 1H, J = 6.0 Hz, J = 12.0 Hz, Glu H-6), 3.36 (t, 1H, J = 8.9 Hz, Glu H-3), 3.32 (m, 1H, Glu H-5), 3.27 (t, 1H, J = 8.8 Hz, Glu H-4), 3.18 (t, 1H, J = 8.0 Hz, Glu H-2), 2.51 (s, 1H, H-9), 1.10 (s, 3H, H-10); ^{13}C NMR (CD_3OD, 600 MHz) δ = 168.1 (C-11), 153.7 (C-3), 114.6 (C-4), 99.7 (C-1′), 93.0 (C-1), 80.6 (C-6), 78.4 (C-3′), 77.3 (C-5′), 74.5 (C-8), 74.3 (C-2′), 73.1 (C-7), 71.6 (C-4′), 66.1 (C-5), 62.8 (C-6′), 58.4 (C-9), 51.9 (OMe), 18.5 (C-10). Comparing with the reported data, the ^{1}H NMR and ^{13}C NMR data are in agreement with that of phloyoside II in the literature [17].

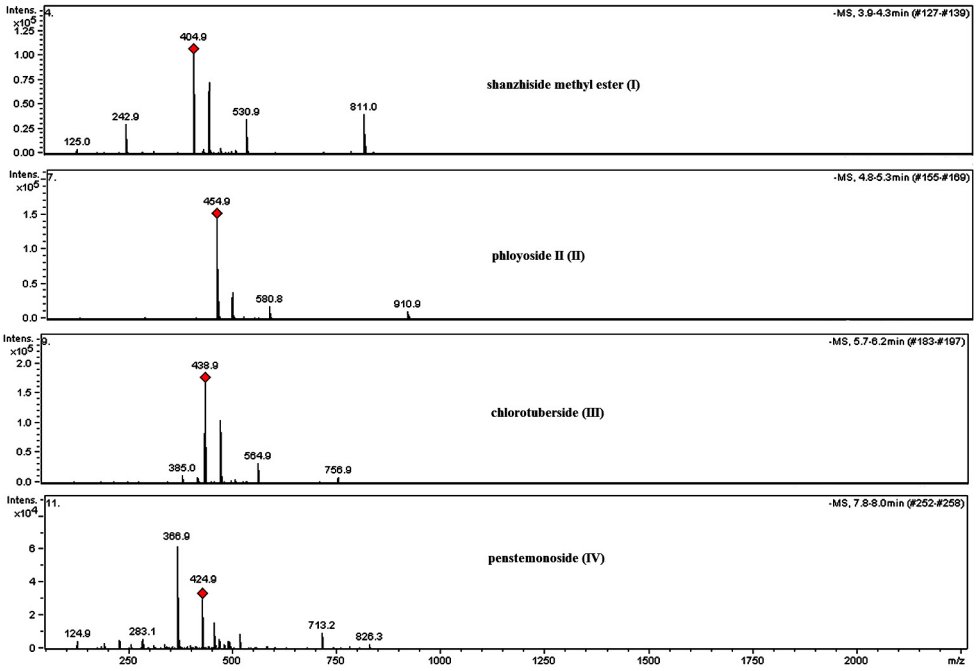

Fig. 4. The mass spectra of shanzhiside methyl ester (I), phloyoside II (II), chlorotuberside (III), and penstemonoside (IV).

Peak fraction III: Chlorotuberside, yellow needles, $[a]_D^{25}$ −98.1° (MeOH, c = 0.9); ESI-MS m/z: 439 $[M-H]^-$; ^{1}H NMR (CD_3OD, 600 MHz) δ = 7.40 (s, H-3), 5.66 (s, 1H, H-1), 4.59 (d, 1H, J = 7.9 Hz, Glu H-1), 3.99 (d, 1H, J = 8.9 Hz, H-7), 3.87 (d, 1H, J = 2.0 Hz, J = 12.0 Hz, Glu H-6), 3.73 (s, 3H, COOMe), 3.67 (dd, 1H, J = 4.3 Hz, J = 7.9 Hz, H-6), 3.64 (dd, 1H, J = 6.2 Hz, J = 11.6 Hz, Glu H-6), 3.34 (t, 1H, J = 8.9 Hz, Glu H-3), 3.32 (m, 1H, Glu H-5), 3.26 (t, 1H, J = 9.0 Hz, Glu H-4), 3.14 (t, 1H, J = 8.1 Hz, Glu H-2), 2.81 (dd, 1H, J = 3.4 Hz, J = 10.6 Hz, H-5), 2.65 (d, 1H, J = 11.5 Hz, H-9), 1.18 (s, 3H, H-10); ^{13}C NMR (CD_3OD, 600 MHz) δ = 169.4 (C-11), 152.3 (C-3), 111.7 (C-4), 99.7 (C-1′), 93.3 (C-1), 82.4 (C-6), 78.3 (C-3′), 77.9 (C-8), 77.6 (C-5′), 74.5 (C-7), 74.5 (C-2′), 71.5 (C-4′), 62.7 (C-6′), 52.0 (OMe), 47.9 (C-9), 36.2 (C-5), 18.9 (C-10). Comparing with the reported data, the ^{1}H NMR and ^{13}C NMR data are in agreement with that of chlorotuberside in the literature [18].

Peak fraction IV: Penstemonoside, yellow needles, $[a]_D^{25}$ −143.2° (MeOH, c = 2.7); ESI-MS m/z: 425 $[M+Cl]^-$; ^{1}H NMR (CD_3OD, 600 MHz) δ = 7.42 (s, 1H, H-3), 5.52 (d, 1H, J = 3.2 Hz, H-1), 4.61 (d, 1H, J = 7.9 Hz, Glu H-1), 4.19 (m, 1H, H-6), 3.89 (d, 1H, J = 1.9 Hz, J = 11.8 Hz, Glu H-6), 3.70 (s, 3H, COOMe), 3.64 (dd, 1H, J = 6.3 Hz, J = 11.9 Hz, Glu H-6), 3.34 (t, 1H, J = 9.1 Hz, Glu H-3), 3.32 (m, 1H, Glu H-5), 3.24 (t, 1H, J = 9.1 Hz, Glu H-4), 3.16 (t, 1H, J = 8.2 Hz, Glu H-2), 2.85 (d, 1H, J = 8.4 Hz, H-5), 2.63 (m, 1H, H-8), 2.57 (m, 1H, H-9), 1.75 (m, 1H, H-7), 1.46 (m, 1H, H-7), 1.05 (d, 3H, J = 7.2 Hz, H-10); ^{13}C NMR (CD_3OD, 600 MHz) δ = 169.5 (C-11), 153.8 (C-3), 111.2 (C-4), 99.8 (C-1′), 96.2 (C-1), 78.4 (C-3′), 78.0 (C-6), 77.9 (C-5′), 74.7 (C-2′), 71.7 (C-4′), 62.8 (C-6′), 51.7 (OMe), 43.1 (C-5), 42.6 (C-9), 41.9 (C-7), 33.9 (C-8), 16.7 (C-10). Comparing with the reported data, the ^{1}H NMR and ^{13}C NMR data are in agreement with that of penstemonoside in the literature [19].

4. Conclusion

In conclusion, a convenient and efficient method for preparative separation and purification of shanzhiside methyl ester, phloyoside II, chlorotuberside, and penstemonoside, from the partially purified extract of *L. rotata* (Benth.) Kudo has been successfully developed by HSCCC in one-step separation within 4 h. The present study firstly indicates that HSCCC is a powerful technique for the preparative separation and purification of iridoid glucosides from an herbal medicinal plant.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.jchromb.2013.08.007.

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