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Application of high-speed counter-current chromatography combined with macroporous resin for rapid enrichment and separation of three anthraquinone glycosides and one stilbene glycoside from *Rheum tanguticum*

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ABSTRACT

In this paper, an efficient method was successfully established by the combination of macroporous resin (MR) and high-speed counter-current chromatography (HSCCC) for rapid enrichment and separation of aloe-emodin 8-*O*-β-D-glucoside, emodin 1-*O*-β-D-glucoside, emodin 8-*O*-β-D-glucoside and piceatannol 4′-*O*-β-D-(6″-O-gallate)-glucoside. Six kinds of macroporous resins were investigated in the first step and X-5 macroporous resin was selected for the enrichment of the target compounds. The recoveries of the target compounds reached 89.0, 85.9, 82.3 and 84.9% respectively after 40% ethanol elution. In the second step, the target compounds were separated by HSCCC with a two-phase solvent system composed of chloroform/ethyl acetate/methanol/water (8:1:6:5, v/v). The established method will be helpful for further characterization and utilization of *Rheum tanguticum*. The results demonstrate that MR coupled with HSCCC is a powerful technique for separation of bioactive compounds from natural products.

1. Introduction

Rheum tanguticum is one of the three genuine rhubarb species reported in the Chinese Pharmacopoeia [1], whose roots and rhizomes have been traditionally used as a folk medicine because of their strong antibacterial, antipyretic and antispasmodic activities [2]. Previous phytochemical studies have demonstrated that *R. tanguticum* contains various constituents, including anthraquinones, dianthrones, stilbenes, anthocyanins, flavonoids, anthraglycosides, polyphenols, essential oils, organic acids, chromenes, chromanone, chromone glycosides and vitamins [3–6]. Pharmacological studies have revealed that the anthraquinone glycosides and stilbene glycosides are the bioactive components because of their pharmacological properties. For example, anthraquinone glycosides have been reported to exhibit antifungal, anti-microbial, cytotoxic and antioxidant activities [7–9], whereas stilbene glycosides have shown anti-HIV, antioxidant, antiplasmodial, antimalarial and antiallergy activities [10–14]. Among the anthraquinone glycosides and stilbene glycosides, aloe-emodin 8-*O*-β-D-glucoside (C1), emodin 1-*O*-β-D-glucoside (C3), emodin 8-*O*-β-D-glucoside (C4) and piceatannol 4′-*O*-β-D-(6″-*O*-gallate)-glucoside (C2) (Fig. 1) are some of the important constituents.

In view of their wide pharmacological activities, large quantities of pure compounds are urgently needed as chemical reference standards and for further pharmacological studies. With regard to the methods available for the separation of organic compounds, silica and gel chromatography are two classical methods due to their different separation principles. Silica chromatography relies on the principles of adsorption and desorption, whereas gel chromatography relies on the principle of molecular sieve. In terms of the above compounds, the similar polarity presents a number of practical difficulties for the separation by silica chromatography. Furthermore, silica has several disadvantages, such as low yields, time-consuming, bulk amount of solvent wastage, poisonous residual solvents (chloroform, ethylacetate, etc.), and is not suitable for large-scale production. The similar polarity and structures of C3 and C4 meant that these two compounds could not be separated using gel chromatography. Based on these, we came to the conclusion that the above compounds could not be well separated by the conventional liquid–solid separation methods. Thus, an efficient

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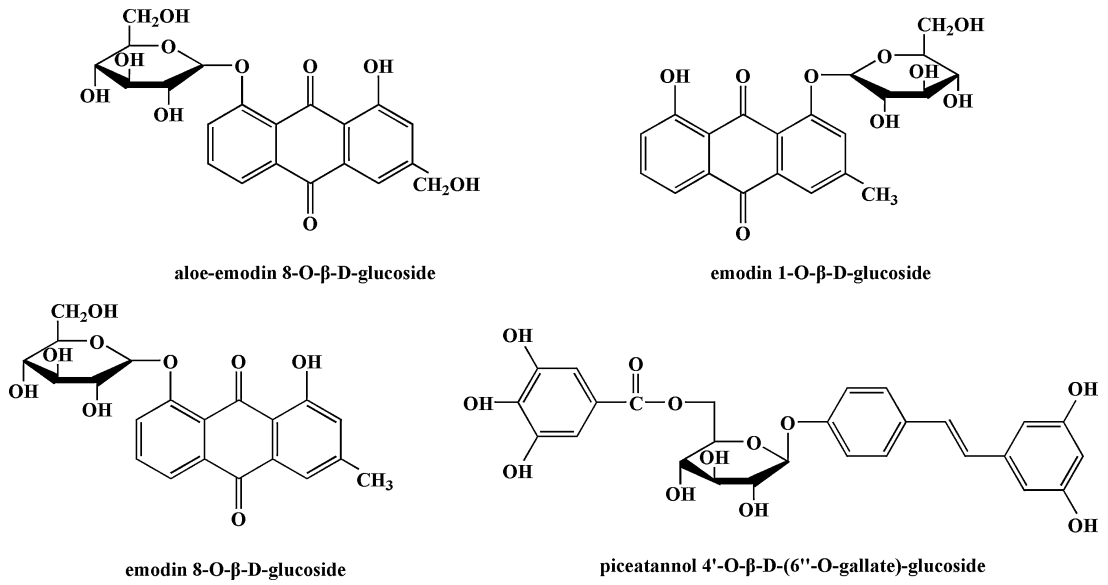

Fig. 1. Chemical structures of aloe-emodin 8-*O*-β-D-glucoside, emodin 1-*O*-β-D-glucoside, emodin 8-*O*-β-D-glucoside and piceatannol 4′-*O*-β-D-(6″-*O*-gallate)-glucoside.

method for separation of the above compounds from *R. tanguticum* becomes necessary.

Macroporous resin (MR) is an adsorption material for column chromatography, which can be reused for thousands of times and is friendly to the environment [15]. MR is durable polar, non-polar, or slightly hydrophilic polymers with high adsorption capacity. It can selectively adsorb targeted constituents through electrostatic force, hydrogen bonding interaction, complexation, and size sieving action. However, low purities of the products were often obtained only by using this single method for pure compound separation. Other separation techniques are needed in combination with MAR to obtain final compounds in a pure form. High-speed counter-current chromatography (HSCCC), a solid support-free liquid–liquid partition chromatography, eliminates the irreversible adsorption of sample onto the solid support matrix that is usually encountered in conventional column chromatography [16]. Furthermore, it can be readily scaled up and provide facile access to high quantity of target compounds. It has been widely applied for separation of bioactive compounds from natural products [17–21]. Thus, HSCCC is common used combination with MR to separate bioactive compounds from natural products [22,23].

In the current paper, an X-5 macroporous resin was used for the enrichment of C1, C2, C3 and C4 from *R. tanguticum*, and an efficient method was successfully established by HSCCC for the separation of these compounds.

2. Experiment

2.1. Apparatus

The HSCCC experiment was conducted on a TBE-300B high-speed counter-current chromatography system (Tauto Biotech, Co. Ltd., Shanghai, China), equipped with three polytetrafluoroethylene (PTFE) preparative coils (i.d. of the tubing = 1.6 mm, total volume = 280 mL) and a 20 mL sample loop. The revolution radius of the apparatus was 5 cm, and the β values of the multilayer coil ranged from 0.5 (at the internal terminal) to 0.8 (at the external terminal). For the apparatus, $\beta = r/R$, where r is the distance from the coil to the holder shaft, and R is the revolution radius or the distance between the holder axis and central axis of the centrifuge. Apparatus revolution speed was regulated with a speed controller within the range of 0–1000 rpm. The system was equipped with a model TBP5002 constant-flow pump (Tauto Biotech, Co. Ltd., Shanghai, China), a model UV-500 detector (XUYUKJ Instruments, Hangzhou, China) operating at 254 nm, and a model N2000 workstation (Zhejiang University, Hangzhou, China). A DC-0506 constant temperature-circulating implement (Shanghai Shunyu Hengping Instruments, Shanghai, China) was used to adjust the experiment temperature.

High-performance liquid chromatography (HPLC) analysis was performed on an Agilent 1200 system (Agilent Technologies Co. Ltd., USA). The Agilent 1200 system was equipped with a G1354A solvent delivery unit, a G1315B UV–vis photodiode array detector, a G1316A column thermostat, a G1313A autosampler, an Agilent Eslipse-XDB C18 (250 mm × 4.6 mm, 5 μm) analytical column, and an Agilent HPLC workstation.

The nuclear magnetic resonance (NMR) spectrometer used in this study was a Mercury-400B NMR spectrometer (Varian Co. Ltd., USA).

2.2. Reagents and materials

All of the solvents used in the HSCCC separation were of analytical grade and purchased from the Jinan Reagent Factory (Jinan, China). The methanol used for HPLC analysis was of chromatographic grade, and was purchased from Yuwang Chemical Ltd. (Shandong, China). Six kinds of MRs, including D101, S-8, HPD-600, X-5, AB-8 and D3520, were purchased from Cangzhou Bon Adsorber Technology Co., Ltd (Cangzhou, China). Dimethyl sulfoxide (DMSO-d_6) was used as the solvent for NMR analysis. Deionized water was used throughout the experiment.

R. tanguticum was collected in Huangzhong County, Qinghai Province, China, in October 2010. The species was identified by Professor Jing Sun (Northwest Institute of Plateau Biology, Chinese Academy of Sciences). Voucher specimens were deposited in the archives of the Northwest Institute of Plateau Biology (HNWP), Chinese Academy of Sciences (QZPMB-0277974).

2.3. Preparation of sample solutions

The dried root (0.5 kg) of *R. tanguticum* was powdered and extracted with 70% ethanol (1 L × 3) under reflux for 2 h. All filtrates were combined and concentrated by rotary evaporation at 60 °C under reduced pressure, producing 0.12 kg of crude extract.

Table 1
Physical properties of macroporous resins.

Type of MR	Surface area (m^2/g)	Average pore diameter (Å)4pc	Polarity
D101	≥400	100–110	Non-polar
S-8	100–120	280–300	Polar
HPD-600	550–600	80	Polar
X-5	500–600	90–100	Weak-polar
AB-8	480–520	130–140	Weak-polar
D3520	480–520	85–90	Non-polar

A 0.12 kg of crude extract was suspended in distilled water (1 L) and extracted with a solvent system composed of petroleum ether (2 L × 3) and ethyl acetate (2 L × 3). The water layer was concentrated to 500 mL to get sample solutions.

2.4. Macroporous resin

2.4.1. Selection of suitable resin for cleaning-up

The physical properties of six kinds of MRs were summarized in Table 1. Before the experiment, these MRs were soaked with 95% ethanol for 24 h to remove some impurities. Then, they were washed once by 4% HCl solution, deionized water, and 4% NaOH solution, respectively. Finally, they were washed by deionized water to remove the monomers and porogenic agents trapped inside the pores during the synthesis process. The wet adsorbents were evaporated under reduced pressure at 60 °C for 12 h to produce dry resins.

All MRs were screened through static adsorption tests as follows: the hydrated resins (equal to 500 mg dry resins) were put into an Erlenmeyer flask and 20.0 mL sample solutions of crude extracts was added. The flasks were then shaken (180 rpm) for 6 h at 25 °C to reach adsorption equilibrium. The solutions after absorption were analyzed by HPLC. The initial concentrations of the target compounds in sample solutions were 7.81, 1.23, 5.28 and 2.08 mg/mL, respectively. The adsorption capacities were calculated based on formula (1). After reaching absorption equilibrium, the resins were firstly washed by deionized water and then desorbed with 10.0 mL 95% ethanol. The flasks were shaken (180 rpm) for 6 h at 25 °C. The desorpted solutions were then analyzed by HPLC to calculate the desorption ratios according to formula (2). All adsorption and desorption experiments were carried out three times, and the results were expressed as mean ± S.D.

$$q_e = \frac{V_0(C_0 - C_e)}{W} \quad (1)$$

where q_e is the adsorption capacity at adsorption equilibrium (mg/g resin); C_0 and C_e are the initial and equilibrium concentrations of target compound, respectively. V_0 is the initial solution volume added into the flask; W is the weight of the dry resin.

$$D = \frac{C_d V_d}{(C_0 - C_e)V_0} \quad (2)$$

where D is the desorption ratio (%); C_d is the concentration of target compound in desorption solutions; V_d is the volume of the desorption solution; C_0, C_e, and V_0 are the same as described above.

2.4.2. Macroporous resin column chromatography

250 mL sample solutions were added into a glass column (120 cm × 5.0 cm, 1 kg X-5 MR). The column was firstly washed by (10 L) water to remove the water-soluble components, and then eluted by 30% ethanol (7.5 L, 5 BV) to remove some undesired compounds, and finally eluted by 40% ethanol (12 L, 8 BV) to enrich the target compounds. The 40% ethanol solutions were collected and evaporated to dryness under reduced pressure at 60 °C. Brown powder (4.8 g) was obtained for further HSCCC separation.

2.5. HSCCC

2.5.1. Selection of two-phase solvent system for HSCCC

The two-phase solvent system was selected according to the partition coefficient (K) of the target compounds. The K value was determined using HPLC as follows. A suitable amount of crude sample was added into a series of pre-equilibrated two-phase solvent systems, and the system 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 then dissolved into 2 mL methanol and analyzed by HPLC. The K value was defined as the peak area of the target compound in the upper phase divided by the peak area of the target compound in the lower phase.

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

The two-phase solvent system used here was composed of chloroform/ethyl acetate/methanol/water (8:1:6:5, v/v). Each solvent was added to a separation funnel and thoroughly equilibrated at room temperature. The upper phase and the lower phase were then separated and degassed via ultrasonic bath for 30 min before use.

Sample solutions for HSCCC separation were prepared as follows: 50 mg of the sample was dissolved in 10 mL of the upper phase.

2.5.3. HSCCC separation procedure

Prior to the introduction of the stationary phase to the column, the column was flushed with methanol to wash out any remaining materials. For each separation, the multilayer column was firstly filled entirely with the lower phase (stationary phase), and the mobile phase was then pumped into the column at a flow rate of 2.2 mL/min while the apparatus was rotated at 800 rpm.

After hydrodynamic equilibrium was reached, as indicated by the emergence of the mobile phase, sample solutions were injected into the separation column through the sample port. The data were immediately collected. The peak fractions were collected manually according to the elution profile, and then evaporated under reduced pressure. Residues were dissolved in methanol for purity analysis by HPLC.

2.6. HPLC analysis and identification of HSCCC peak fractions

An Agilent Eclipse-XDB C18 column (250 mm × 4.6 mm, 5 μm) was used to analyze the crude sample, as well as each fraction from the MR columns and HSCCC. The mobile phase was composed of water (solvent A) and methanol (solvent B). A gradient elution program was performed as follows: 0 min, 10% B; 60 min, 80% B. The flow rate was 1.0 mL/min, the column temperature was 25 °C, and the detection wavelength was 280 nm. Identification of HSCCC fractions was carried out by ^{1}H NMR and ^{13}C NMR.

3. Results and discussions

3.1. Optimization of suitable macroporous resin for cleaning-up

It is a critical step to select the suitable MR for efficient and rapid cleaning-up. A good adsorbent should have a large adsorption capacity and desorption ratios toward the target compounds. Resin screening was carried out according to the chemical characteristic of the target compounds as well as the physical properties of the resins such as polarity, surface areas, and average pore diameter. In this study, six kinds of MRs including D101, AB-8, HPD-600, S-8, X-5 and D3520 were investigated. The adsorption capacities and desorption ratios of the target compounds were shown in

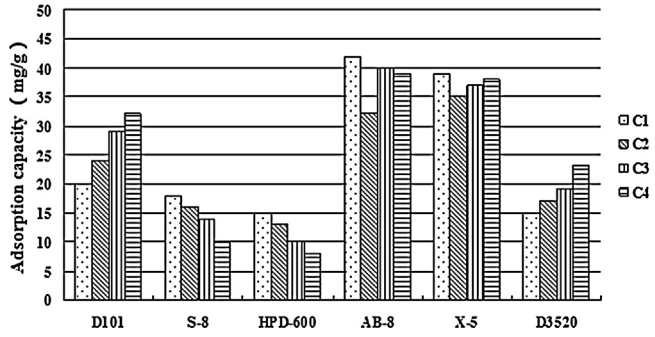

Fig. 2. Adsorption capacities of the target compounds on six different macroporous resins.

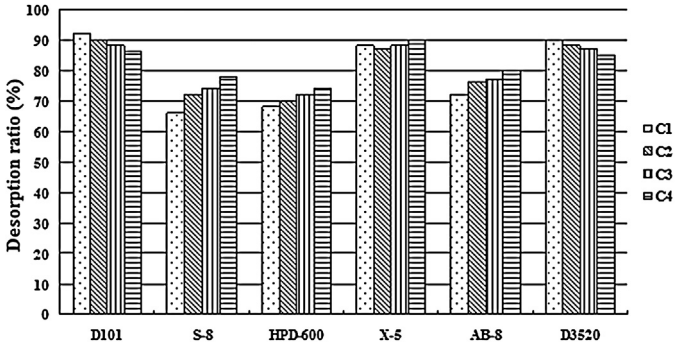

Fig. 3. Desorption ratios of the target compounds on six different macroporous resins.

Figs. 2 and 3, respectively. From the data, it was found that AB-8 and X-5 resins exhibited higher adsorption capabilities than the other resins. Besides, D101, X-5 and D3520 resins exhibited higher desorption ratios than the other resins. That is to say, X-5 resin exhibited both higher adsorption capacity and desorption ratio. Therefore, X-5 resin was selected for the subsequent cleaning-up experiments.

3.2. X-5 macroporous resin column chromatography

In X-5 MR column chromatography, water and ethanol solutions of different concentrations (10–90%) were used to elute the column in series. Each concentration was washed using six times resin bed volume. Fifty elution fractions were collected individually and analyzed by HPLC. The mass of the four compounds in each fraction were calculated (Table 2). As can be seen in Table 2, the target compounds distributed predominantly in 40% ethanol. But, there was 10% of C1 distributing in 30% ethanol and 15% of C4 in 50% ethanol. Thus, it was necessary to choose proper volume of 30% ethanol for removing undesired compounds and 40% ethanol for eluting the targets.

Firstly, the resin was eluted by water to near colorless, then 30% ethanol of different bed volumes (BV) was used to wash the

Table 2
The mass of the target compounds in different ethanol fractions from X-5.

Ethanol concentration (%)	Mass (mg)			
	C1	C2	C3	C4
0, 10	Non	Non	Non	Non
20	Non	Non	Non	Non
30	14.68	Non	Non	Non
40	132.15	22.14	97.15	31.47
50	Non	Non	Non	5.55
60, 70, 80, 90	Non	Non	Non	Non

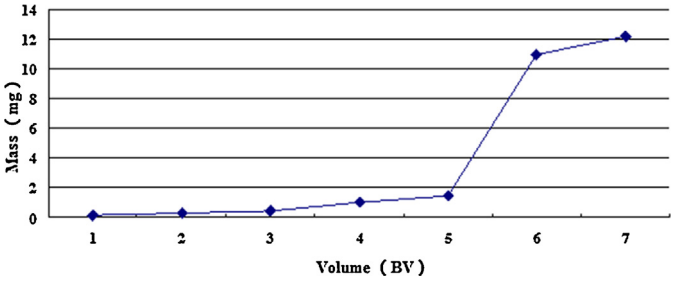

Fig. 4. The elution curve of C1 on X-5 column.

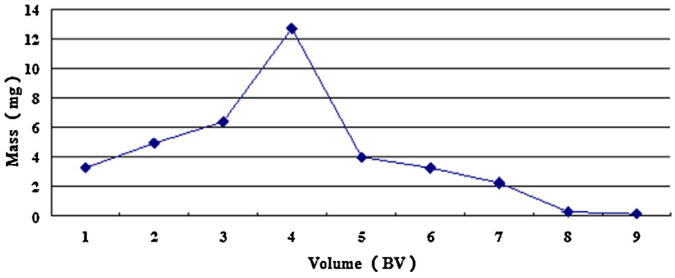

Fig. 5. The elution curve of C4 on X-5 column.

MR column, and the elution solutions were collected and analyzed by HPLC. The elution curve was shown in Fig. 4. It was found that the mass of C1 increased great when the volume of 30% ethanol increased from five BV to six BV. Thus, five BV of 30% ethanol was used to remove undesired compounds. Secondly, 40% ethanol of different BV was used to elute the targets, and elution curve was shown in Fig. 5. It was found that the mass of C4 decreased nearly zero at eight BV. Thus, eight BV of 40% ethanol was used to elute the targets.

Under the optimized conditions, 4.8 g of brown powder was obtained from 250 g raw materials. The HPLC chromatogram was shown in Fig. 6, in which the target compounds showed the contents of 36.2, 5.5, 22.6 and 9.2%, respectively. The recoveries of the target compounds reached 89.0, 85.9, 82.3 and 84.9% respectively after 40% ethanol elution.

3.3. Selection of solvent system and other HSCCC conditions

A successful separation of target compounds using HSCCC requires a careful search for a suitable two-phase solvent system that could provide an ideal range of partition coefficients (K). K values in the range of 0.2 to 5.0 are generally considered to be appropriate for HSCCC separation [24,25]. Besides, the separation

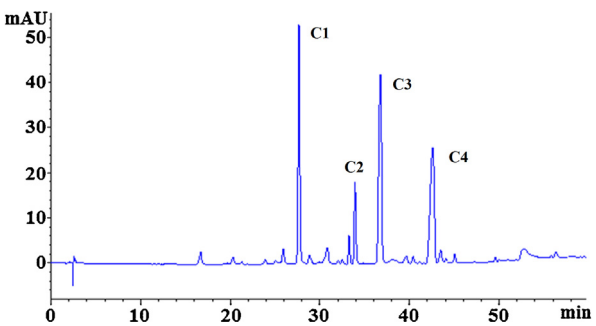

Fig. 6. HPLC chromatograms of the sample from *R. tanguticum*. Conditions: column: Agilent Eslipse-XDB C18 (250 mm × 4.6 mm, 5 μm); mobile phase: methanol/water (methanol: 0–60 min, 10–80%); flow-rate: 1.0 mL/min; column temperature: 25 °C; and detection wavelength: 280 nm.

Table 3
The *K*-values of the target compounds measured in several solvent systems.

Solvent system	Partition coefficient (*K*)			
	C1	C2	C3	C4
Chloroform/methanol/water (8:6:5, v/v)	0.20	0.41	0.48	1.15
Chloroform/methanol/water (8:8:5, v/v)	0.29	0.55	0.60	1.22
Chloroform/methanol/water (8:10:5, v/v)	0.39	0.68	0.72	1.27
Chloroform/*n*-butanol/methanol/water (8:1:6:5, v/v)	0.52	0.93	1.05	1.79
Chloroform/*n*-butanol/methanol/water (8:2:6:5, v/v)	0.83	1.43	1.56	2.50
Chloroform/ethyl acetate/methanol/water (8:0.5:6:5, v/v)	0.17	0.33	0.40	0.68
Chloroform/ethyl acetate/methanol/water (8:1:6:5, v/v)	0.16	0.29	0.37	0.60
Chloroform/ethyl acetate/methanol/water (8:2:6:5, v/v)	0.12	0.21	0.34	0.46

factor between the two components ($a = K1/K2$, $K1 > K2$) should be greater than 1.5 [26]. Furthermore, the target compounds must be stable and soluble in such a solvent system, and the solvent system must separate clearly and quickly into two phases.

In the present paper, a common used solvent system composed of chloroform/methanol/water was selected as a basic system. The results were shown in Table 3. A two-phase solvent system composed of chloroform/methanol/water (8:6:5, v/v) was tested initially, and good *K* values could be obtained. However, it was impossible to separate C**2** and C**3** using this system for the separation factor was only 1.16. Methanol was then added to separate these two compounds. Unfortunately, the separation factor tended to be much smaller with the increasement of the methanol ratio. The same results were obtained by adding *n*-butanol. Besides, the setting time of this solvent system tended to be longer when adding *n*-butanol, which was bad for a high retention of the stationary phase. The addition of ethyl acetate to the solvent system was then evaluated. As shown in Table 3, when the ratio of chloroform/methanol/water was fixed at 8:6:5, the separation factor between C2 and C3 increased from 1.15 to 1.61 as the ratio of ethyl acetate increased from 0 to 2, while the separation factor between C3 and C4 decreased from 1.71 to 1.34; besides, the *K* value of C1 tended to be much smaller. All things considered, the solvent system chloroform/ethyl acetate/methanol/water (8:1:6:5, v/v) was finally selected for the HSCCC separation. The results demonstrated that ethyl acetate could be a good regulator for the chloroform/methanol/water solvent system.

In addition to the *K* values, other important parameters, including the flow rate of the mobile phase and the revolution speed of the apparatus, were studied. Different flow rates of the mobile phase (1.5, 2.2 and 3.0 mL/min) were tested to determine their effect on the separation time and peak resolution properties. The results revealed that a low flow rate led to a lengthy separation time with good peak resolution, whereas a high flow rate produced the opposite effect. Based on these, a flow rate of 2.2 mL/min was selected in the subsequent HSCCC separation procedures. In addition, the revolution speed could have an effect on the stationary phase retention, and the use of a high revolution speed could cause emulsification [27]. Thus, a revolution speed of 800 rpm was used.

3.4. HSCCC separation

Under the optimized conditions, 16 mg of C**1**, 2.6 mg of C**2**, 11.2 mg of C**3**, and 4.5 mg of C**4** were obtained in one step separation within 2 h. The HSCCC chromatogram was shown in Fig. 7. HPLC analysis (Fig. 8) showed that the purities were 96.4%, 98.6%, 97.2% and 95.3% respectively.

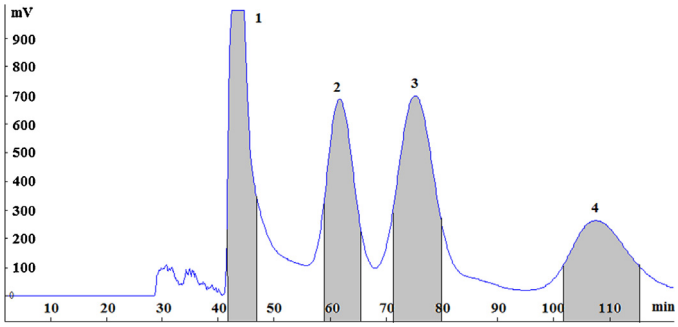

Fig. 7. HSCCC chromatogram of the sample using the chloroform/ethyl acetate/methanol/water (8:1:6:5, v/v) solvent system. Conditions: stationary phase, lowerer phase; flow rate, 2.2 mL/min; revolution speed, 800 rpm; sample amount, 50 mg; separation temperature, 25 °C; detection wavelength, 280 nm; retention of the stationary phase: 75%.

3.5. Structural identification

The identification of the HSCCC fractions was confirmed by ^{1}H NMR and ^{13}C NMR. The detailed data were as follows.

Peak 1 in Fig. 6: ^{1}H NMR (400 MHz, DMSO-d_6): 7.88 (1H, d, J = 7.6 Hz, H-5), 7.72 (1H, m, H-6), 7.71 (1H, d, J = 8.0 Hz, H-7), 7.66 (1H, s, H-4), 7.28 (1H, s, H-2), 5.15 (1H, d, J = 8.0 Hz, anomeric-H), 4.62 (2H, s, Ar-CH_2-OH), 4.62–3.17 (sugar-H). ^{13}C NMR (100 MHz, DMSO-d_6): 187.6 (C-9), 182.1 (C-10), 161.6 (C-1), 158.2 (C-8), 152.3 (C-3), 140.0 (C-6), 134.8 (C-10a), 132.2 (C-4a), 122.4 (C-2), 120.7 (C-4), 120.6 (C-7), 116.0 (C-5, 8a), 115.4 (C-9a), 62.0 (C-CH_2OH), 100.5 (C-1′), 77.3 (C-3′), 76.5 (C-5′), 73.3 (C-2′), 69.5 (C-4′), 60.6 (C-6′). Compared with the data given in ref. [28], peak 1 corresponded to aloe-emodin 8-*O*-β-D-glucoside.

Peak 2 in Fig. 6: ^{1}H NMR (400 MHz, DMSO-d_6): 7.45 (2H, d, J = 8.8 Hz, H-2′, 6′), 7.03 (2H, d, J = 7.6 Hz, H-3′, 5′), 6.98 (2H, s, galloyl-H), 6.94, 6.87 (each 1H, d, J = 16.4 Hz, transolefinic H), 6.45 (2H, d, J = 1.6 Hz, H-2, 6), 6.16 (1H, s, H-4), 4.94 (1H, d, J = 7.2 Hz, H-1″), 4.50 (1H, d, J = 8.8 Hz, H-6″), 4.24 (1H, m, H-6″), 3.77–3.34 (4H, H-2″, 3″, 4″, 5″). ^{13}C NMR (100 MHz, DMSO-d_6): 165.8 (C-7‴), 158.5 (C-3, 5), 156.8 (C-4′), 145.7 (C-3‴, 5‴), 139.0 (C-1), 138.9 (C-4‴), 130.9 (C-1′), 127.6 (C-2′, 6′), 127.3 (C-α, β), 119.2 (C-1‴), 116.4 (C-3′, 5′), 108.8 (C-2‴, 6‴), 104.6 (C-2, 6), 102.1 (C-4), 100.3 (C-1″), 76.5 (C-3″), 73.9 (C-5″), 73.3 (C-2″), 70.0 (C-4″), 63.6 (C-6″). Compared with the data given in ref. [29], peak 2 corresponded to piceatannol 4′-*O*-β-D-(6″-*O*-gallate)-glucoside.

Peak 3 in Fig. 6: ^{1}H NMR (400 MHz, DMSO-d_6): 7.66 (1H, s, H-2), 7.51 (1H, s, H-5), 7.00 (1H, d, J = 2.4 Hz, H-4), 6.48 (1H, d, J = 2.4 Hz, H-7), 5.06 (1H, d, J = 7.6 Hz, anomeric-H), 3.72–3.18 (sugar-H), 2.43 (3H, s, Ar-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): 185.5 (C-9), 182.4 (C-10), 164.8 (C-3), 158.2 (C-8, 1), 146.3 (C-6), 134.4 (C-4a), 134.0 (C-10a), 123.4 (C-7), 121.5 (C-9a), 118.7 (C-5), 109.3 (C-8a), 108.2 (C-4, 2), 101.0 (C-1′), 77.3 (C-3′), 76.4 (C-5′), 73.3 (C-2′), 69.6 (C-4′), 60.6 (C-6′), 21.6 (Ar-CH_3). Compared with the data given in ref. [30], peak 3 corresponded to emodin 1-*O*-β-D-glucoside.

Peak 4 in Fig. 6: ^{1}H NMR (400 MHz, DMSO-d_6): 7.42 (1H, s, H-4), 7.12 (1H, s, H-5), 6.80 (1H, s, H-7), 4.96 (1H, d, J = 7.6 Hz, anomeric-H), 4.66–3.17 (sugar-H), 2.39 (3H, s, Ar-CH_3). ^{13}C NMR (100 MHz, DMSO-d_6): 186.4 (C-9), 182.9 (C-10), 164.8 (C-3), 161.7 (C-1), 161.2 (C-8), 146.1 (C-6), 136.2 (C-10a), 132.2 (C-4a), 124.0 (C-7), 118.9 (C-5), 114.4 (C-8a), 109.4 (C-2, 4), 101.6 (C-1′), 77.3 (C-3′), 76.2 (C-5′), 73.3 (C-2′), 69.5 (C-4′), 60.6 (C-6′), 21.3 (Ar-CH_3). Compared with the data given in ref. [31], peak 4 corresponded to emodin 8-*O*-β-D-glucoside.

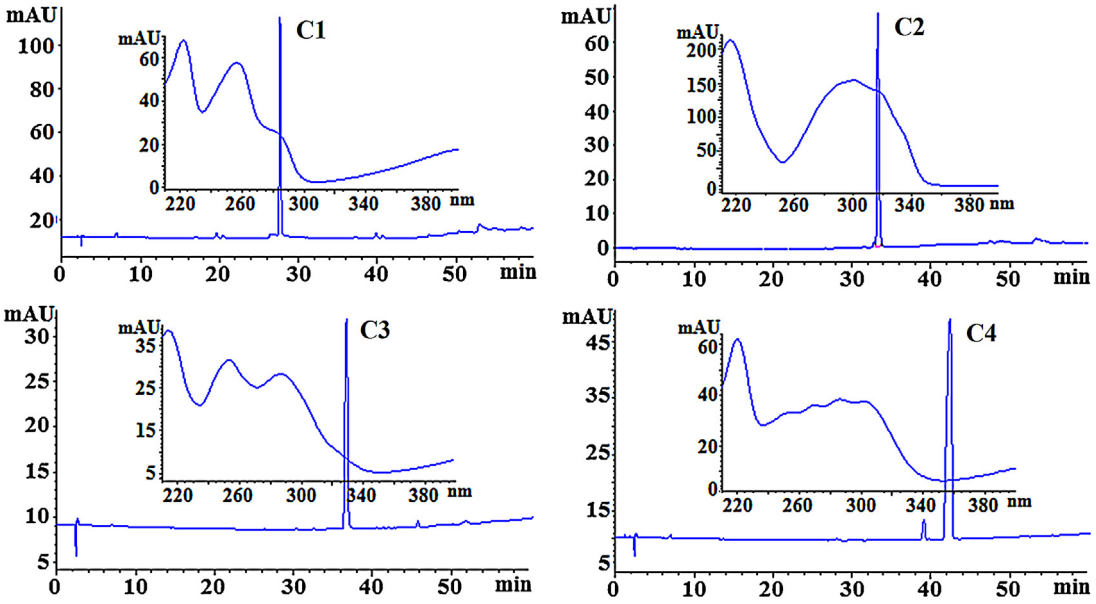

Fig. 8. HPLC chromatograms of the target compounds purified by HSCCC. Conditions: column: Agilent Eslipse-XDB C18 (250 mm × 4.6 mm, 5 μm); mobile phase: methanol/water (methanol: 0–60 min, 10–80%); flow-rate: 1.0 mL/min; column temperature: 25 °C; and detection wavelength: 280 nm.

4. Conclusion

In our paper, an efficient method for rapid enrichment and separation of aloe-emodin 8-*O*-β-D-glucoside, emodin 1-*O*-β-D-glucoside, emodin 8-*O*-β-D-glucoside and piceatannol 4′-*O*-β-D-(6″-O-gallate)-glucoside from *Rheum tanguticum* was established. The results demonstrated that MR coupled with HSCCC could be a powerful technique for separation of bioactive compounds from natural products.

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