Research Article

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A green strategy for obtaining anthraquinones from *Rheum tanguticum* by subcritical water

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Abstract: *Rheum tanguticum* is a traditional Chinese herbal medicine, which contains abundant anthraquinones. In this study, anthraquinones were efficiently extracted from *Rheum tanguticum* by subcritical water extraction (SWE). The parameters of extraction time (33–67 min), temperature (100–200°C), and SW flow rate (1.4–4.6 mL/min) were optimized so as to achieve a high yield of the target product. A high yield of the total anthraquinones was achieved under the optimized SWE conditions of extraction time 54 min, extraction temperature 170°C, and the flow rate 2.0 mL/min. The comparison between the SWE and traditional extraction techniques implied that the SWE is an efficient and green alternative method for the extraction of anthraquinones. Four anthraquinone glycosides were purified from the SWE extract by high-speed counter-current chromatography and identified as emodin-1-O-β-D-glucoside, physcion-8-O-β-D-glucopyranoside, chrysophanol-1-O-β-D-glucoside, and chrysophanol-8-O-β-D-glucoside.

Keywords: *Rheum tanguticum*, anthraquinones, subcritical water extraction, high-speed counter-current chromatography

1 Introduction

Rheum tanguticum (family: Polygonaceae) is widely used for its curative effects against bacterial dysentery, gastric problems, and renal disorders; the herb also cools and detoxifies the body and promotes blood circulation [1]. *R. tanguticum* is included in the Chinese Pharmacopoeia [2]. The herb is distributed mainly in Qinghai–Tibetan Plateau at an altitude of 2,300–4,200 m [3]. Many compounds have been isolated from *R. tanguticum*, including anthraquinone derivatives, anthranone derivatives, distyrene derivatives, tannins, and acyl glycosides, of which anthraquinone derivatives are the main active components [4,5].

Anthraquinones derivatives extracted from *Rheum* possess diverse biological activities, including antibacterial, anti-inflammatory, antipyretic, antitumor, and cardioprotective efficacy [6–8]. Traditionally, anthraquinones were obtained by solvent extraction methods such as reflux extraction (RE), ultrasound-assisted extraction (UAE), and microwave-assisted extraction (MAE) [9–12], and disadvantages such as usage of poisonous solvents, low recovery, and large time consumption were accompanied. Till now, no studies have reported "green" and efficient methods for extracting anthraquinones.

The subcritical water extraction (SWE) is a green and environmental technology, extensively applied in the extraction of environmental pollutants, phytochemicals, and foods [13–15]. Under subcritical conditions, when the temperature is increased, the dielectric constant of water can be reduced, which causes a gradual change in the water polarity, and the medium- and low-polarity components can be extracted [13]. The SWE is advantageous due to its short extraction time, low cost, and the absence of residual toxic solvents in the extracts [16]. The SWE can be directly used in the preparation of food or pharmaceutical products [15].

Separation is another important step in the isolation process of the natural products from the herbal medicine [17]. Till now, the anthraquinones are obtained from the genus *Rheum* mainly using column chromatography with gradient elution [18,19]. However, this separation technique is associated with the following drawbacks: low yield, long operational time, and usage of toxic solvents. High-speed counter-current chromatography (HSCCC) offers high recovery of target components and is widely used for the separation and purification of the natural products [20]. Compared to the traditional approach, HSCCC offers the advantages of wide application range, good adaptability, good reproducibility, high recovery rate, and high separation efficiency [21–23].

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A combination of SWE and HSCCC offers "green extraction" and effective separation of anthraquinones from *R. tanguticum*. In the current study, the effects of the extraction parameters on the recovery of anthraquinones were evaluated by the response surface methodology (RSM). Furthermore, four highly pure anthraquinone glycosides were isolated on a large-scale basis using HSCCC.

2 Materials and methods

2.1 Materials

R. tanguticum was collected from Yushu, Qinghai Province. After air-drying, the roots were ground to a powder using an electric grinder. The pulverized material was screened through a mesh of 20–40 (850–425 μm) maintaining a humidity of 6.4%.

2.2 Chemicals

The standard 1, 8-dihydroxyanthraquinone (purity > 98%) was obtained from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Acetonitrile (HPLC grade) was purchased from Xinlanjing Chemical Company (Yunnan, China). Deionized water was prepared using UPH-II-20T ultra-pure water system (Chengdu Youpu Technology Co. Ltd., Sichuan, China).

2.3 SWE

A laboratory-built SWE apparatus (Figure 1) was employed in this study [24]. The system included a LC-20 AT pump (SHIMADZU, Japan), a GC9800 oven (Shanghai Kechuang Chromatograph Instruments, China), a stainless coil preheater (0.3 mm of inner diameter and 2.0 m of length), a stainless extraction vessel (50 mL), a 26–1,764 back-pressure regulator (TESCOM, USA), and a SS-0006WT-B-P microchannel heat exchanger (Hangzhou Shenshi Energy Conservation Technology Co., Ltd, China). During the SWE process, 10 g of pulverized dry *R. tanguticum* roots was placed in the extraction vessel. The SWE process was conducted in a continuous mode, and the parameters were set by the research needs. The total amount of the anthraquinones was determined by a spectrophotometric method using 1,8-dihydroxyanthraquinone as the reference standard [25]. High-performance liquid chromatography (HPLC) was employed to analyze the SWE extract.

2.4 RSM design

The RSM is a fast and economical mathematical tool used to study the effect of independent factors on the response variables. In the current study, experiments were optimized by the RSM coupled with central composite design (CCD). The key variables selected in this research (Table 1) were extraction time (min, X_1), extraction temperature (°C, X_2), and SW flow rate (mL/min, X_3). Minitab 16 software (Minitab Inc. Pennsylvania, USA) was employed for analyzing the RSM data for modeling and optimizing the anthraquinone extraction process. The yield of the total anthraquinones was calculated by the following equation.

$$\text{Yield (\%)} = \left(\frac{\text{Amount of total anthraquinones in the SWE extract}}{\text{Amount of } R.\ tanguticum}\right) \times 100$$

2.5 HSCCC

A TBE-300A HSCCC system (Shanghai Tauto Biotechnique, China) was employed for further purification and identification of the compounds in the SWE extract. The two-phase solvent system of chloroform–methanol–water (5:3:4, v/v/v) was chosen for HSCCC separation. The rotational speed of HSCCC system was set at 800 rpm. The lower phase of chloroform–methanol–water (5:3:4, v/v/v) system was used as the mobile phase in the separation procedure at 2.0 mL/min. The detector was set at 280 nm. The impure fractions were further purified using an NP7000 preparative liquid chromatograph (Jiangsu Hanbon Sci. & Tech., China). The separation was conducted on a Megres C_{18} column (5 μm, 10.0 × 250 mm), with methanol–water (52:48, v/v) at 15.0 mL/min and the detection wavelength of 280 nm.

2.6 HPLC analysis

Agilent 1260 HPLC (Agilent Technologies, USA) was used to analyze the SWE extract and HSCCC fractions on an XDB-C18 (5 μm, 4.6 × 250 mm) column with the detection wavelength being set at 280 nm. The mobile phase was comprised of solvents A (methanol) and B (deionized water)

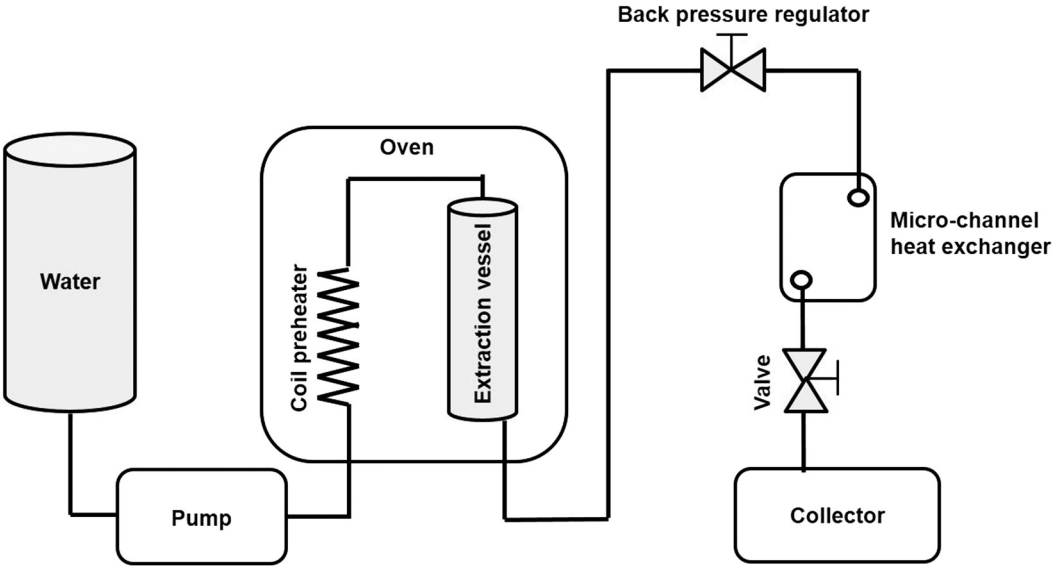

Figure 1: Schematic diagram of the SWE apparatus.

Table 1: Coded and uncoded levels of the independent variables used in the RSM design

X_1 (min)	X_2 (°C)	X_3 (mL/min)	Coded variables
33.0	100.0	1.3	−1.6818
40.0	120.0	2.0	−1
50.0	150.0	3.0	0
60.0	180.0	4.0	1
67.0	200.0	4.7	1.6818

by linear gradient elution: 0–25 min 45–55% A (v/v), 25–45 min 55–80% A (v/v), with the flow rate being set to 1.0 mL/min.

2.7 Structure identification

The structures of the compounds isolated from the SWE extract were elucidated based on the ^{1}H-NMR and ^{13}C-NMR spectrometry (Avance II 400 MHz, Bruker Corporation, Germany).

Ethical approval: The conducted research is not related to either human or animal use.

3 Results and discussion

3.1 Evaluation of the RSM model

The details of CCD matrix that provides the response values used to develop the model are shown in Table 2,

Table 2: CCD matrix and response values for the yield of the total anthraquinones.

Run	Independent variable X_1 (min)	Yield of the total anthraquinones (%)			
		X_2 (°C)	X_3 (mL/min)	Experimental (n = 3)	Predicted
1	40	120	2.0	2.03 ± 0.21	2.12
2	34	150	3.0	3.46 ± 0.17	3.09
3	50	150	4.6	1.55 ± 0.43	1.74
4	60	120	4.0	1.95 ± 0.24	1.74
5	50	199	3.0	2.65 ± 0.29	2.71
6	50	150	3.0	3.54 ± 0.14	3.51
7	50	150	3.0	2.29 ± 0.19	2.12
8	50	150	3.0	2.73 ± 0.23	2.71
9	50	150	1.4	3.48 ± 0.20	3.51
10	40	180	4.0	2.95 ± 0.17	3.09
11	60	180	2.0	3.79 ± 0.12	3.51
12	50	101	3.0	3.38 ± 0.22	3.51
13	50	150	3.0	3.02 ± 0.34	3.16
14	66	150	3.0	2.61 ± 0.27	2.54
15	60	120	2.0	3.31 ± 0.16	3.51
16	40	120	4.0	3.26 ± 0.20	3.32
17	40	180	2.0	2.18 ± 0.19	2.22
18	50	150	3.0	3.63 ± 0.25	3.51
19	60	180	4.0	1.73 ± 0.34	1.74
20	50	500	3.0	2.19 ± 0.27	2.22

while ANOVA of the quadratic model is shown in Table 3. A highly significant (p = 0.000) regression model and a nonsignificant (p = 0.550) lack of fitting were obtained, indicating that the regression model possessed the high accuracy and reliability. The regression coefficient (R^2) was 96.60%, while the adjusted regression coefficient (adj. R^2) was 92.81%, suggesting that the model

Table 3: Analysis of variance (ANOVA) of the fitted quadratic polynomial model

Source	Degree of freedom	Sum of squares	Mean square	*F* value	*p*-value
Model	9	8.76727	0.97414	28.28	0.000
Linear	3	3.70733	1.23578	35.88	0
X_1	1	0.47193	0.47193	13.7	0.005
X_2	1	3.14823	3.14823	91.4	0
X_3	1	0.08717	0.08717	2.53	0.146
Square	3	5.02095	1.67365	48.59	0
X_1^2	1	0.4845	0.80725	23.44	0.001
X_2^2	1	1.47325	1.78408	51.79	0
X_3^2	1	3.06321	3.06321	88.93	0
Two-way interactions	3	0.03898	0.01299	0.38	0.772
X_1X_2	1	0.00543	0.00543	0.16	0.701
X_1X_3	1	0.02626	0.02626	0.76	0.405
X_2X_3	1	0.0073	0.0073	0.21	0.656
Error	9	0.31002	0.03445	—	—
Lack of fit	5	0.16535	0.03307	0.91	0.55
Pure error	5	0.14467	0.03617	—	—
Total	19	9.10504	—	—	—

can reflect the actual experimental data. The linear terms of the extraction time and temperature together with all the quadratic terms were significant ($p < 0.05$). However, insignificance ($p > 0.05$) was detected in the interactions between all terms. Temperature was the most significant parameter, while the SW flow rate was not significant. The yield of the total anthraquinones (Y) after the irrelevancy was eliminated can be expressed as:

$$Y = 3.510 + 0.188X_1 + 0.486X_2 - 0.247X_1^2 - 0.368X_2^2 - 0.482X_3^2$$

The predicted highest yield of the total anthraquinones was 3.717% when the extraction time was 54.124 min, the temperature was set at 170.289°C, the flow rate was maintained at 2.082 mL/min. The optimal conditions were modified as 54 min of extraction time, 170°C of temperature, and 2.0 mL/min of flow rate for convenience. According to the modified conditions, a confirmation experiment of the model accuracy was conducted. The average yield of the total anthraquinones was calculated as 3.581 ± 0.246% ($n = 3$), suggesting that the established model accurately and adequately reflected the extraction process.

3.2 The influence of the operating parameters

Lots factors involving pressure, time, temperature, and flow rate influence the selectivity and extraction efficiency of the SWE [14,15,17]. At a constant temperature, the water was kept in the liquid phase by maintaining pressure at 1–8 MPa. Regulating the pressure did not to improve the extraction efficiency and the yield of the SWE [13,15]. So, the pressure was maintained at 5 MPa throughout the extraction process in our research.

Temperature significantly influenced the selectivity and efficiency of extraction [24,26–29]. High temperature can decrease the dielectric constant of water, improve the nonpolar compounds solubility in water, and improve the target product yield. Nevertheless, compounds may be degraded at a higher temperature by hydrolysis or oxidation [30]. The extraction temperature immensely influenced the yield of the total anthraquinones in the current study. A higher yield of the total anthraquinones can be obtained by maintaining the temperature at the range of 150–180°C (Figure 2a). Previous studies suggested that the recovery of the target product could be enhanced at higher temperatures [24,26]. The higher temperature increased the diffusion coefficient of the solvent, which was conducive to the penetration of the solvent into the matrix, and hence the components diffusion velocity and solubility were improved, and the solvent surface tension and viscosity were reduced [14–16].

Extraction time was an important parameter in the dynamic extraction mode of SWE, which may be associated with extraction temperature, properties of the matrix, and extracts [15]. In the current study, the yield of the compounds increased significantly with the extension of extraction time (Figure 2b). However, the yield did not continue to increase with the increase in the extraction time. In addition, with the increase in extraction time, the volume of extraction solvent increased. The maximum yield was obtained when the dynamic extraction was performed for 54 min.

At a constant extraction time, the yield of the target compound was proportional to the SW flow rate in the dynamic mode of SWE [24]. As can be seen from Figure 2c, the yield of the total anthraquinones improved while the flow rate of SW was increased. However, when the SW flow exceeded 3 mL/min, the yield decreased, for the solvent and matrix contacting time was too short.

3.3 Comparison of SWE with traditional extraction methods

Five methods including reflux extraction (RE), ultrasound-assisted extraction (UAE), microwave-assisted

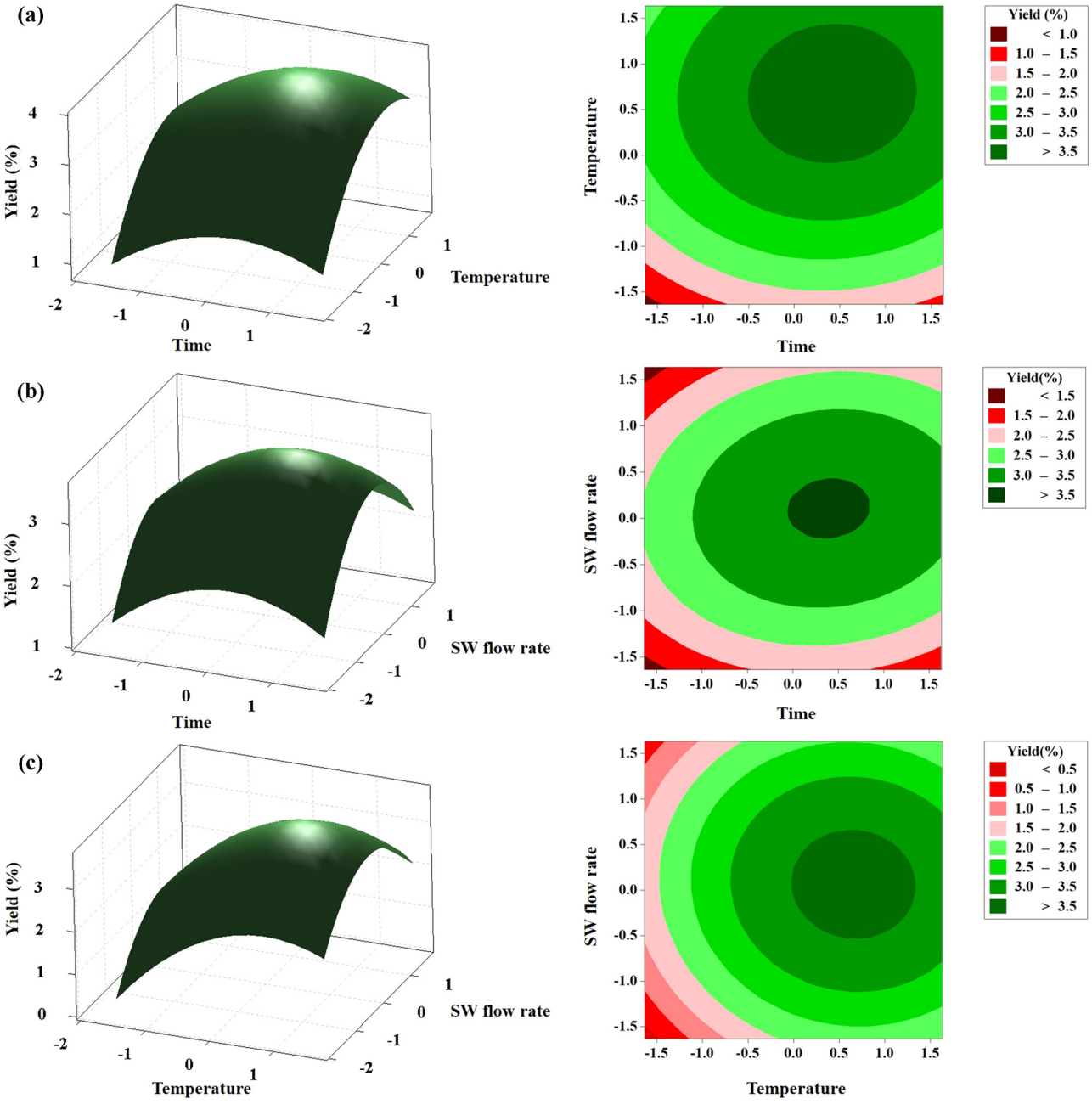

Figure 2: Response surface and contour plots of the yield of the total anthraquinones affected by extraction time, extraction temperature, and SW flow rate. (a) The interactive effect of extraction temperature and time on the yield of the total anthraquinones; (b) the interactive effect of the SW flow rate and extraction time on the yield of the total anthraquinones; (c) the interactive effect of the SW flow rate and extraction temperature on the yield of the total anthraquinones.

extraction (MAE), supercritical fluid extraction (SFE), and SWE for anthraquinones extraction from *R. tanguticum* were compared (Table 4). Jin et al. [9] reported that the extraction of anthraquinones by RE with water as the solvent achieved a yield of 0.672%, while Wu et al. [10] obtained a higher yield of 1.121% by using 95% petroleum ether as the extraction solvent. The extraction frequency of the RE was all three times in the two studies, which prolonged the extraction time and increased the solvent dosage. When it comes to UAE and MAE, a higher yield (1.775% and 1.970%, respectively) was achieved in a shorter time (33 min and 10 min, respectively) than in RE, and with a less solvent consumption [11,12]. Supercritical fluid extraction was conducted by using 95% petroleum ether as dosage of entrainer, and a yield of 2.630% was achieved in 60 min

Table 4: Comparison of subcritical water extraction of anthraquinones with previously reported extraction techniques.

Methods	Solvent	Extraction parameters	(%)	Reference
Reflux extraction	Water	Extraction temperature 100°C; extraction time 20 min; solid–liquid ratio 1:15; extraction frequency three times	0.672	[9]
Reflux extraction	95% petroleum ether	Extraction temperature 60°C; extraction time 30 min; solid–liquid ratio 1:5; extraction frequency three times.	1.121	[10]
Ultrasound-assisted extraction	84% methanol	Extraction temperature 67°C; extraction time 33 min; solid–liquid ratio 1:15; ultrasound power 250 W	1.755	[11]
Microwave-assisted dynamic extraction	60% petroleum ether	Extraction time 10 min; microwave power 520 W; solvent flow rate 4 mL/min	1.970	[12]
Supercritical fluid extraction	CO_2 and 95% petroleum ether (dosage of entrainer)	Extraction time 60 min; extraction pressure 18 MPa; extraction temperature 60°C; dosage of entrainer 450 mL	2.630	[31]
Subcritical water extraction	Water	Extraction pressure 5 MPa; extraction time 54 min; extraction temperature 170°C; SW flow rate 2.0 mL/min	3.581	The present study

[31], which was much higher than that of RE, UAE, and MAE, but a large amount of dosage of entrainer (450 mL) was used. In the present study, SWE was conducted for 54 min with the highest yield (3.581%), which may be because more conjugated anthraquinone was extracted at a certain pressure and temperature. Compared to RE, a higher extraction efficiency was obtained in SWE. Compared to UAE, MAE, and SFE, a higher yield was acquired in SWE. Therefore, SWE is an efficient and green alternative method for anthraquinones extraction.

3.4 Screening HSCCC two-phase solvent system

Three solvent systems were tried in this experiment, and the distribution coefficients (*K* values) of the target compounds were calculated (Table 5). The distribution coefficients were relatively high for the *n*-hexane/ethyl acetate/methanol/water system (5:5:5:5, 2:5:2:5, 2:5:3:5, 2:5:3:2, v/v/v/v) so as to the ethyl acetate/*n*-butanol/methanol/water system (5:1:2:5, 5:0.2:4:4, 4:0.5:3:4, v/v/v/v). Although the allocation coefficient can be reduced gradually by adjusting the proportion of the components, the delamination time of the two systems increased gradually, which diminished the retention rate of the fixed phase. Therefore, these two systems were not suitable for the separation. However, the distribution coefficients of the target components significantly improved on the chloroform/methanol/water (4:3:2, 4:2:5, 5:3:4, v/v/v) system. Therefore, the chloroform/methanol/water system (5:3:4, v/v/v) was selected. The *K* values of the four components were obtained between 0.56 and 2.81, which means that the selected system was suitable for the target components separation.

3.5 HSCCC separation and structure elucidation

HSCCC was employed for separating and purifying the crude samples from the SWE extract of *R. tanguticum* (Figure 3). In this study, 260 mg of crude extract was injected at a time, and three components were obtained within 7 h, namely compound I (13 mg), compound II (26 mg), and compounds III and IV (56 mg). The purity of the compounds I and II was calculated as 93.6% and 97.9%, respectively. Compounds III (16 mg) and IV (19 mg) were further purified by *prep*-HPLC, and the

Table 5: The *K* values of the target compounds in different solvent systems

Two-phase solvent system	Ratio (v/v)	Partition coefficient (*K* value)			
		I	II	III	IV
n-Hexane/ethyl acetate/methanol/water	5:5:5:5	−∞	−∞	−∞	−∞
n-Hexane/ethyl acetate/methanol/water	2:5:2:5	18.25	36.55	77.65	78.24
n-Hexane/ethyl acetate/methanol/water	2:5:3:5	10.50	18.06	37.45	36.00
n-Hexane/ethyl acetate/methanol/water	2:5:3:2	5.30	8.35	15.87	14.15
Ethyl acetate/*n*-butanol/methanol/water	5:1:2:5	39.65	66.54	120.28	105.25
Ethyl acetate/*n*-butanol/methanol/water	5:0.2:4:4	35.12	45.45	87.32	76.80
Ethyl acetate/*n*-butanol/methanol/water	4:0.5:3:4	5.08	9.65	19.35	18.28
Chloroform/methanol/water	4:3:2	4.85	7.45	11.90	12.10
Chloroform/methanol/water	4:2:5	1.59	3.45	6.48	7.90
Chloroform/methanol/water	5:3:4	0.56	1.44	2.72	2.81

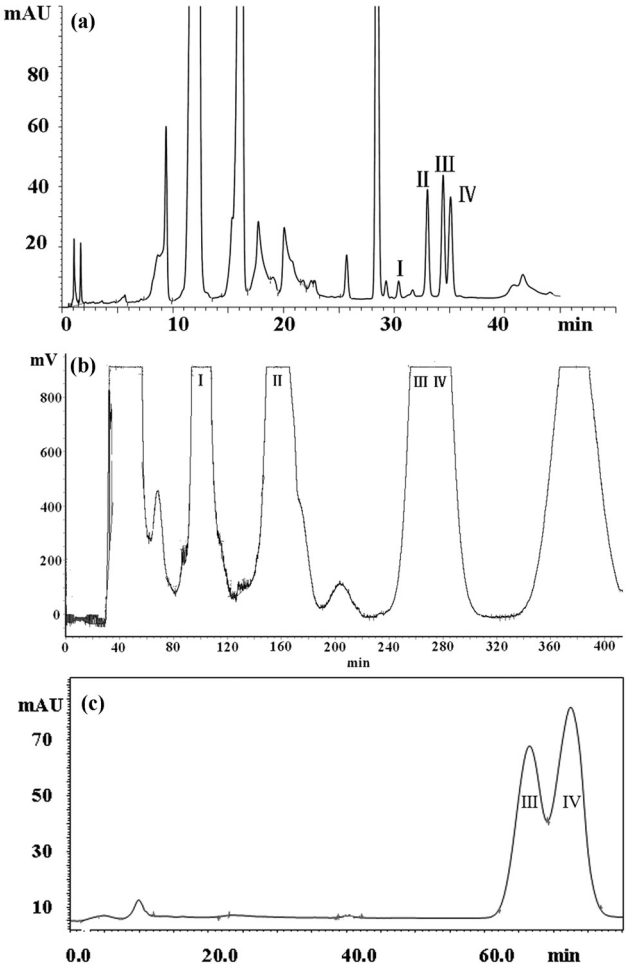

Figure 3: Chromatograms. (a) Crude extracts of *R. tanguticum* by SWE; (b) HSCCC chromatogram of the sample using the chloroform/methanol/water (5:3:4, v/v) solvent system; (c) *prep*-HPLC separation of compounds III and IV; compounds I, II, III, and IV were emodin-1-O-β-D-glucoside, physcion-8-O-β-D-glucopyranoside, chrysophanol-1-O-β-D-glucoside, and chrysophanol-8-O-β-D-glucoside, respectively.

purity of the two substances was 98.9 and 98.3%, respectively.

Data of ^{1}H NMR and ^{13}C NMR obtained in this study were compared with those of the published literature [32,33], and the four fractions were identified (Figure 4). The spectroscopic data for each compound are as follows.

Emodin-1-O-β-D-glucoside (compound I): ^{1}H-NMR (400 MHz, DMSO-d6) δH, ppm: 2.48 (3H, s, Ar-CH_3), 3.72–3.17 (sugar-H), 5.07 (1H, d, J = 7.5 Hz, H-1′), 6.48 (1H, d, J = 2.5 Hz, H-2), 7.00 (1H, d, J = 2.5 Hz, H-5), 7.51 (1H, br.s, H-4), 7.66 (1H, s, H-7), 13.27 (1H, s, OH-8); ^{13}C-NMR (100 MHz, DMSO-d6, TMS) δ, ppm: 21.6 (Ar-CH_3), 60.6(C-6′), 69.6(C-4′), 73.3(C-2′), 76.4(C-5′), 77.3(C-3′), 101.0(C-1′), 108.2(C-2, 4), 109.3(C-8a), 118.7(C-5), 121.5(C-9a), 123.4(C-7), 134.0(C-10a), 134.4(C-4a), 146.3(C-6), 158.2(C-8), 164.8(C-1), 166.3(C-3), 182.4(C-10), 185.5(C-9).

Physcion-8-O-β-D-glucopyranoside (compound II): ^{1}H-NMR (400 MHz, DMSO-d6) δH, ppm: 2.46 (3H, s, A r-CH_3), 3.69–3.27 (sugar-H), 3.91 (3H, s, OCH_3), 5.16 (1H, d, J = 7.6 Hz, anomeric-H), 7.16 (2H, d, J = 2 Hz, H-2, 7), 7.33 (1H, d, J = 2.4 Hz, H-5), 7.46 (1H, br.s, H-4), 13.07 (1H, s, α-OH); ^{13}C-NMR (100 MHz, DMSO-d6, TMS) δ, ppm: 21.4(CH3), 56.1(OCH3), 60.7(C-6′), 76.4(C-5′), 69.7(C-4′), 73.1(C-2′), 77.4(C-3′), 100.7(C-1′), 106.5(C-8a), 106.5(C-7), 107.4(C-5), 114.5(C-9a), 119.2(C-2), 124.1(C-4), 132.1(C-4a), 136.3(C-10a), 147.1(C-3), 160.7(C-6), 161.4(C-8), 164.7(C-1), 181.9(C-10), 186.4(C-9).

Chrysophanol-1-O-β-D-glucoside (compound III): ^{1}H-NMR (400 MHz, DMSO-d6) δH, ppm: 2.41 (3H, s, 3-CH_3), 3.44–3.20 (sugar-H), 5.15 (1H, d, J = 7.0 Hz, 1′-H), 7.17 (1H, br, s, 2-H), 7.47 (1H, d, J = 7.9 Hz, 7-H), 7.68 (1H, br, s, 4-H), 7.83 (1H, dd, J = 7.4, 7.9 Hz, 6-H), 7.85 (1H, d, J = 7.4 Hz, 5-H), 12.8 (1H, s, 1-OH); ^{13}C-NMR (100 MHz, DMSO-d6, TMS) δ, ppm: 21.9(CH_3), 61.1(C-6′), 70.0(C-4′),

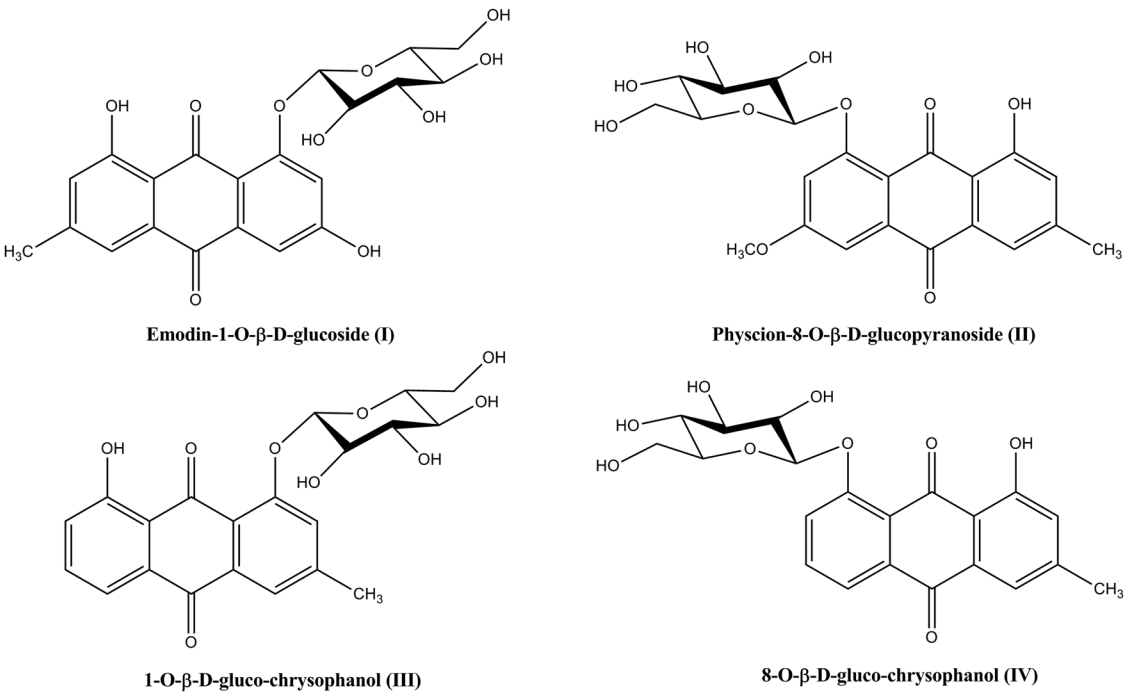

Figure 4: Molecular structures of the four anthraquinone glycosides.

73.8(C-2′), 76.9(C-5′), 77.8(C-3′), 101.1(C-1′), 115.3(C-1a), 119.6(C-8a), 119.9(C-5), 121.0(C-4), 122.9(C-7), 123.9(C-2), 132.7(C-4a), 135.2(C-5a), 136.3(C-6), 148.0(C-3), 158.7(C-8), 161.2(C-1), 182.6(C-10), 187.9(C-9).

Chrysophanol-8-O-β-D-glucoside (compound IV): ^{1}H-NMR (400 MHz, DMSO-d6) δH, ppm: 2.48 (3H, s, 3-CH_3), 3.71–3.20 (sugar-H), 5.13 (1H, d, J = 7.0 Hz, 1′-H), 7.33 (1H, br, s, 2-H), 7.52 (1H, d, J = 7.9 Hz, 7-H), 7.65 (1H, br, s, 4-H), 7.71 (1H, dd, J = 7.4, 7.9 Hz, 6-H), 7.73 (1H, d, J = 7.4 Hz, 5-H), 12.9 (1H, s, 1-OH); ^{13}C-NMR (100 MHz, DMSO-d6, TMS) δ, ppm: 22.3(CH_3), 61.1(C-6′), 70.0(C-4′), 73.8(C-2′), 77.0(C-5′), 77.7(C-3′), 100.9(C-1′), 117.2(C-1a), 118.7(C-5), 121.7(C-4), 123.2(C-7), 124.7(C-2), 132.9(C-4a), 134.9(C-5a), 136.6(C-6), 147.8(C-3), 158.8(C-1), 161.8(C-8), 182.6(C-10), 188.1(C-9).

4 Conclusions

In this study, we used the SWE to obtain anthraquinones from *R. tanguticum*. The SWE parameters including extraction time, temperature, and the SW flow rate were optimized by the RSM coupled with CCD. The results demonstrated that the yield of anthraquinones was significantly affected by the extraction temperature and extraction time. The maximum recovery was obtained with the following SWE conditions: extraction time of 54 min, extraction temperature of 170°C, and SW flow rate of 2.0 mL/min. Four anthraquinone glycosides were further isolated and purified using the HSCCC. This study exhibited that the combined application of SWE and HSCCC can effectively obtain active products from medical plants, providing a novel and effective approach to isolating the natural products from herbs.

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