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Orthogonal test design for optimization of supercritical fluid extraction of daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone from *Stellera chamaejasme* L. and subsequent isolation by high-speed counter-current chromatography

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Abstract

Supercritical fluid extraction (SFE) of daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1- pentanone from *Stellera chamaejasme* L. was performed. An orthogonal L_9 $(3)^4$ test design was applied to select the optimum extraction parameters including pressure, temperature, modifier and sample particle size on yield using an analytical-scale SFE system. The process was then scaled up by 100 times using a preparative SFE system under the optimized conditions of 25 MPa of pressure, 45 °C of temperature, 40–60 mesh of sample particle size and modified CO_2 with 20% methanol. The yield of the crude extract from preparative SFE was 2.65%, which contained daphnoretin 25.2%, 7-methoxy-daphnoretin 22.8% and 1,5-diphenyl-1-pentanone 21.1%, respectively. Then the crude extract was successfully isolated and separated by preparative high-speed counter-current chromatography (HSCCC) with a two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water (10:13:13:10, v/v) by increasing the flow-rate of the mobile phase stepwise from 1.0 to 2.0 ml/min after 90 min. The target compounds isolated and purified by HSCCC were analyzed by high-performance liquid chromatography (HPLC). The separation produced total of 69.2 mg of daphnoretin at 99.2% purity, 63.4 mg of 7-methoxy-daphnoretin at 98.7% purity and 58.3 mg of 1,5-diphenyl-1-pentanone at 98.1% purity from 300 mg of the crude extract in one-step separation. The recoveries of daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone were 90.8, 91.5 and 90.4%, respectively, in HSCCC isolation step and the chemical structure identification was carried out by MS, ^{1}H NMR and ^{13}C NMR.

Keywords: *Stellera chamaejasme* L.; Counter-current chromatography; SFE; Preparative chromatography

1. Introduction

Stellera chamaejasme L. (*Ruixianglangdu* in Chinese), family of *Thymelaeaceae*, is widely distributed in the provinces of northern and western China as well as the regions along the Yellow River. It was firstly recorded at *Sheng Nong's Herbal Classic*, a famous ancient medicine literature. The radix of the genus has been used as the herbal remedy for treating mange, arthritis and asthma [1,2]. Now pharmacological studies have revealed that the roots of *S. chamaejasme* L. possessed antivirus, antitumor, antibacterial and immunomodulatory activities [3,4], and it has been used for clinical treatment of stubborn skin ulcer, chronic tracheitis, tuberculosis and sciatica for many years [5,6].

With regard to the chemical constituents of this genus, the major active chemical constituents are considered to be diterpenoids, coumarin, lignans and flavonoids [7–9], which are often isolated and purified by some conventional protocols of extraction and separation techniques, such as using organic solvents to extract and column chromatography including silica gel and polymide to isolate, in which organic solvents are unfriendly to our environment and the conventional separation

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methods are tedious, time consuming, needing multiple steps, and worse still the sample are adsorbed onto the stationary phase irreversibly.

Two new techniques, supercritical fluid extraction (SFE) and high-speed counter-current chromatography (HSCCC) are widely used to extract and separate natural products from medicinal plants [10–16]. The former uses CO_2 possessed unusual properties such as high compressibility, liquid-like density, high diffusivity, low viscosity and low surface tension. So, supercritical fluid shows a greater ability to diffuse into the ultrafine matrix than the conventional organic solvents, thus improving extraction yield of desired materials from complex matrices. The later, a support free liquid–liquid partition chromatographic technique, eliminates irreversible adsorption of the sample onto solid support [17], has an excellent sample recovery and permits directly introduction of crude samples into the column without more preparation. But there have no reports of using SFE to extract and HSCCC to isolate chemical compounds from *S. chamaejasme* L.

The aim of the present paper, therefore, was first to optimize the suitable extraction conditions of three compounds including daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone (shown in Fig. 1) by an analytical-scale SFE system using an orthogonal test design L_9 $(3)^4$. Then, the extraction was scaled up by 100 times using a preparative-scale SFE system. Subsequently, a preparative HSCCC was used to isolate and separate the three targets from the crude extract by increasing the flow-rate of the mobile phase. A literature search did not yield any early reference about using SFE and HSCCC to extract and separate daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone from the medicinal plant *S. chamaejasme* L.

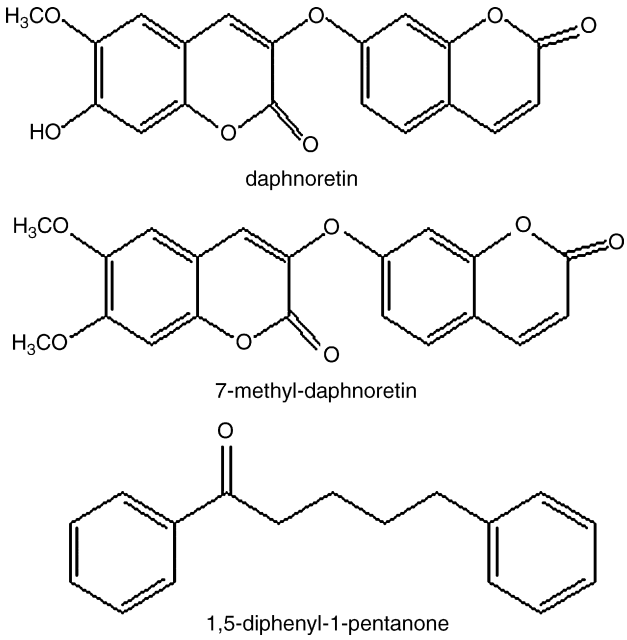

Fig. 1. The chemical structures of daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone.

2. Experimental

2.1. Reagents

Carbon dioxide (CO_2, 99.95%) was obtained from Beijing Analytical Instrument Factory. All solvents and other chemicals including *n*-hexane, ethyl acetate, methanol and acetic acid were analytical grade and purchased from Wulian Chemical Factory, Shanghai, China. While acetonitrile used for high-performance liquid chromatography (HPLC) was HPLC grade (Merck, Germany). Reverse osmosis Milli-Q water (18 MΩ) (Millipore, USA) was used for all solutions and dilutions. The *S. chamaejasme* L. was purchased from a local drug store (Dalian, China).

2.2. Optimization of SFE extraction

A Suprex HA system (Hua An SFE Company, Nan Tong, Jiang Su Province, China) in the SFE mode was used for optimization the extraction conditions. In this study, extraction was accomplished with 100 ml volume extraction vessel. Nine extractions were carried out at temperature of 45, 55 and 65 °C, pressure of 15, 25 and 35 MPa, sample particle size of 10–20, 20–40, 40–60 mesh and two different concentrations of methanol (10 and 20%) were used as modifier. Table 1 shows the SFE experimental conditions for the extraction of daphnoretin, 7-methoxy- daphnoretin and 1,5-diphenyl-1-pentanone from *S. chamaejasme* L. After 1 h of static extraction (no liquid flow), the sample was subjected to dynamic extraction for 1 h by flowing

Table 1
L_9 $(3)^4$ orthogonal test design

Test no.	Factors							
	(A) Pressure (MPa)		(B) Temperature (°C)		(C) Particle size (mesh)		(D) Modifier (methanol %)[a]	
Matrix 1	A_1	15	B_1	45	C_1	10–20	D_1	0
2	A_1	15	B_2	55	C_2	20–40	D_2	10%
3	A_1	15	B_3	65	C_3	40–60	D_3	20%
4	A_2	25	B_1	45	C_2	20–40	D_3	20%
5	A_2	25	B_2	55	C_3	40–60	D_1	0
6	A_2	25	B_3	65	C_1	10–20	D_2	10%
7	A_3	35	B_1	45	C_3	40–60	D_2	10%
8	A_3	35	B_2	55	C_1	10–20	D_3	20%
9	A_3	35	B_3	65	C_2	20–40	D_1	0

[a] Modifier (methanol %) = volume of added methanol (ml)/sample mass (g).

liquid CO_2 at a rate of 0.4 ml/min. The extract was collected and evaporated to dryness at 60 °C under reduced pressure, and weighted. Then 100 ml methanol was used to dissolve the extract for analysis and the contents of the three compounds were determined by HPLC.

2.3. Comparison with Soxhlet extraction (SE)

After extraction conditions were optimized, 50 g of the material (40–60 mesh) was extracted by the analytical-scale SFE system at conditions of 25 MPa of pressure, 45 °C of temperature and modified CO_2 with 20% methanol. At the same time, 10.0 g and 150 ml of ethanol were refluxed by 10 h using Soxhlet apparatus. After extraction, the extract was filtered and analyzed by HPLC. The experiments of SFE and SE were performed in triplicate.

2.4. Scaling-up SFE and preparation of the crude extract

After the SFE conditions were optimized, the extraction was scaled up by 100 times using a preparative system. Five kilograms amount of sample (40–60 mesh) was placed into an extraction vessel with a 1.0×10^4 ml capacity, and extracted statically for 1 h followed by another 5 h dynamically under the optimized conditions at 45 °C, 25 MPa. The flow-rate of carbon dioxide supercritical fluid was set at 40 kg/h, and the extract in supercritical fluid was depressed directly into a separate vessel. The SFE extract (crude extract) was light yellow semi-solid and evaporated to dryness under reduced pressure at 60 °C, which was subjected to subsequent HSCCC isolation and separation.

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

In the present paper, we selected several kinds of two-phase solvent systems. Each solvent system was thoroughly equilibrated in a separatory funnel at room temperature and the two phases were separated shortly before use. The sample solution was prepared by dissolving the crude extract in the solvent mixture of lower phase and upper phase (1:1, v/v) of the solvent system for isolation because the sample was not easily dissolved in either phase.

2.6. HSCCC separation procedure

Preparative HSCCC was carried out with a Model TBE-300A high-speed counter-current chromatography system manufactured by Tauto Biotech, Shanghai, China. The apparatus equipped with a polytetrafluoroethylene three preparative coils (diameter of tube, 1.6 mm, total volume, 260 ml) and a 20 ml sample loop. The β value varied from 0.47 at the internal terminal to 0.73 at the external terminal ($\beta = r/R$, $R = 7.5$ cm, where r is the distance from the coil to the holder shaft, and R, the revolution radius or the distance between the hold axis and central axis of the centrifuge). The HSCCC system was equipped with a model S constant-flow pump, a model 8823A UV detector (Beijing Institute of New Technology Application) operating at 280 nm, and a model N2010 workstation (Zhejiang University, Hangzhou, China).

In each separation, the coil column was first entirely filled with the upper phase (stationary phase), and then the apparatus was rotated at 900 rpm, while the lower phase (mobile phase) was pumped into the column at a flow-rate of 1.0 ml/min. After the mobile phase front emerged and hydrodynamic equilibrium was established in the column, approximately 10 ml of the sample solution containing 300 mg of the crude extract was injected into the head of the column through the injection valve. After 90 min, the flow-rate of the mobile phase was increased to 2.0 ml/min. The effluent of the column was continuously monitored with a UV–vis detector at 280 nm. Peak fractions were collected according to the elution profile.

2.7. HPLC analysis and identification of HSCCC fractions

The analytical HPLC system used throughout this study consisted of one 515 pump (Waters, Milford, MA, USA), a sample injector (Rheodyne, Cotati, CA, USA) with a 20 μl loop, a Waters 996 photodiode array detector, and a model N2000 workstation (Zhejiang University, Hangzhou, China). The crude sample and peak fractions obtained by HSCCC were all analyzed by HPLC. The column used was a reversed-phase Lichrospher C_{18} (250 mm × 4.6 mm I.D., 5 μm) (Hanbang Science, Jiang Su Province, China) with a pre-column equipped with the same stationary phase, the mobile phase was CH_3CN–H_2O–HAC (55:45:2, v/v/v). The flow rate was 1.0 ml/min, and the effluent was monitored at 280 nm.

Identification of HSCCC fractions was carried out by MS (Finnigan MAT 711), ^{1}H NMR and ^{13}C NMR spectra (Varian Unity Inova-500).

3. Results and discussion

3.1. Optimization of the SFE conditions

The first step in the SFE is to optimize the operating conditions to obtain an efficient extraction of the target compounds and avoid the co-extraction of undesired compounds such as fatty acids and their esters.

Since various parameters potentially affect the extraction process, the optimization of the experimental conditions is a critical step in the development of a SFE method. In fact, the fluid pressure, temperature, sample particle size and modifier are generally considered to be the most important factors. Optimization the suitable extraction conditions in SFE can be carried out step-by-step or by using an experimental design. In the present study, all selected factors were examined using an orthogonal L_9 $(3)^4$ test design.

The extract obtained from each test in SFE was weighted and quantitatively analyzed by HPLC to determine the contents of daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone and then the extraction yields of the crude extract and each compound were calculated. The results of experiments presented in Table 2 indicated that the maximum extraction yield of the crude extract was 2.35%, and the maximum extraction

Table 2
L_9 $(3)^4$ test results

Test no.	Parameters				Yield (%)[a]	Yield (mg/g)[b]		
	A	B	C	D		Compound 1	Compound 2	Compound 3
1	A_1	B_1	C_1	D_1	0.38	0.56	0.47	0.45
2	A_1	B_2	C_2	D_2	0.68	2.65	2.32	2.03
3	A_1	B_3	C_3	D_3	1.26	5.02	4.65	4.57
4	A_2	B_1	C_2	D_3	2.35	5.87	5.12	4.88
5	A_2	B_2	C_3	D_1	1.12	2.13	1.56	1.26
6	A_2	B_3	C_1	D_2	1.25	2.56	1.87	1.65
7	A_3	B_1	C_3	D_2	1.87	4.32	3.67	3.26
8	A_3	B_2	C_1	D_3	1.54	3.75	3.35	3.18
9	A_3	B_3	C_2	D_1	0.12	0.23	0.19	0.16

[a] Extraction yield (%) = (the amount of crude extract/sample mass) × 100%.
[b] Extraction yield (mg/g) = the amount of the target compounds/sample mass.

yields of the three compounds were 5.87, 5.12 and 4.88 mg/g sample mass, respectively. In each test, the extraction yields of 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone were lower than that of daphnoretin, and the great yield differences among each set of SFE conditions were obvious. However, we cannot select the best extraction conditions only based on these outcomes in Table 2, and a further orthogonal analysis was warranted. Thus, the K, k and R values were calculated and listed in Table 3.

As seen from Table 3, we can find that the influence to the mean extraction yields of the compounds decreases in the order: D > C > B > A according to the R values. The modifier was found to be the most important determinant of the yield. The extraction yields of the three compounds significantly increased 5.03, 5.91 and 10.02 times as the concentration of the modifier increased from 0 to 20% shown in Fig. 2. The sample particle size was also effect the outcomes, and the extraction yields increased 1.67, 1.73 and 1.72 times when the sample particle size changed from 10–20 to 40–60 mesh. Small size of the raw material would lead to high yield of the compound. The yields decreased 21.3, 15.8 and 15.4% when the pressure increased from 25 to 35 MPa, and 27.4, 27.5 and 25.5% when the extraction temperature increased from 45 to 65 °C, respectively. However, pressure, temperature and sample particle size have no significant influence on the yield of the three targets compared with modifier, and 25 MPa of pressure, 45 °C of temperature and 40–60 mesh of sample particle size seem favorable for the extraction of the compounds shown in Fig. 2 and Table 3. High temperature, large sample particle size and no modifier were not satisfactory. Moderate pressure was favorable to our aim. These results indicated that the optimal conditions for extraction of the three compounds by SFE were 25 MPa of pressure, 45 °C of temperature, 40–60 mesh of sample particle size and modified with 20% methanol.

3.2. Comparison with SFE and SE

Comparison with SFE and SE, they had comparable extraction yields of the three compounds shown in Fig. 3. However, the contents of the three compounds were lower and more co-existed constituents were extracted in SE than that of SFE shown in Figs. 3B and 4, which were hostile for HSCCC separation. Furthermore, the extraction time in SE was longer and more organic solvent was required than that of in SFE. Therefore, SFE was selected to extract the compounds from *S. chamaejasme* L. in our research.

3.3. Preparative-scale SFE

Under the optimal conditions, 5 kg of the powder was extracted by SFE and 132.5 g (extraction yield 2.65%)

Table 3
Analysis of L_9 $(3)^4$ test results

	Compound 1 (mg/g)				Compound 2 (mg/g)				Compound 3 (mg/g)			
	A	B	C	D	A	B	C	D	A	B	C	D
K_1	8.23 [a]	10.75	6.87	2.92	7.44	9.26	5.69	2.22	7.05	8.59	5.28	1.87
K_2	10.56	8.53	8.75	9.53	8.55	7.23	7.63	7.86	7.79	6.47	7.07	6.94
K_3	8.30	7.81	11.47	14.64	7.21	6.71	9.88	13.12	6.60	6.38	9.09	12.63
k_1	2.74 [b]	3.58	2.29	0.97	2.48	3.09	1.90	0.74	2.35	2.86	1.76	0.42
k_2	3.52	2.84	2.92	3.18	2.85	2.41	2.54	2.62	2.60	2.16	2.36	2.31
k_3	2.77	2.60	3.82	4.88	2.40	2.24	3.29	4.37	2.20	2.13	3.03	4.21
R	0.78 [c]	0.98	1.53	3.91	0.45	0.85	1.39	3.63	0.40	0.73	1.27	3.79
Optimal level	A_2	B_1	C_3	D_3	A_2	B_1	C_3	D_3	A_2	B_1	C_3	D_3

[a] $K_i^A = \Sigma$ the amount of target compounds at A_i.
[b] $k_i^A = K_i^A/3$.
[c] $R_i^A = \max\{k_i^A\} - \min\{k_i^A\}$.

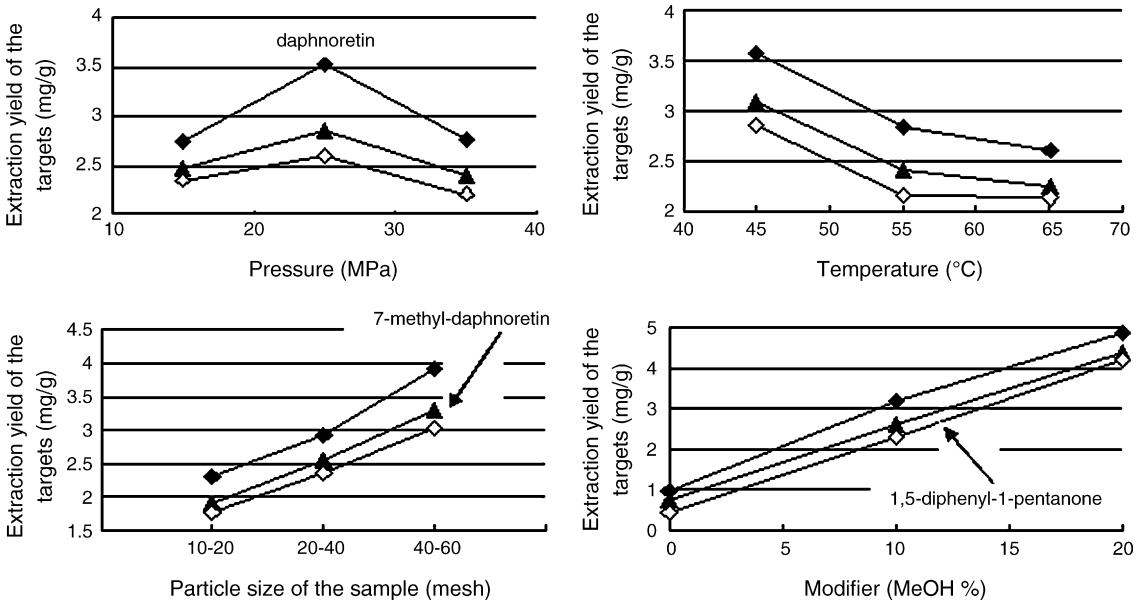

Fig. 2. Effects of pressure, temperature, sample particle size and modifier on yield of daphnoretin, 7-methoxy- daphnoretin and 1,5-diphenyl-1-pentanone from *S. chamaejasme* L.

crude extract was obtained, which mainly contained three peaks including daphnoretin (25.2%), 7-methoxy-daphnoretin (22.8%) and 1,5-diphenyl-1-pentanone (21.1%), and the HPLC chromatogram is shown in Fig. 4A. Subsequently, the crude extract was directly subjected to purification by HSCCC without any preparation.

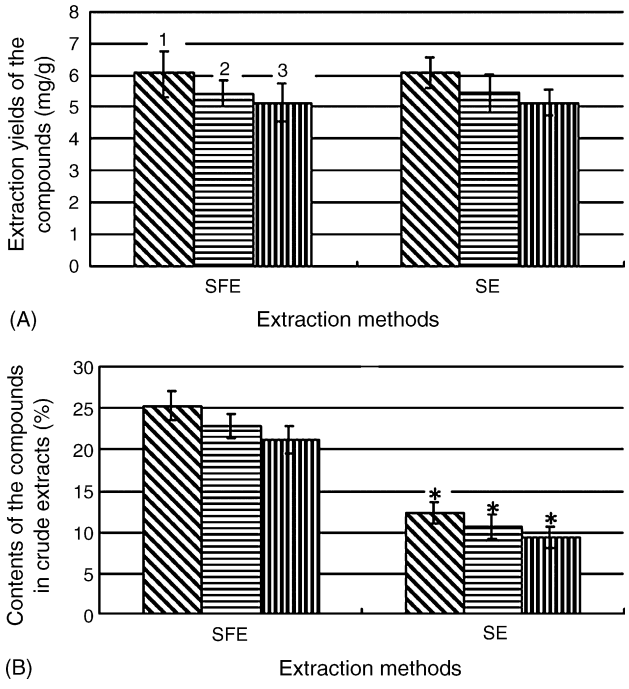

Fig. 3. The comparison of SFE and SE on yields of daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl- 1-pentanone from *S. chamaejasme* L. and their contents in crude extract. 1, 2 and 3 correspond to daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone. The data was expressed as mean ± SD ($n = 3$); $^{*}P < 0.01$.

3.4. Selection HSCCC separation conditions

In HSCCC, the selection of two-phase solvent system is the most important for successful separation, and is also the most difficult step; it is estimated that about 90% of the

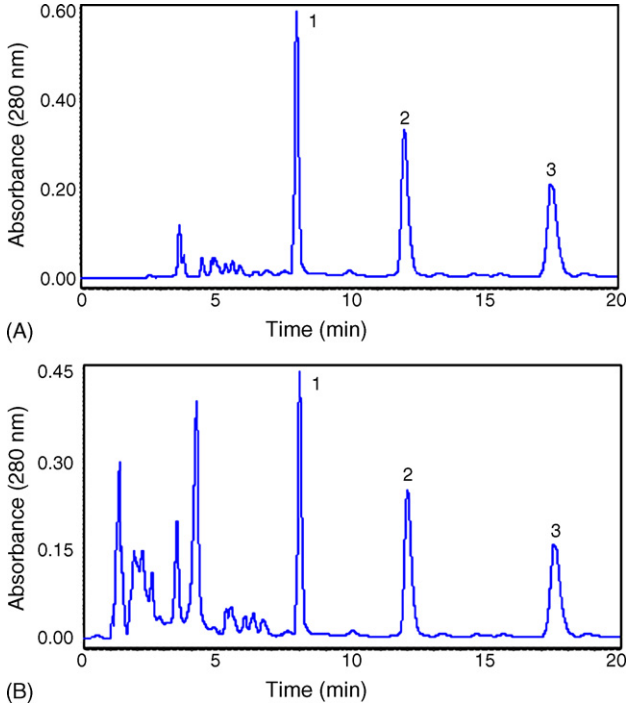

Fig. 4. HPLC chromatograms of the crude extracts from preparative SFE and SE. Column: reversed-phase Lichrospher C_{18} (250 mm × 4.6 mm I.D., 5 μm); mobile phase: CH_3CN–H_2O–HAC (55:45:2, v/v/v); flow rate: 1.0 ml/min; UV wavelength: 280 nm; column temperature: 25 °C; Peaks 1, 2 and 3 correspond to daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone, respectively. (A): SFE extract; (B): SE extract.

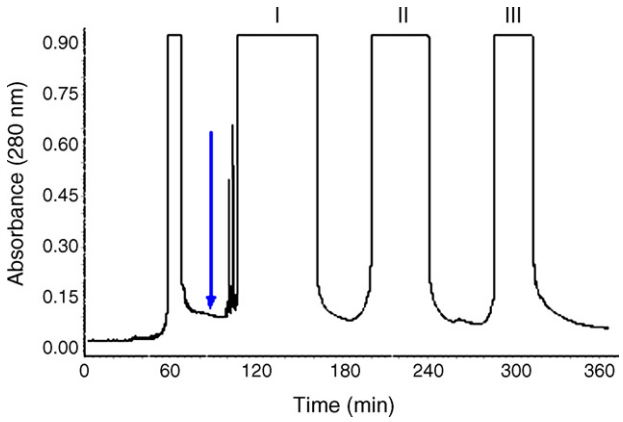

Fig. 5. HSCCC chromatogram of the crude extract from *S. chamaejasme* L. Solvent system: *n*-hexane–ethyl acetate–methanol–water (10:13:13:10, v/v); stationary phase: upper phase; mobile phase: lower phase; flow rate, 0–1.5 h, 1.0 ml/min and 1.5–6 h, 2.0 ml/min; detection wavelength: 280 nm; sample size: 300 mg; retention of stationary phase: 55%; separation temperature: 30 °C; revolution speed: 900 rpm; sample loop: 20 ml. The arrow indicates the flow-rate of the mobile phase was increased stepwise from 1.0 to 2.0 ml/min after 1.5 h.

entire work in HSCCC is spent on that. If only one component requires to be isolated from others, the standard HSCCC method, which uses a constant flow-rate of the mobile phase, could be used. In order to isolate more different compounds, stepwise elution or stepwise increasing the flow-rate of the mobile phase might be adopted [18–20]. In the present paper, several kinds of two-phase solvent systems were investigated. The K and α values of the three compounds were all determined according to the literature [21,22]. The result indicated that the solvent systems composed of *n*-hexane–methanol (1:1, v/v), *n*-hexane–methanol–water (10:4:6, 10:3:7, v/v/v) and *n*-hexane–ethyl acetate–methanol–water (6:4:4:6, v/v/v) had small K values, and the solvent system composed of *n*-hexane–ethyl acetate–methanol–water (1:1:3:3, v/v/v) had large K values, which were all not suitable for our isolation and separation. A two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water at a volume ratio of 10:13:13:10 (v/v) had suitable K values to the compounds 1 and 2, and large K value to the compound 3, which was suitable to purify the three compounds by stepwise increasing the flow-rate of the mobile phase. First, the flow rate of the mobile phase was set at 1.0 ml/min. Although daphnoretin and 7-methoxy-daphnoretin were purified and separated, 1,5-diphenyl-1-pentanone was retained in the column for a long time (10 h) and more mobile phase was required. Then, the flow-rate of the mobile phase was increased to 2.0 ml/min, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone were obtained from other constituents, but daphnoretin could not be separated. Finally, the method with stepwise increasing the flow-rate of the mobile phase was attempted with this two-phase solvent system. That is, the flow-rate of the mobile phase was kept at 1.0 ml/min before 1.5 h, and then increased to 2.0 ml/min after 90 min.

At the same time, the influence of the separation temperature and the revolution speed were also investigated. The temperature has significant effect on K values, the retention of stationary phase and the mutual solvency of the two-phase. After tested at 15, 20, 25, 30, 35 and 40 °C, it can be seen that good result can be obtained when the separation temperature was controlled at 30 °C. The revolution speed has a great influence to the retention of the stationary phase, high rotary speed can increase the retention of the stationary phase. In our experiment, the revolution speed was set at 900 rpm.

Under the above optimized separation conditions, the isolation of the target compounds was achieved with good resolution and the retention of the stationary phase was satisfactory

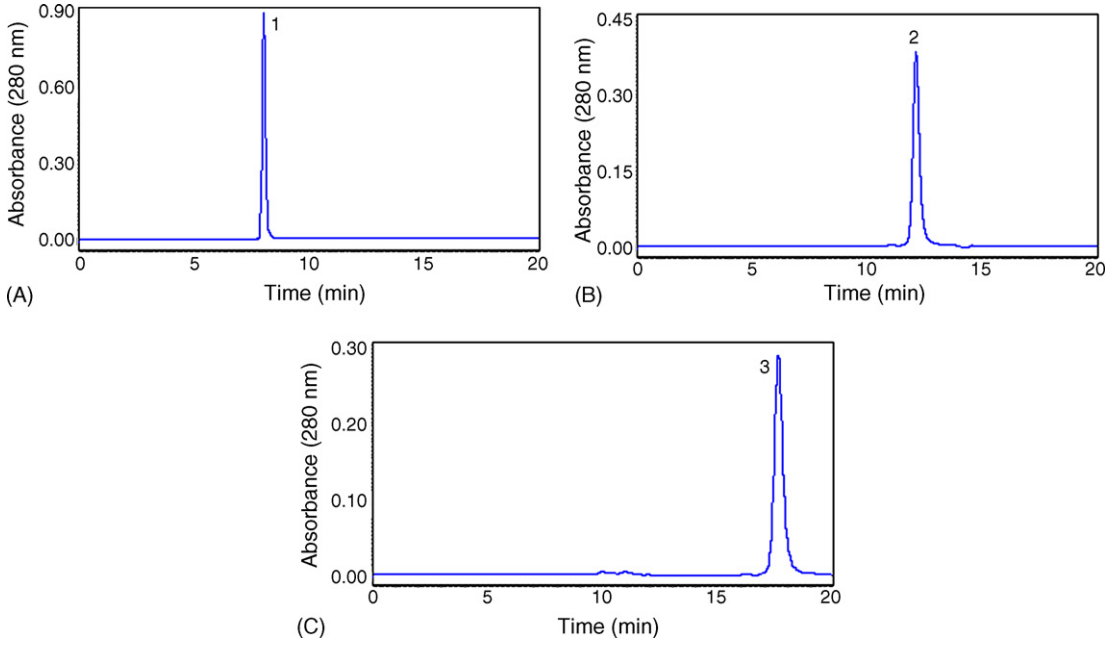

Fig. 6. HPLC chromatograms of daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone purified from *S. chamaejasme* L. by HSCCC. HPLC conditions and the peaks were the same as showed in Fig. 4. (A) Fraction I purified by HSCCC; (B) fraction II purified by HSCCC; (C) fraction III purified by HSCCC.

(55%), and the purification lasted approximately 360 min (HSCCC chromatogram is shown in Fig. 5). After daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone were eluted out, in order to save solvents and time, the remaining compounds in the column were removed by forcing out the stationary phase with pressurized nitrogen gas instead of eluting them with the mobile phase because the stationary phase was not to be reused. Fig. 5 shows the preparative HSCCC isolation of 300 mg of crude extract using the solvent system composed of *n*-hexane–ethyl acetate–methanol–water at a volume ratio of 10:13:13:10 (v/v) by increasing the flow-rate of the mobile phase stepwise from 1.0 to 2.0 ml/min after 3 h. This separation yielded 69.2 mg of daphnoretin at 99.2% purity, 63.4 mg of 7-methoxy-daphnoretin at 98.7% purity and 58.3 mg of 1,5-diphenyl-1-pentanone at 98.1% purity according to HPLC analysis (HPLC chromatograms are shown in Fig. 6). The recoveries of the three compounds were 90.8, 91.5 and 90.4%, respectively in HSCCC isolation step.

3.5. Chemical structure identification

The chemical structure identification of the three compounds was carried out by MS, ^{1}H NMR and ^{13}C NMR spectra as follows. Fraction I: light yellow oil. HR-ESI-MS (*m/z*): 351.1342 $[M-H]^-$ for $C_{19}H_{12}O_7$ (calcd. 351.1349) with ^{1}H NMR and ^{13}C NMR data are in agreement with daphnoretin in the literature [23]. Fraction II: light yellow oil. HR-ESI-MS (*m/z*): 365.1538 $[M-H]^-$ for $C_{20}H_{14}O_7$ (calcd. 365.1536) with ^{1}H NMR and ^{13}C NMR data are in agreement with 7-methoxy-daphnoretin in the literature [24]. Fraction III: yellow oil. HR-ESI-MS (*m/z*): 235.1202 $[M-H]^-$ for $C_{17}H_{16}O$ (calcd. 235.1208) with ^{1}H NMR and ^{13}C NMR data are in agreement with 1,5-diphenyl-1-pentanone in the literature [25].

4. Conclusion

Three compounds including daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone from the traditional Chinese medicinal herb *S. chamaejasme* L. were extracted, separated and purified by SFE and HSCCC. Under optimal conditions i.e., a pressure of 25 MPa, a temperature of 45 °C, a sample particle size of 40–60 mesh and modifier of 20% methanol, the extraction yield of the crude extract was 2.65%, in which daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1- pentanone presented at 25.2%, 22.8% and 21.1%, respectively. At last, pure compounds with purities of over 97% were obtained by HSCCC with a two-phase solvent system composed of *n*-hexane–ethyl acetate–methanol–water at a volume ratio of 10:13:13:10 (v/v) by increasing the flow-rate of the mobile phase stepwise from 1.0 to 2.0 ml/min after 90 min. The results of the present paper demonstrated that SFE and HSCCC are very useful techniques for the extraction, isolation and purification of daphnoretin, 7-methoxy-daphnoretin and 1,5-diphenyl-1-pentanone from *S. chamaejasme* L.

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