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ORIGINAL ARTICLE

Preparation of Sesquiterpenoids from *Tussilago farfara* L. by High-speed Counter-current Chromatography

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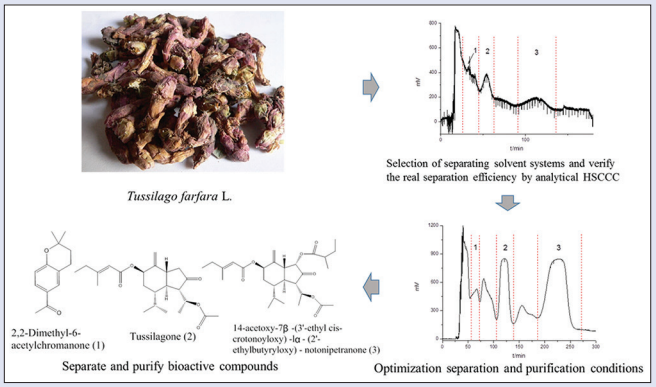
ABSTRACT

Background: Sesquiterpenoids, such as tussilagone, has effects of raising blood pressure, antiplatelet aggregation, and anti-inflammation activities, which is regarded as index compound for quality control of *Tussilago farfara* L. **Objective:** This study was aimed to obtain an effective method for fast isolation of sesquiterpenoids from *T. farfara* L. by high-speed counter-current chromatography (HSCCC). **Materials and Methods:** A solvent optimization method for HSCCC was presented, i.e., the separation factors of compounds after the *K* values of solvent system should be investigated. **Results:** A ternary solvent system of n-hexane:methanol:water (5:8:2, v/v/v) was selected and applied for the HSCCC, and 56 mg of tussilagone (2) was isolated from *T. farfara* L., along with two other sesquiterpenoids 5.6 mg of 2,2-dimethyl-6-acetylchromanone (1) and 22 mg of 14-acetoxy-7 β-(3′-ethyl cis-crotonoyloxy)-lα-(2′-methylbutyryloxy)-notonipetranone (3) by HSCCC with high purities. Their chemical structures were elucidated by liquid chromatography-mass spectrometry and nuclear magnetic resonance experiments. **Conclusion:** These results offered an efficient strategy for preparation of potentially health-relevant phytochemicals from *T. farfara* L., which might be used for further chemical research and pharmacological studies by preparative HSCCC.

Key words: High-speed counter-current chromatography, preparation, sesquiterpenoids, solvent selection, *Tussilago farfara* L.

SUMMARY

- The real separation efficiency has been verified by analytical HSCCC.
- A solvent optimization method for HSCCC was presented and applied to separate and prepare active compounds.
- A method for rapid and effective separation of target compound Tussilagone with high yield and purity from the flower buds of Tussilago farfara.
- Two other compounds 2,2-Dimethyl-6-acetylchromanone and 14-acetoxy-7β-(3′-ethyl cis-crotonoyloxy) -lα- (2′-methylbutyryloxy) - notonipetranone has been obtained with high purities from flower buds of Tussilago farfara.



Abbreviations used: HSCCC: High-Speed Counter-Current Chromatography; LC-MS: Liquid Chromatograph-Mass Spectrometer; NMR: Nuclear Magnetic Resonance; TCM: Traditional Chinese Medicine; HPLC: High Performance Liquid Chromatography; ESI-MS: Electrospray Ionization Mass Spectrometry; PE: petroleum ether

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INTRODUCTION

Kuan Donghua, the dried flower buds of *Tussilago farfara* L., was traditionally used in China, Europe, South Africa, and Siberia for the treatment of asthma, bronchitis, cough, and phlegm.[1-3] Sesquiterpenoids, such as tussilagone, were representative active compounds of *T. farfara* L. It displayed multiple biological activities such as raising blood pressure, antiplatelet aggregation, and anti-inflammation.[4-6] Moreover, they are also the major index compounds for the quality control of *T. farfara* L.[5]

So far, quite a few literatures have been developed for the analysis of sesquiterpenoids *T. farfara* L., but they only focus on the leaf of *T. farfara* L.[3] Moreover, very few data on chemical components of the flower of *T. farfara* L. are available.

Traditional separation methods such as gel column chromatography or thin-layer chromatography are often high costing, time-consuming, low recovery yields, and adsorption behavior on the stationary phase.[7,8] High-speed counter-current chromatography (HSCCC),[9] as a unique form of free liquid–liquid partition chromatography, eliminates irreversible adsorption of sample on stationary phase in conventional column chromatography. This technology has recently been used to effectively separate and purify a number of natural products.[10-12] HSCCC solvent systems of these separations were determined only according to the partition coefficient (K) which was analyzed by high-performance liquid chromatography (HPLC). However, no report has been published on the factors of the solvent system, revolution speed, flow rate, and

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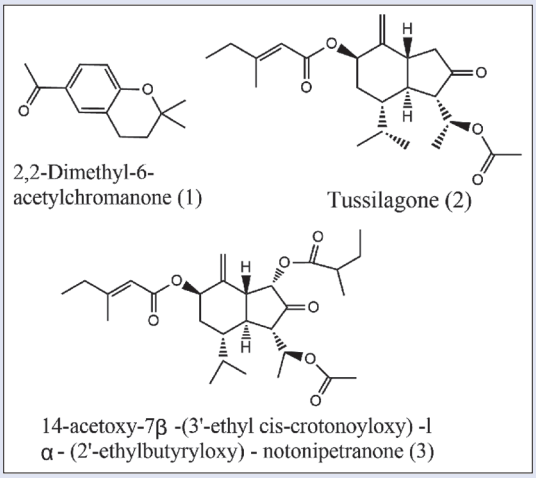

Figure 1: Chemical structures of three compounds isolated from *Tussilago farfara* L.

other factors in depth during the separation, which were the guidance for solvent system. Thus, it is necessary to develop a more useful method to verify optimal solvent system and actual separation effect to instead of the partition coefficient (K).

In the present study, an efficient HSCCC method was developed for fast isolation of sesquiterpenoids from *T. farfara* L. A solvent system optimization method of HSCCC was established, combined partition coefficient (K) determination with HSCCC analysis. Based on this approach, a ternary solvent system was selected and applied for the HSCCC, and index compound tussilagone (2) was isolated from *T. farfara* L., along with two other sesquiterpenoids 2,2-dimethyl-6-acetylchromanone (1), and 14-acetoxy-7 β-(3'-ethylcis-crotonoyloxy)-lα-(2'-methylbutyryloxy)-notonipetranone (3) [Figure 1]. Their chemical structures were elucidated by nuclear magnetic resonance (NMR) and high-resolution mass spectrometry (MS) experiments. The results were discussed herein.

MATERIALS AND METHODS

Apparatus

Preparative HSCCC was realized on the model OptiChrome™- a double-action column semi-preparation HSCCC (Counter-Current Technology Co., Ltd., Jiangsu, China). The instrument was a fully automated system consisting of a 2 polytetrafluoroethylene (PTFE) preparative coils (i.d of tube, 1.59 mm; revolution radius, 80 cm; range of β 0.50–0.80; total volume, 360 mL) and a 20 mL sample loop. Separating effect could be predicted by a FastChrome-30 analytical HSCCC (Counter-Current Technology Co., Ltd., Jiangsu, China) with a PTFE preparative coils (total volume, 25 mL; range of β, 0.56–0.91), a 1 mL sample loop. The HSCCC system was equipped with a model ultraviolet (UV)-3000 detector (Beijing Chuang Xin Tong Heng Science and Technology Co., Ltd), a model CXTH3000 workstation. The analytical HPLC equipment was measured on an Agilent 1260 system (Agilent Technologies Inc., USA). NMR spectra were obtained on a Bruker500 MHz DD2 NMR spectrometer (Bruker, Swiss); and the electrospray ionization (ESI)-MS was operated on Waters SQD (Waters, USA).

Reagents and materials

All chemicals including organic solvents used for sample preparation and HSCCC were of analytical grade and purchased from Chuandong Chemical Factory (Chongqing, China). Methanol and acetonitrile used for HPLC analysis were of chromatographic grade (Adamas-beta Chemical reagent Co., Ltd. Switzerland) and water was distilled. Tussilagone (purity >98%) was purchased from the National Institutes for Food and Drug Control (Beijing, China).

The flower buds of *T. farfara* L. were collected in Wuxi, Chongqing, China in 2012 and identified by Dr. Junjiang Lv, Kunming Institute of Botany, Chinese Academy of Sciences. A voucher specimen (No. 2012006) was deposited at the herbarium of the Department of Pharmacy, University of Chongqing, Chongqing.

Preparation of the crude extract

Dried buds of *T. farfara* L. (400 g) were crushed to powder and extracted with 80% methanol (1:5 w/v, 3×) under reflux for 3 h, and the combined aqueous methanol extract was evaporated at 60°C under reduced pressure. The methanolic extract (81 g) was subsequently dissolved in water, partitioned with equal volume petroleum ether (PE) (60–90°C, 3×). Solvent was evaporated, and the residue was freeze-dried to afford the PE fraction (8.10 g), which was subjected to subsequent HSCCC separation. The crude sample and extract fraction were analyzed by HPLC with an SB-C_{18} column (4.6 mm × 150 mm, 5 μm) at 25°C. Methanol: water (85:15, v/v) was used as the mobile phase. The flow rate was 1.0 mL/min and the injection volume of sample was 20 μL. Tussilagone was monitored by the use of the diode array detector (DAD) at 220 nm. Commercially available standard of tussilagone (98%) was used as a standard stock solution for preparing calibration curve. The calibration curve was $Y = 28221X + 112.63$ ($r^2 = 0.9993$). Where Y was the peak area of Tussilagone, and X was the content of Tussilagone (mg/mL).

Measurement of partition coefficients (*K*)

The partition coefficient (K) of tussilagone in different solvent systems was determined by HPLC as follows: 20 mg of crude extract was put in a 4 mL centrifuge tube, to which 10 mL of each phase of the preequilibrated two-phase solvent were added. After the tube was shaken vigorously, the solution was quickly separated; then the upper and the lower phases were analyzed by HPLC to obtain the partition coefficient of the target compound. The peak area of the upper phase was recorded as A_U and that of the lower phase was recorded as A_L. The K value was calculated according to the following equation: $K = A_U/A_L$.

Verification of solvent systems by analytical high-speed counter-current chromatography

Three solvent systems were determined primarily including normal hexane:methanol:water (5:8:2, v/v/v), PE:methanol:water (5:8:2, v/v/v), and PE ethyl acetate (EtAC):methanol:water (1:0.5:1.1:0.3, v/v/v/v). Three solvent systems were prepared, respectively, by adding the solvents to a separation funnel according to the volume ratios and thoroughly equilibrated by vigorously shaking. Then, it was left overnight, and two phases were separated and degassed by sonication for 30 min before use. The sample solution for HSCCC was prepared by dissolving 100 mg crude extract into 5 mL solvent mixture of upper and lower phases (1:1, v/v).

Anal-HSCCC separation was performed as follows: The multilayer coil column was first filled with upper phase as a stationary phase. The lower aqueous mobile phase was then pumped into the head end of the column inlet at a flow rate of 1 mL/min, whereas the apparatus was rotated at 1800 rpm. About 1 mL of the sample solution was loaded via the injection valve after the system reached hydrodynamic equilibrium, as indicated by the mobile phase eluting from the tail outlet. The whole separation experiment was conducted at 25°C. The UV detector was set at 220 nm and collected with a fraction collector set at 5 min for each tube. The

optimal solvent system was determined by comparing resolution and equilibration time of two-phase solvent system.

Prehigh-speed counter-current chromatography separation procedure

For the pre-HSCCC separation, an optimal solvent system consisting of normal hexane:methanol:water (5:8:2, v/v/v) was shaken vigorously in a separating funnel and equilibrated at room temperature for overnight. After each layer was degassed by sonication for 30 min, the lower phase was used as the mobile phase, and the upper phase was used as the stationary phase.

The multilayer coil column was first entirely filled with the upper phase at the flow rate of 20 mL/min. The lower phase was then pumped into the head end of the column inlet at a flow rate of 3 mL/min, whereas the apparatus was rotated at 800 rpm at 25°C. When the hydrodynamic equilibrium was established, 800 mg of crude extract was dissolved in 20 mL mixture solution of lower phase and upper phase of 1:1, v/v was injected through the sample loop. Peak fractions were concentrated and dried according to the chromatogram recorded at 220 nm in the monitor. After the separation was completed, the solvents in the column were pushed out, and the retention of stationary phase was measured. The purified compounds were stored at −20°C before HPLC and NMR analyses. Resolution was computed according to the chromatogram.

Structure analysis of preparation product

HPLC analyses of the crude sample and the HSCCC peak fractions were performed with an SB-C18 column (150 mm × 4.6 mm, 5 μm) at 25°C. Methanol:water (85:15, v/v) was used as the mobile phase. The flow rate was 1 mL/min and the column effluent was monitored by the use of the DAD at 220 nm. ESI-MS data were measured on an acquity SQD MS. Identification of each isolated target compound was carried out by ^{1}H-NMR and ^{13}C-NMR.

RESULTS

Preparation of the crude sample

Isolation and purification of the main constitutes are important to provide a better understanding of their bioactivity. *T. farfara* L. contains mainly sesquiterpenoids, flavonoids, etc., Considering the weak polarity of target compounds, the crude extract was successively extracted with PE, EtAC, and n-butyl alcohol with the increasing polarity.[13,14] The contents of tussilagone in different extracts were summarized in Table 1. After the extraction partitioning, the major tussilagone (87.3%) was enriched in PE extract. The tussilagone content in PE extract is five times more than that in ethanol (ET). The enriched sample was subjected to the HSCCC for further separation [Figure 2 and Table 1].

Table 1: The contents of tussilagone in different extracts

	Extract (g)	Peak area of tussilagone	Tussilagone content of extract (mg/g)	Tussilagone mass (mg)	Proportion (%)
Methanol extract	80.9	2595.4	8.21	664	
Petroleum ether extract	8.1	14121	71.6	580	87.3
Ethyl acetate extract	8.5	59.7	-	-	

Table 2: The partition coefficients (*K*) of tussilagone in different solvent systems

Solvent system	Ratio (v/v)	*K* value of tussilagone
I Petroleum ether:methanol:water	5:8:2	2.35
II n-hexane:methanol:water	5:8:2	2.18
III n-hexane:methanol:water	5:7:2	5.16
IV n-hexane:ethyl acetate:methanol:water	1:0.5:1:0.5	3.56
V n-hexane:ethyl acetate:methanol:water	1:0.5:1.1:0.3	1.57

Measurement of partition coefficient (*K*)

The optimization of two-phase solvent system is critical in the HSCCC process, and it may consume up to 90% of the time in procedure development. Moreover, partition coefficient (*K*) was the first and the most important parameter in the selection of a suitable solvent system. The suitable *K* value for HSCCC should be within the range of 0.5–2.0.[15] A small *K* value elutes the solute closer to the solvent front with lower resolution, whereas a larger *K* value tends to give better resolution but broader, more dilute peaks due to a longer elution time. In this study, a series of experiments were performed to determine the optimal solvent systems for the HSCCC. As shown in Table 2, five different compositions of two-phase solvent system were listed for the *K* values of the target compound tussilagone. Results indicated that tussilagone provided satisfactory *K* values in I, II, and V, which had small *K* values. Solvent system III and IV were abandoned because *K* values of tussilagone were too large, which might result in long elution time and board peak. Hence, the solvent system I, II, and V was chosen as the two-phase solvent systems to further investigate actual separation effect by anal-HSCCC.

Verification of the solvent systems by analytical high-speed counter-current chromatography

As the most important parameter in solvent system, *K* value describes the polarity and particular tendency of the solvent system. However, in actual separation, the influences of separation conditions such as temperature, retention of the stationary phase, and flow rate cannot be accurately reflected via *K* value of solvent systems, let alone the existence of multiple solvents emulsification, equilibrium time of multiple solvent systems, and resolution under high-speed rotation. Therefore, it is necessary to verify the separation effect of solvent systems which had preliminary screened by *K* values. Furthermore, as another important parameter in HSCCC separation, a good resolution between the peaks which will benefit for the separation of active compounds from traditional

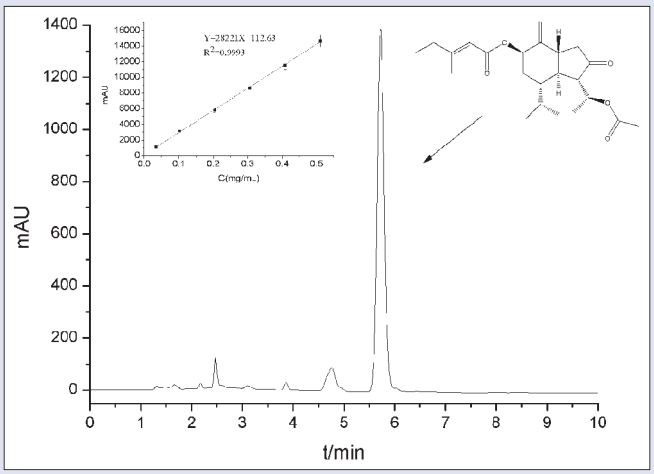

Figure 2: High performance liquid chromatography chromatograms of tussilagone in *Tussilago farfara* L. and calibration curve of standard substance tussilagone (RSD < 0.94% [*n* = 5]) (80 mm × 56.6 mm)

Chinese medicine should be ensured rapidly. Because of the advantages of small column volume and low solvent consumption, anal-HSCCC was used to verify the preliminary systems and obtain separation conditions in this study. This selection method based on the combination of the selection of partition coefficient (*K*) and verification of solvent system by anal-HSCCC was of obvious advantages and prospective application.

The influence of separation temperature, revolution speed, and flow rate was investigated as well. In this experiment, the separation temperature was set 25°C, the revolution speed was set 1800 rpm, and the flow rate was kept at 1 mL/min. To obtain the optimal solvent system, three solvent systems screened preliminary were investigated by anal-HSCCC. The results indicated that tussilagone can be separate effectively from PE extract of *Tussilago farfara* L. in solvent system I (PE: methanol: water with [5:8:2, v/v/v]), II (n-hexane:methanol:water [5:8:2, v/v/v]), and V (n-hexane:EtAC:methanol:water [1:0.5:1.1:0.3]). Although tussilagone in solvent system V had a smaller *K* value than solvent system I and II, it needed more time (120 min) and more mobile phase, and the chromatogram peak was extended in the actual separation. The separation results in solvent system II, which can be separated completely within 80 min were similar to solvent system I, superior to others. On the other side, emulsification induced by PE of the solvent system I under high-speed rotation will affect solvent system equilibrium and stability of the baseline. Compared with the solvent system **I**, quadruple and multisystems can avoid emulsification caused by PE of the solvent system, but more equilibrium time was needed, and the solvent system became complex and unstable in the actual separation process. Only in system II, a good resolution between three peaks can be obtained, and peak 1 can be successfully separated. Considering these aspects, solvent system II was selected to separate PE extract sample. Under the optimized HSCCC condition, peak 1, 2, and 3 were successfully separated with stationary retention ratio of 70% and resolution of 3.5 between peak 2 and 3. The elution curve of the anal-HSCCC was shown in Figure 3.

Prehigh-speed counter-current chromatography separation of target compounds

Based on the optimal solvent system, the influences of flow rate and rotation speed were also investigated. The results showed that 3.0 mL/min and 800 rpm were satisfactory for the separation of target compounds from PE extract.

Under the optimal pre-HSCCC conditions, index compound tussilagone was isolated and purified from PE extract of *T. farfara* L., along with two other sesquiterpenoids [Figure 4]. The purity of compound 1, 2, and 3 was 97.3%, 98%, and 98.2%, respectively, as determined by HPLC [Figure 5]. The amount of three separated products in one-step preparation process could reach 5–10 mg, 50–60 mg, and 20–30 mg, respectively. The retention of stationary phase reached 73%, and total separation time was about 260 min. Two other compounds were also obtained in this process. The resolution of peak 2 and 3 was 2.3. This method takes short equilibrium time of the solvent system and total separation time.

Structure analysis of isolated compounds

The chemical structure identification of the two compounds was carried out by MS, ^{1}H-NMR, ^{13}C-NMR spectra as followers. Compound 1: Colorless gum; EI-MS m/z 218[M]+, 203, 163, 147, 43; UV λ_{max}: 239 nm, IRγ_{max} (KBr) CM$^{-1:}$ 3430, 2960, 2935, 2865, 2370, 1455, 1375, 1060; ^{1}H-NMR (500 MHz, $CDCl_3$) δ: 1.49 (6H, s, $CH_3\times2$), 2.59 (3H, s,-$COCH_3$), 2.77 (2H, s, H-2), 7.01 (1H, d, J = 8.8 Hz, H-8), 8.13 (1H, dd, J = 8.8,2.2 Hz, H-7), 8.44 (1H, d, J = 2.2 Hz, H-5); ^{13}C-NMR (125 MHz, $CDCl_3$)δ; 80.5 (C-1), 48.7 (C-2), 191.8 (C-3), 130.4 (C-4), 135.6 (C-5),

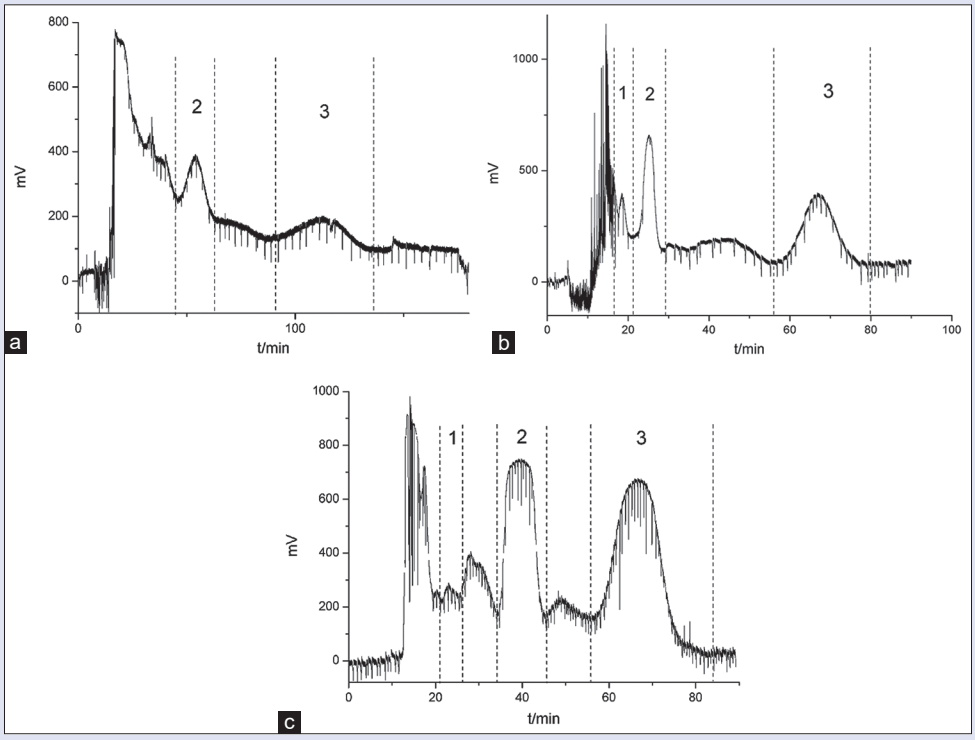

Figure 3: Analytical high-speed counter-current chromatography chromatogram of petroleum ether extract of *Tussilago farfara* L. Two-phase solvent system: (a) (n-hexane: ethyl acetate:methanol:water [1:0.5:1.1:0.3]), (b) (petroleum ether:methanol:water [5:8:2, v/v/v]), and (c) (n-hexane:methanol: water [5:8:2, v/v/v]); 1: 2,2-Dimethyl-6-acetylchromanone, 2: tussilagone, 3: 14-acetoxy-7 β-(3′-ethylcis-crotonoyloxy)-lα-(2′-methylbutyryloxy)-notonipetranone (80 mm × 56.6 mm)

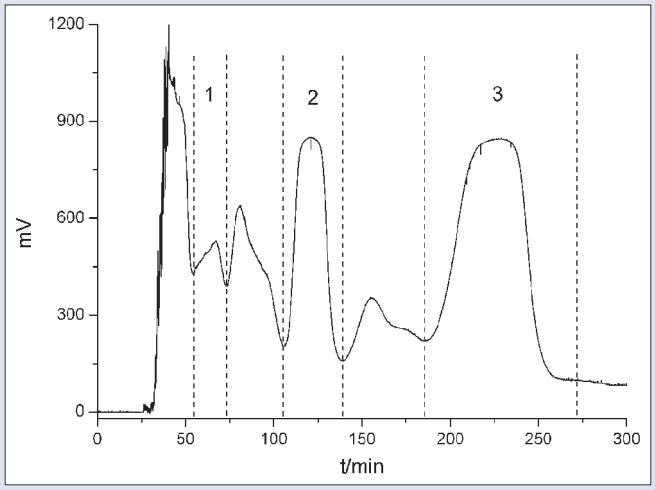

Figure 4: Pre high-speed counter-current chromatography chromatogram of petroleum ether extract of *Tussilago farfara* L. Two-phase solvent system: (n-hexane:methanol:water [5:8:2, v/v/v]), sample size 800 mg crude sample dissolved in 10 mL of the upper phase and 10 mL of the lower phase; 1: 2,2-dimethyl-6-acetylchromanone, 2: tussilagone, 3: 14-acetoxy-7 β-(3′-ethylcis-crotonoyloxy)-lα-(2′-methylbutyryloxy)-notonipetranone (80 mm × 56.6 mm)

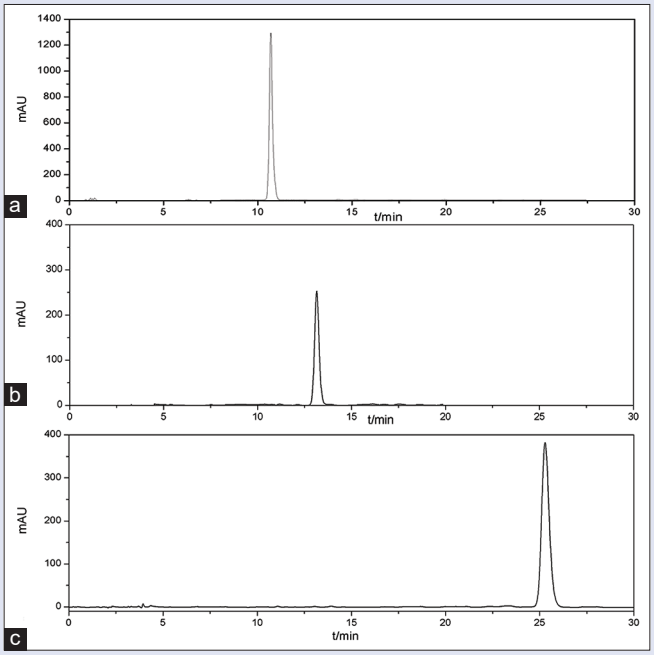

Figure 5: High performance liquid chromatography chromatograms of three fractions obtained by high-speed counter-current chromatography preparation. (a) 1: 2,2-Dimethyl-6-acetylchromanone: (b) 2: tussilagone, (c) 3: 14-acetoxy-7 β-(3′-ethylcis-crotonoyloxy)-lα-(2′-methylbutyryloxy)-notonipetranone (80 mm × 80 mm)

119.3 (C-6), 128.3 (C-7), 119.2 (C-8), 163.6 (C-9), 26.7 (CH_3 × 2), 26.5 (-$COCH_3$), 196.5 (-$COCH_3$). The data above were identical with 2,2-dimethyl-6-acetylchromanone in literature.[7]

Compound 2: Colorless prism-like crystal; EI-MS m/z 390 [M] +, 330,276,216,97; ^{1}H-NMR (500 MHz, $CDCl_3$)δ: 2.17 (1H, dd, *J* = 16.9, 13.9 Hz, H-1α), 2.40 (1H, ddd, *J* = 16.9, 1.0, 5.5 Hz, H-1 β), 2.51 (1H, dd, *J* = 3.0, 11.0 Hz, H-3), 1.48 (1H, m, H-4), 1.97 (1H, dddd, *J* = 2.0, 2.0, 11.0, 14.0 Hz, H-5), 1.45 (1H, ddd, *J* = 2.0,11.0,14.0 Hz, H-6α), 2.07 (1H, dt, *J* = 2.0, 14.0 Hz, H-6 β), 5.58 (1H, t, *J* = 3.0 Hz, H-7), 2.59 (1H, ddddd, *J* = 2.0, 2.0, 5.9, 11.5, 13.9 Hz, H-9), 5.14 (1H, s-like, H-10Z), 4.79 (1H, d, *J* = 1.0 Hz, H-10E), 2.30 (1H, dqq, *J* = 3.0, 6.8, 6.9 Hz, H-11), 0.99 (3H, d, *J* = 6.5 Hz, H12), 0.79 (3H, d, *J* = 7.0 Hz, H13), 5.10 (1H, dq, *J* = 3.2,6.6 Hz, H-14), 1.22 (3H, d, *J* = 6.5 Hz, H3-15), 5.63 (1H, qt, *J* = 1.3,1.3 Hz, H-2'), 2.18 (2H, dq, *J* = 1.3,7.5 Hz, H-4'), 1.07 (3H, t, *J* = 7.5 Hz, H3-5'), 2.15 (3H, d, *J* = 1.3 Hz, H6'), 2.10 (3H, s, $OCOCH_3$); ^{13}C-NMR (125 MHz, $CDCl_3$) δ:42.4 (C-1), 215.0 (C-2), 57.3 (C-3), 49.2 (C-4), 44.0 (C-5), 31.3 (C-6), 73.0 (C-7), 146.2 (C-8), 42.4 (C-9), 110.2 (C-10), 27.7 (C-11), 21.5 (C-12), 15.5 (C-13), 69.7 (C-14), 15.3 (C-15), 166.0 (C-1'), 114.7 (C-2'), 162.1 (C-3'), 33.9 (C-4'), 12.0 (C-5'), 19.0 (C-6'), 171.0 ($OCOCH_3$), 21.5 ($OCOCH_3$). These data were in agreement with tussilagone in literature.[11]

Compound 3: EI-MS m/z 513 [M + Na] +. ^{1}H (500 MHz, $CDCl_3$) δ: 5.46 (1H, H-1 β), 2.60 (1H, H-3 β), 2.03 (1H, H-4α), 1.91 (1H, H-5 β), 5.52 (1H, H-7α), 2.60 (1H, H-9 β), 4.80 (1H, H-10), 5.20 (1H, H-10'), 2.37 (1H, H-11), 1.01 (2H, H-12), 0.82 (2H, H-13), 1.23 (3H, H-15), 5.59 (1H, H-2'), 2.16 (2H, H-4'), 1.07 (3H, H-5'), 2.10 (3H, H-6'), 2.37 (1H, H-2"), 0.89 (3H, H-4"), 1.15 (3H, H-5"), 2.14 (3H, $COCH_3$). ^{13}C-NMR (125 MHz, $CDCl_3$) δ:72.4 (C-1), 208.1 (C-2), 56.6 (C-3), 44.1 (C-4), 44.2 (C-5), 30.3 (C-6), 73.5 (C-7), 140.8 (C-8), 47.2 (C-9), 113.4 (C-10), 27.6 (C-11), 21.6 (C-12), 15.4 (C-13), 69.6 (C-14), 15.8 (C-15), 165.8 (C-1'), 114.5 (C-2'), 162.3 (C-3'), 33.8 (C-4'), 11.7 (C-5'), 18.9 (C-6'), 174.6 (C-1"), 44.3 (C-2"), 26.8 (C-3"), 11.9 (C-4"), 16.8 (C-5"), 21.3 ($COCH_3$), 170.9 ($COCH_3$). The data above were identical with 14-acetoxy-7 β-(3'-ethyl cis-crotonoyloxy)-lα-(2'-m ethylbutyryloxy)-notonipetranone in literature.[7]

DISCUSSION

In the solvent selection, the partition coefficient (K) was used to determine whether a solvent system was suitable for separation of target compound. However, experimental factors such as emulsification and equilibrium time under high-speed rotation during the actual separation cannot be determined by *K* value. Moreover, the partition coefficient (*K*) was the only parameter in the present HSCCC separation. Therefore, it is necessary to measure partition coefficient (*K*) and actual separation efficiency by anal-HSCCC. Furthermore, the anal-HSCCC experiments visually displayed the separating process, and it can help to predict separation efficiency by pre-HSCCC more accurately. Hence, the separation of object products should screen solvent systems with partition coefficient (*K*) measured by HPLC at first, and then verify the feasibility of solvent system which *K* value was in the range of 0.5–5 by anal-HSCCC; finally select the best solvent system for pre-HSCCC. This method has been successfully used in the preparation of sesquiterpenoids from *T. farfara* L., and also can be widely used for preparative isolation and purification of various natural products.

CONCLUSION

In this paper, a solvent optimization method for HSCCC was presented, combined partition coefficient (*K*) determination with HSCCC analysis. Based on this approach, the partition coefficients (*K*) of target compound in different solvent systems were measured by HPLC at first, and then the real effects of different solvent systems were verified by anal-HSCCC; finally, the optimal solvent was selected via comprehensive analysis based on the partition coefficients (*K*) and the

real effects in different solvent systems. Using this method, a ternary solvent system of n-hexane:methanol:water (5:8:2, v/v/v) was selected and applied for HSCCC, 56 mg index compound tussilagone (2) was isolated from *T. farfara* L., along with two other sesquiterpenoids 5.6 mg 2,2-Dimethyl-6-acetylchromanone (1) and 22 mg 14-acetoxy-7 β-(3'-ethylcis-crotonoyloxy)-lα-(2'-methylbutyryloxy)-notonipetranone (3) were obtained with purities of 97.3%, 98%, and 98.2% from 800 mg PE extract, respectively. These result offered an efficient strategy for isolation of potentially bioactivity phytochemicals from natural products.

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Conflicts of interest

There are no conflicts of interest.

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