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Ionic liquid based ultrasonic assisted extraction of isoflavones from *Iris tectorum* Maxim and subsequently separation and purification by high-speed counter-current chromatography

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ABSTRACT

We developed an ionic liquid based ultrasonic assisted extraction (ILUAE) method for the extraction of the three isoflavones, namely tectoridin, iristectorin B and iristectorin A from *Iris tectorum* Maxim of the Iridaceae family. Three kinds of 1-alkyl-3-methylimidazolium ionic liquids with different alkyl chain and anion were investigated. The results indicated that ionic liquids (ILs) showed remarkable effects on the extraction yield of isoflavones. In addition, the ILUAE, including several ultrasonic parameters, such as the concentration, extraction time and solvent to solid ratio have been optimized. Under these optimal conditions (e.g., with 30 min extraction time and the solvent to solid ratio of 30 ml/g), this approach gained the highest extraction yields of tectoridin (37.45 mg/g), iristectorin B (2.88 mg/g) and iristectorin A (5.28 mg/g). Meanwhile, tectoridin, iristectorin B and iristectorin A in the ILUAE extract were separated and purified successfully through the high-speed counter-current chromatography (HSCCC) with a two-phase solvent system consisting of *n*-butanol–water (1:1, v/v). The additional advantage of this approach is that 60.21 mg tectoridin, 4.33 mg iristectorin B and 8.24 mg iristectorin A with more than 95.0% purities have been obtained from 400 mg ILUAE extract of *I. tectorum* within 5 h and one-step elution under the most optimized conditions (e.g., a flow rate of 2.0 ml/min, 900 rpm and the wavelengh of 280 nm). The obtained fractions were successfully analyzed by HPLC and identified by ^{1}H-NMR and ^{13}C-NMR.

1. Introduction

Iris tectorum Maxim, a very popular Chinese traditional medicinal herb belongs to the Iridaceae family, is widely distributed in China. Traditionally, its root and rhizome have been used for the treatment of inflammation, cough, tonsillitis and pharyngitis [1]. More recently, it has been used to fight against severe acute respiratory syndrome (SARS). Therefore, *I. tectorum* has been listed as therapeutical treatments appeared on the list of treatment therapy, and has caught some doctor's attention as it helps people fight against this new infectious disease. Many studies on chemical compounds have revealed that *I. tectorum* contains a variety of important chemicals, such as isoflavones [2–4], quinones [5] and triterpenoids [6–8]. These chemical compounds are believed to be responsible for a number of biological activities of anti-atherosclerosis [9], anti-oxidant [10] and anti-tumor [11]. Meanwhile, these isoflavone compounds are also often used for the quality control of *I. Tectorum*.

Ionic liquids (ILs) are low melting salts that form liquids composed entirely of ions. They have negligible volatility, low flammability, large liquid range, high thermal, chemical stability, strong solubility power and a number of possible variations in cation and anion features which allow the fine tuning of the IL properties [12]. Recently, ILs have been used extensively in areas of sample enrichment [13], sample preparation [14], chemical synthesis [15], catalysis [16], process of green chemistry [17] and analytical as well as separation processes [18,19]. Among them, alkylammoniums are the most important types of ILs, such as 1-butyl-3-methylimidazolium tetrafluoroborate, 1-hexyl-3-methylimidazolium bromide and 1-octyl-3-methylimidazolium bromide (Table 1), which have characteristics of hydrophilic and miscible in any proportion with water.

High-speed counter-current chromatography (HSCCC) is a liquid–liquid partition chromatography technique. The stationary phase of HSCCC is retained in the separation columns by gravity and centrifugal force field. Therefore, HSCCC possesses unusual properties such as low solvent consuming, rapid separation and high recovery [20,21]. Additionally, the significant feature of HSCCC is that it can separate several target compounds in a

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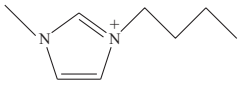
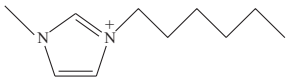
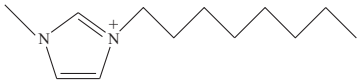
Table 1
Some physicochemical properties of the studied ILs.

ILs	Cation	Anion	MW	Density (25 °C, g/ml)[a]	Surface tension (mN/m, 25 °C)[a]	From (25 °C)	Solubility in H_2O
$[C_4MIM]BF_4$		BF_4^-	226	1.26	40.68	Liquid	Miscible
$[C_6MIM]Br$		Br^-	247	1.14	26.20	Liquid	Miscible
$[C_8MIM]Br$		Br^-	275	1.04	21.25	Liquid	Miscible

[a] Ref. [34].

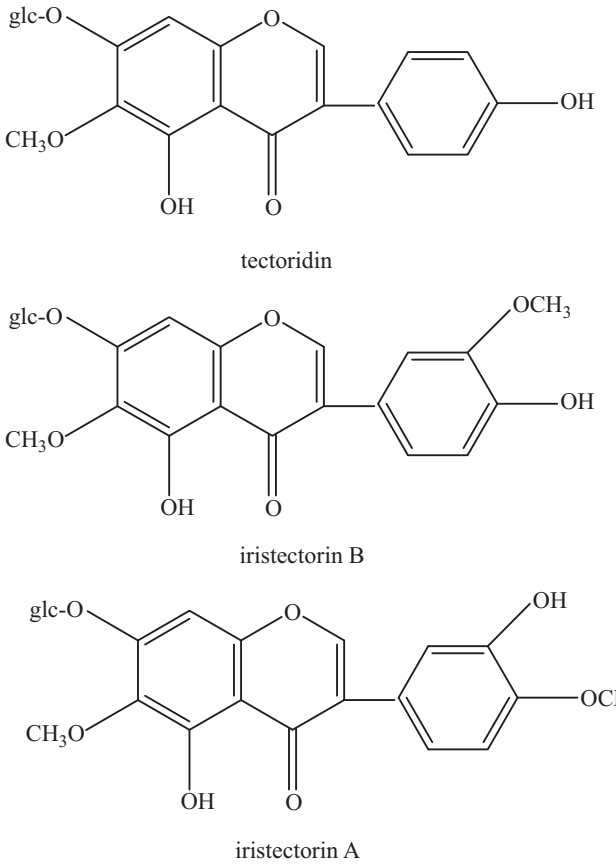


Fig. 1. The chemical structure of tectoridin, iristectorin B and iristectorin A.

large-scale simultaneously [22–24]. This method has been widely applied to the separation and purification of many herb medicines [25–27].

The present report describes a convenient, efficient and environmentally friendly ionic liquids based on ultrasonic-assisted extraction (ILUAE) method. This method includes the extraction of three isoflavones: tectoridin, iristectorin B and iristectorin A from *I. tectorum*, and the separation and purification of these three compounds from the obtained ILUAE extract by HSCCC. To our knowledge, this is the first report on application, in combining ILUAE and HSCCC, of extraction, isolation and purification of the active components from *I. tectorum* (the chemical structures of tectoridin, iristectorin B and iristectorin A were shown in Fig. 1).

2. Experimental

2.1. Chemicals and materials

All organic solvents used for HSCCC were of analytical grade and purchased from Tianjin Chemical Factory, Tianjin, China. Acetonitrile used for HPLC was of chromatographic grade (Yongda Chemical Factory, Tianjin, China), and water used was redistilled water. Dimethyl sulfoxide (DMSO-d_6) was used as the solvent for NMR determination. All ionic liquids were purchased from Chengjie Chemical Company, Shanghai, China.

Iris tectorum Maxim dried roots were collected from the Medicinal Plant Farm of Shandong Agricultural University in August 2009, Taian, China, and were identified by Doctor Jianhua Wang (College of Agronomy, Shandong Agricultural University, Taian, China).

2.2. Apparatus

For the ultrasound assisted extraction experiments, an ultrasonic bath with electric power of 250 W was used as an ultrasound source. The bath (KQ-250DE, Kunshan Ultrasound Co. Ltd., China) was a rectangular container (300 × 240 × 150 mm), to which 40 kHz transducers were annealed at the bottom. The operating temperature was set at 25 °C.

The HSCCC instrument employed in this study is a Model GS-10A high-speed counter-current chromatograph (Beijing Institute of New Technology Application, Beijing, China). The apparatus was equipped with a polytetrafluoroethylene multilayer coil (i.d. of the tubing = 1.6 mm, total volume = 230 ml) and a manual sample injection valve with a 10 ml loop. The distance between the holder axis and central axis of the centrifuge (R) was 5 cm, and the β value varied from 0.5 to 0.8 at the external terminal ($\beta = r/R$, 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). The revolution speed of the apparatus could be regulated with a speed controller in the range between 0 and 1000 rpm. The HSCCC system used in the present study was equipped with a Model NS-1007A constant-flow pump, a Model 8823B-UV Monitor at 280 nm and BF-2002 CT11 Signal collection cell (Chromatography Center of Beifenruili Group Company, Beijing, China). The data were collected with HW-2000 chromatography workstation (Qianpu Software Co. Ltd., Shanghai, China).

For HPLC, the Waters 600E (USA) HPLC system was used. This equipment includes a 4-Solvent delivery system 600E start-up kit, a 600 pump (0–20 ml/min), a 2996 photodiode array detector, an Empower Add-on single system, a Degasser in-line 4 chamber and a 600E controller.

Nuclear magnetic resonance (NMR) spectrometer used is AVANCE 600 (Bruker, Switzerland).

2.3. Ionic liquids based ultrasound assisted extraction

The extraction of three isoflavones from *I. tectorum* by means of UAE was performed by employing various different extraction conditions including solvents: methanol, NaCl, [C_4MIM][BF_4], [C_6MIM]Br and [C_8MIM]Br; concentration: 0.1, 0.3, 0.5, 1.0, 1.5 and 2.0 M; extraction time: 10, 20, 30 and 50 min; solvent to solid ratio: 20, 30, 40 and 50 ml/g. All samples were prepared and analyzed in triplicate.

The extraction efficiency of UAE method was evaluated using the extraction yield as index, which were calculated according to the following equation:

$$\text{Yield (mg/g)} = \frac{\text{weight of isoflavones extracted (mg)}}{\text{weight of dried sample (g)}}$$

2.4. Selection of the two-phase solvent systems of HSCCC

N-butanol–water was used as the two-phase stepwise solvent system of HSCCC. The composition of the solvent system was selected according to the partition coefficients (*K*) of the target compounds. The *K*-values were determined by HPLC as follows: a small amount (5 mg) of ILUAE extract was added into a test tube to which 2 ml of each phase of the two-phase solvent system was added. The test tube was shaken vigorously for 1 min. The peak areas of the upper phase analyzed by HPLC were recorded as *A*, whereas the peak areas of lower phase as *B*. The partition coefficients (*K*) of all components based on the peak areas were generated using the equation of $K = A/B$.

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

N-butanol–water solvent system with the volume ratio of 1:1 were prepared by adding the solvents to a separation funnel according to the volume ratios and thoroughly equilibrated by shaking repeatedly. Then, the upper phase and the lower phase were separated and degassed by sonication for 60 min prior to use. ILUAE extract (400 mg) was dissolved in 2 ml of the mixture solvent consisting of equal volumes of both upper and lower phases.

2.6. HSCCC separation procedure

Preparative HSCCC was performed with a two-phase solvent system composed of *n*-butanol–water 1:1 (v/v). The multilayer coil column was first entirely filled with upper phase as the stationary phase, the lower phase as the mobile phase was then pumped at a flow rate of 2.0 ml/min, and the HSCCC apparatus was rotated at 900 rpm. While after the mobile phase front emerged and hydrodynamic equilibrium was established in the column (about 1.2 h later), 2 ml sample solution containing 400 mg of the ILUAE extract was injected into the head of the column through the sample port (with 2 ml loop). The effluent from the outlet of the column was continuously monitored with a UV detector (Model UV-8823B) at 280 nm. All fractions of the same pure compound were combined and evaporated under the reduced pressure.

After the separation, the retention of stationary phase (Sr) is expressed as a percentage of a volume of the stationary phase (Vs) relative to the total column capacity (Vc), that is $Sr = [Vs/Vc] \times 100\%$.

2.7. HPLC analysis and identification of HSCCC peak fractions

HPLC analysis of the ILUAE extract, *K*-values and each HSCCC peak fraction were performed with Hypersil C_{18} column (250 mm × 4.6 mm, i.d., 5 μm) at room temperature. The gradient elution system consisted of A (acetonitrile) and B (water), 17% A at 0–10 min, 17–46% A at 10–25 min, 46% A at 25–30 min. All solvents were filtered through a 0.22 μm filter prior to use. The sample injection volume was 20 μl. The flow rate was kept constant at 1.0 ml/min, and the effluents were monitored at 265 nm by a photodiode array detector. The typical chromatogram of ultrasonically extracted *I. tectorum* was shown in Fig. 2. Identification of peak fractions from HSCCC was carried out by ^{1}H NMR and ^{13}C NMR. NMR spectra were recorded on AVANCE 600 NMR with tetramethylsilane (TMS) as the internal standard.

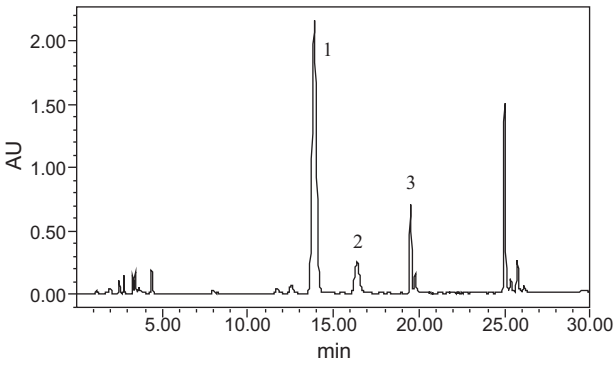


Fig. 2. HPLC chromatogram of ILUAE extract from *I. tectorum*. Peaks 1, 2 and 3 correspond to tectoridin, iristectorin B and iristectorin A, respectively.

3. Results and discussions

3.1. Selection of solvents in UAE

ILs is one kind of the new solvent applied in the extraction and purification field since that the structure of ILs has significant influence on its physicochemical properties, which might greatly affect the extraction efficiency of the target compounds [19]. Considering the hydrophobicity of the three isoflavones, which was similar to the alkylammoniums types of ILs incorporating the imidazolium cation, [C_4MIM]BF_4, [C_6MIM]Br and [C_8MIM]Br were selected (Table 1) in ILUAE process. The five different solvents (methanol; NaCl, 0.5 M; [C_4MIM]BF_4,0.5 M; [C_6MIM]Br, 0.5 M and [C_8MIM]Br, 0.5 M) were tested under the same condition using the same protocol (dried roots: 0.2 g, extraction time: 30 min, solvent to solid ratio: 50 ml/g). The results were summarized in Fig. 3. Clearly, [C_8MIM]Br, 0.5 M produced the highest extraction yields of tectoridin (34.12 mg/g), iristectorin B (2.64 mg/g) and iristectorin A (5.09 mg/g) derived from the dried roots, and the [C_6MIM]Br, 0.5 M and methanol ran the second and third best with respect to the efficiency of the extraction yields, respectively. These results could

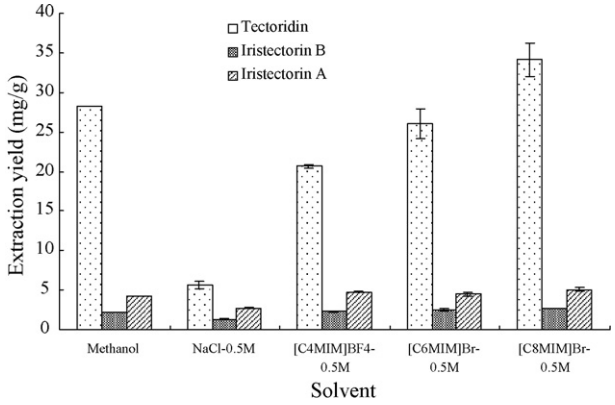


Fig. 3. Effect of the different solvents on the extraction yield of isoflavones from *I. tectorum*. Among them, 0.5 M NaCl is selected as blank experiment.

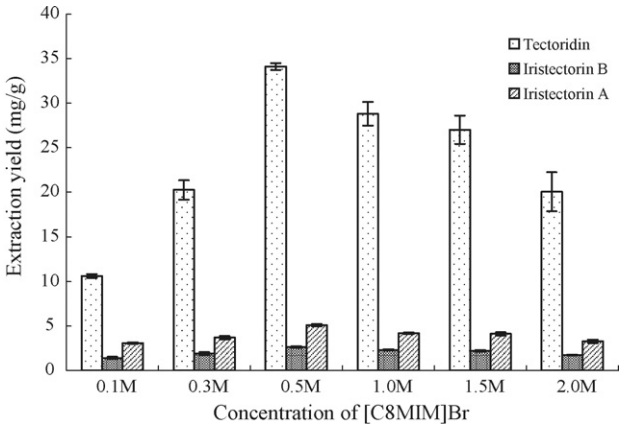


Fig. 4. Effect of the concentration of [C_8MIM]Br on the extraction yield of isoflavones from *I. tectorum*.

be attributed to the fact that increasing the alkyl chain length from butyl to octyl decreased the water miscibility of the ILs [28]. Therefore, [C_8MIM]Br was selected as the best solvent for the subsequent extraction experiments.

3.2. Effect of IL concentration

The concentration of [C_8MIM]Br is very important as it has the dramatic effect on the extraction yields. The six different solvents concentration have been studied for extracting isoflavones from the matrix of *I. tectorum* dried roots. The results illustrated in Fig. 4 showed that the highest extraction yields of tectoridin, iristectorin B and iristectorin A were obtained when 0.5 M [C_8MIM]Br solvent was used. Higher than 0.5 M of [C_8MIM]Br resulted in the reduced extraction yields of these three isoflavones (Fig. 4). Thus, 0.5 M was considered as the most proper concentration and used for the later experiments.

3.3. Effect of extraction time

Traditionally, higher extraction yield requires a longer extraction time. To investigate the best extraction time for isolating higher yield of isoflavones, 0.2 g of *I. tectorum* dried roots sample was extracted under the conditions of 10 ml of 0.5 M [C_8MIM]Br for 10, 20, 30 and 50 min, respectively. The results shown in Fig. 5 clearly indicated that when extraction time increased from 10 to 30 min, the extraction yields of three isoflavones

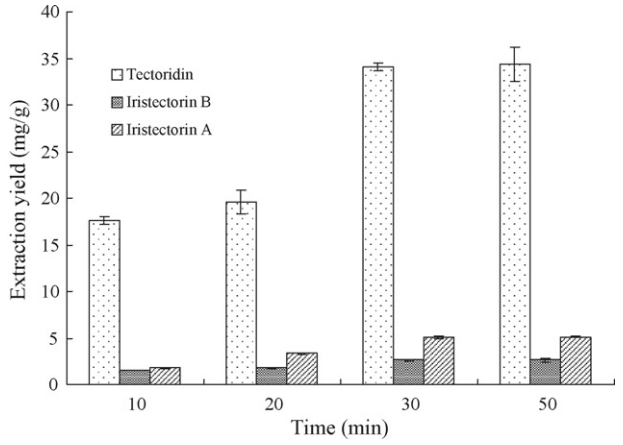


Fig. 5. Effect of the extraction time of [C_8MIM]Br on extraction yield of isoflavones from *I. tectorum*.

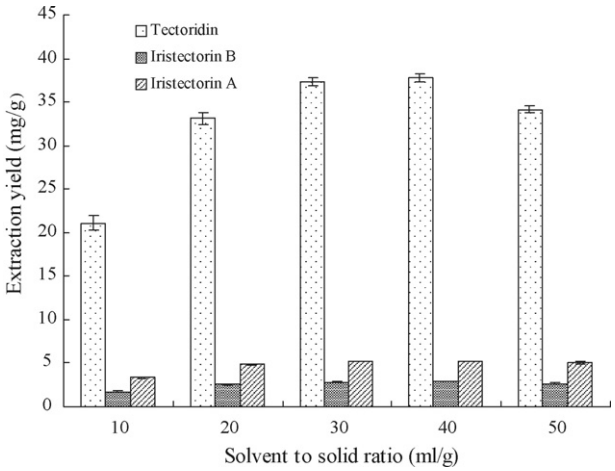


Fig. 6. Effect of the solvent to solid ratio of [C_8MIM]Br on extraction yield of isoflavones from *I. tectorum*.

increased. After ILUAE from 10 to 30 min, the yields of tectoridin, iristectorin B, iristectorin A increased from 21.0 to 37.32, 1.75 to 2.80, 3.34 to 5.23 mg/g, respectively. However, the differences in the yields of tectoridin, iristectorin B and iristectorin A were not significant ($p > 0.05$) when the extraction time went up from 30 to 50 min. Because the diffusion front moved towards the interior of the tissues of *I. tectorum* dried roots, the diffusion area was reduced, but diffusion distance was increased, leading to the lower diffusion rate [29]. As a result, there was no obvious yield change following the extended extraction time. Hence, 30 min was determined as the optimum extraction time.

3.4. Effect of solvent to solid ratio

In general, a higher solvent volume can dissolve the target compounds more efficiently and result in a better extraction yield. 0.2 g of *I. tectorum* dried roots sample was extracted with 0.5 M [C_8MIM]Br and 30 min at five different solvent to solid ratios of 10, 20, 30, 40 and 50 ml/g, respectively. Data shown in Fig. 6 indicated an obvious increase of extraction yield of the three major isoflavones when the solvent to solid ratio was increased from 10 to 40 ml/g. When the solvent to solid ratio was increased from 30 to 40 ml/g, however, no significant differences in the extraction yields of three isoflavones were detected ($p > 0.05$). Further increasing the solvent to solid ratio from 40 to 50 ml/g reduced the extraction yields dramatically (Fig. 6). These results suggest that, from the commercial application point of view, the solvent to solid ratio of 30 ml/g is optimal for the best extraction yields of the three isoflavones, and reducing the waste of solvent and minimizing the bulky handling during the subsequent processes would be another additional advantage.

This ILUAE experiment confirms that the optimum conditions for the efficient extraction of isoflavones were the extraction solvent of [C_8MIM]Br with the concentration of 0.5 M, extraction time of 30 min and solvent to solid ratio of 30 ml/g.

3.5. Measurement of partition coefficient of HSCCC

Previous studies have indicated that the proper two-phase system includes: the target compounds in the solvent system should be soluble and stable; the settling time should be short (<30 s); the ideal *K*-values of the target compounds should be close to 0.5–2 and the retention of the stationary phase should be satisfactory (>50%) [30,31].

Table 2
The solvent polarities (ε) of different two-phase solvent systems and the *K*-values of the three components in different two-phase solvent systems.

Solvent system (v/v)	Solvent polarity (ε)[a]	*K*-value		
		Tectoridin	Iristectorin B	Iristectorin A
Light petroleum–ethyl acetate–methanol–water (2:4:3:3)	5.64	0.04	0.11	0.03
Light petroleum–ethyl acetate–methanol–water (1:5:3:3)	5.99	0.20	0.49	0.31
Light petroleum–ethyl acetate–methanol–water (1:5:2:4)	5.44	0.17	0.02	0.11
Ethyl acetate–water (1:1)	7.25	0.22	0.21	0.30
Ethyl acetate–methanol–water (3:1:3)	7.16	0.19	0.27	0.23
N-butanol–water (1:1)	6.95	0.79	1.43	1.34

[a] Ref. [35].

A series of experiments were performed to optimize the two-phase solvent system for HSCCC separation. Table 2 listed the solvent polarities (ε) of different two-phase solvent systems and the *K*-values of the three isoflavones determined by HPLC. These results indicate that the three systems, all involving in light petroleum, have small *K*-values of three isoflavones (<0.5). This might be due to the fact that the organic polarity effects were negligible and the capability of distribution of three isoflavones in the upper was too small. Then, solvent with increased polarity of ethyl acetate–methanol–water (ε 7.16) and *n*-butanol–water (ε 6.95) were tried. Eventually, *n*-butanol–water (1:1, v/v) has proper *K*-values of three isoflavones and was used as the two-phase in the experiment.

3.6. HSCCC separation of three isoflavones

Under the optimized ILUAE conditions, sample of *I. tectorum* dried roots was extracted with 75 ml of 0.5 M [C_8MIM]Br. The suspension was removed by centrifugation at 10,000 × *g* for 30 min, and the supernatant was evaporated under reduced pressure. Finally 400 mg of extract was obtained from 2.5 g sample of *I. tectorum* dried roots. Fig. 7 illustrates the separation of the sample by the preparative HSCCC. The extraction yields of the three isoflavones, tectoridin, iristectorin B and iristectorin A, were 60.21 mg, 4.33 mg and 8.24 mg, and purities of peak fractions 1, 2 and 3 were 95.3%, 95.9% and 97.0%, respectively, as determined by HPLC (Fig. 8). The retention value of the stationary phase of HSCCC was 53% and the separation time was about 5 h in each separation run. Separation procedure fluidogram of crude extract used for HSCCC separation was summarized in Fig. 9. These results imply that experimental conditions selected for the ILUAE isolation and HSCCC purification of considered here structurally similar three isoflavones from dried roots of *I. tectorum* were suitable for routine application.

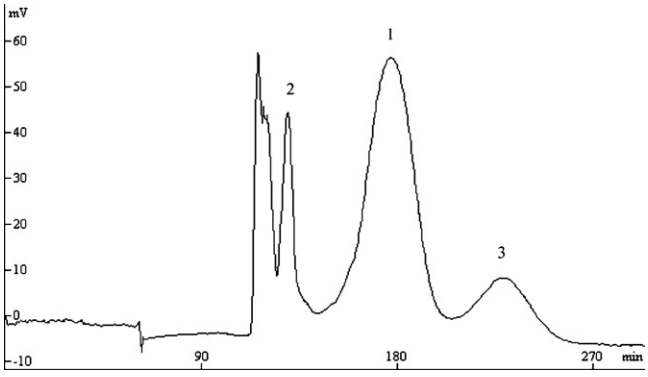


Fig. 7. HSCCC chromatogram of ILUAE extract from *I. tectorum*. Peaks 1, 2 and 3 correspond to tectoridin, iristectorin B and iristectorin A, respectively.

3.7. Identification of the separated peaks

The structures of isolated compounds were identified on the basis of their particular ^{1}H NMR and ^{13}C NMR data. The data of each peak fraction were given as follows:

Compound 1. White needles (methanol), ^{1}H NMR (600 MHz, DMSO-d_6) δ 13.06 (1H, s, 5-OH), 9.62 (1H, s, 4′-OH), 8.46 (1H, s,

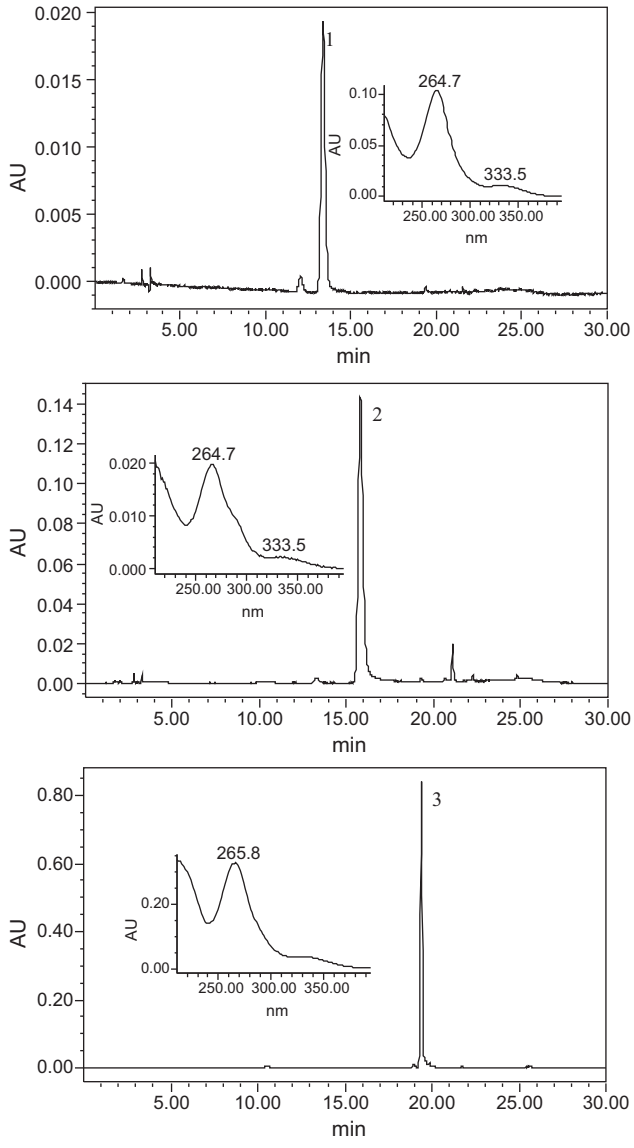


Fig. 8. HPLC chromatograms and UV of HSCCC peak fractions (1–3). Peaks 1, 2 and 3 correspond to tectoridin, iristectorin B and iristectorin A, respectively.

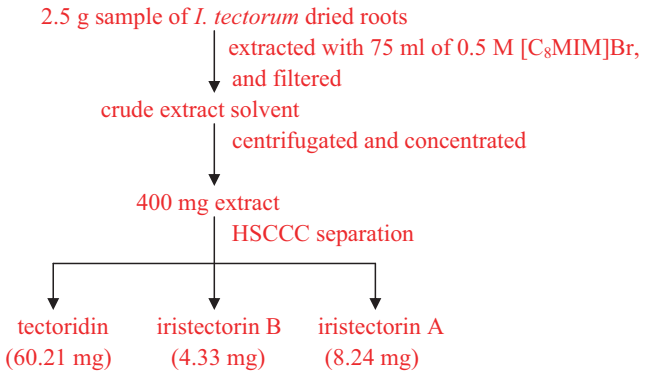


Fig. 9. Separation procedure fluidogram of crude extract used for HSCCC separation.

H-2), 7.40 (2H, d, J = 8.4 Hz, H-2′, H-6′), 6.89 (2H, d, J = 8.4 Hz, H-3′, H-5′), 6.83 (1H, s, H-8), 3.77 (3H, s, 6-OCH_3), 5.46 (1H, d, J = 5.1 Hz, H-1″). ^{13}C NMR (150 MHz, DMSO-d_6) δ 180.7 (C-4), 157.4 (C-4′), 156.5(C-7), 154.6 (C-9), 152.8 (C-2), 152.4 (C-5), 132.3 (C-6), 130.1 (C-2′, 6′), 121.9 (C-1′), 120.9 (C-3), 115.0 (C-3′, 5′), 106.4 (C-10), 100.0 (C-1″), 94.0 (C-8), 77.1 (C-3″), 76.5 (C-5″), 73.0 (C-2″), 69.5 (C-4″), 60.5 (6-OCH_3), 60.1 (C-6″). Comparing the above data with reference [32], compound 1 was identified as tectoridin.

Compound 2. White needles (methanol), ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.95 (1H, s, 5-OH), 9.19 (1H, s, 4′-OH), 8.49 (1H, s, H-2), 7.17 (1H, d, J = 2.4 Hz, H-2′), 7.01 (1H, dd, J = 2.4, 8.4 Hz, H-6′), 6.90 (1H, s, H-8), 6.84 (1H, d, J = 8.4 Hz, H-5′), 3.80 (3H, s, 6-OCH_3), 3.77 (3H, s, 3′-OCH_3), 5.10 (1H, d, J = 5.4 Hz, H-1″). ^{13}C NMR (150 MHz, DMSO-d_6) δ 180.6 (C-4), 156.5 (C-7), 154.8 (C-5), 152.8 (C-9), 152.3 (C-2), 147.2 (C-3′), 146.7 (C-4′), 132.3 (C-6), 122.0 (C-3), 121.6 (C-6′), 121.4 (C-1′), 115.2 (C-5′), 113.1 (C-2′), 106.3 (C-10), 99.9 (C-1″), 93.9 (C-8), 77.2 (C-3″), 76.6 (C-5″), 73.0 (C-2″), 69.5 (C-4″), 60.5(C-6″), 60.2 (6-OCH_3), 55.6 (3′-OCH_3). Comparing the above data with reference [4], compound 2 was identified as iristectorin B.

Compound 3. White needles (methanol), ^{1}H NMR (600 MHz, DMSO-d_6) δ 12.94 (1H, s, 5-OH), 9.09 (1H, s, 3′-OH), 8.48 (1H, s, H-2), 7.06 (1H, d, J = 1.8 Hz, H-2′), 6.97 (1H, d, J = 1.8 Hz, H-6′), 6.96 (1H, s, H-8), 3.80 (3H, s, 5′-OCH_3), 3.78 (3H, s, 4′-OCH_3), 3.72 (3H, s, 6-OCH_3), 5.11 (1H, d, J = 5.4 Hz, H-1″). ^{13}C NMR (150 MHz, DMSO-d_6) δ 180.6 (C-4), 156.5 (C-7), 154.9 (C-5), 152.8 (C-9), 152.3 (C-2), 147.7 (C-3′), 146.0 (C-4′), 132.4 (C-6), 123.0 (C-3), 121.8 (C-6′), 119.7 (C-1′), 116.3 (C-5′), 111.9 (C-2′), 106.4 (C-10), 100.0 (C-1″), 94.0 (C-8), 77.2 (C-3″), 76.6 (C-5″), 73.0 (C-2″), 69.5 (C-4″), 60.5 (C-6″), 60.2 (6-OCH_3), 55.5 (4′-OCH_3). Comparing the above data with reference [33], compound 3 was identified as iristectorin A.

4. Conclusion

An environmental protecting and effective method based on the ILUAE has been developed for the extraction of three isoflavones from *I. tectorum*. Under the optimized ILUAE conditions, the highest extraction yields of tectoridin, iristectorin B and iristectorin A were 37.45, 2.88 and 5.28 mg/g, respectively. Tectoridin, iristectorin B and iristectorin A in the ILUAE extracts have been separated and purified successfully by HSCCC. This approach resulted in 60.21 mg tectoridin, 4.33 mg iristectorin B and 8.24 mg iristectorin A isolated from 400 mg ILUAE extract of *I. tectorum* dried roots using one-step elution within 5 h. These results indicated that the combination of application of ILUAE and HSCCC is a very powerful technique for the preparative extraction and purification of tectoridin, iristectorin B and iristectorin A from *I. tectorum* dried roots.

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