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Ionic liquid-based microwave-assisted extraction of rutin from Chinese medicinal plants

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ABSTRACT

An ionic liquid-based microwave-assisted extraction (ILMAE) method has been developed for the effective extraction of rutin from Chinese medicinal plants including Saururus chinensis (Lour.) Bail. (S. chinensis) and Flos Sophorae. A series of 1-butyl-3-methylimidazolium ionic liquids with different anions were investigated. The results indicated that the characteristics of anions have remarkable effects on the extraction efficiency of rutin and among the investigated ionic liquids, 1-butyl-3-methylimidazolium bromide ([bmim]Br) aqueous solution was the best. In addition, the ILMAE procedures for the two kinds of medicinal herbs were also optimized by means of a series of single factor experiments and an $L_9(3^4)$ orthogonal design. Compared with the optimal ionic liquid-based heating extraction (ILHE), marinated extraction (ILME), ultrasonic-assisted extraction (ILUAE), the optimized approach of ILMAE gained higher extraction efficiency which is 4.879 mg/g in S. chinensis with RSD 1.33% and 171.82 mg/g in Flos Sophorae with RSD 1.47% within the shortest extraction time. Reversed phase high performance liquid chromatography (RP-HPLC) with ultraviolet detection was employed for the analysis of rutin in Chinese medicinal plants. Under the optimum conditions, the average recoveries of rutin from S. chinensis and Flos Sophorae were 101.23% and 99.62% with RSD lower than 3%, respectively. The developed approach is linear at concentrations from 42 to 252 mg L^{-1} of rutin solution, with the regression coefficient (r) at 0.99917. Moreover, the extraction mechanism of ILMAE and the microstructures and chemical structures of the two researched samples before and after extraction were also investigated. With the help of LC–MS, it was future demonstrated that the two researched herbs do contain active ingredient of rutin and ionic liquids would not influence the structure of rutin.

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1. Introduction

Ionic liquids (ILs), which are composed of organic cations and inorganic or organic anions, are liquid near room temperature (or by convention below 100 °C) [1]. The main advantage of ionic liquids is that they are a new class of solvents by their non-molecular nature. The environment of ionic liquids is very different from that of all molecular polar or non-polar organic solvents. Besides the intrinsic non-molecular nature of ILs giving them unique solvent properties, the major advantages of ILs are their extremely low vapor pressure associated to a high thermal stability, very wide liquidus range, good dissolving and extracting ability, excellent microwave-absorbing ability, designable structures and so on [2–5].

Kenneth R. Seddon of Queen University (Belfast, Northern Ireland) recently stated: "Years ago, I predicted that ionic liquids would change the face of organic chemistry. It is clear now that they have the potential to revolutionize all activities where liquids can be used" [6]. ILs will soon be produced on an industrial scale and it will be necessary to develop reliable analytical procedures for their analysis and control.

Recent years, as extraction solvents in liquid–liquid extraction, liquid-phase microextraction, solid-phase microextraction and aqueous two-phase systems extraction, ILs aqueous solutions have shown great promising prospect [7–11]. Comparing with conventional organic solvents, ILs are green solvents because their vapour pressure was so lower that ILs are very difficult to evaporate into the environment. In some cases, they could even be well recycled. They can effectively improve the selectivity and the extraction efficiency of the being investigated compounds from complicated samples such as traditional Chinese medicine.

Saururus chinensis (Lour.) Bail. (S. chinensis) is a medicinal plant of Saururaceae family which is so common in all provinces in the south of the Yangtze River of China. All parts of it can be used as medicine which has been used to treat difficulty in micturition, leucorrhea, urinary tract infection, nephritis, edema, pyocutaneous disease, eczema, and so on [12]. S. chinensis contains various bioactive compounds including volatile oil, hydrolysable tannins

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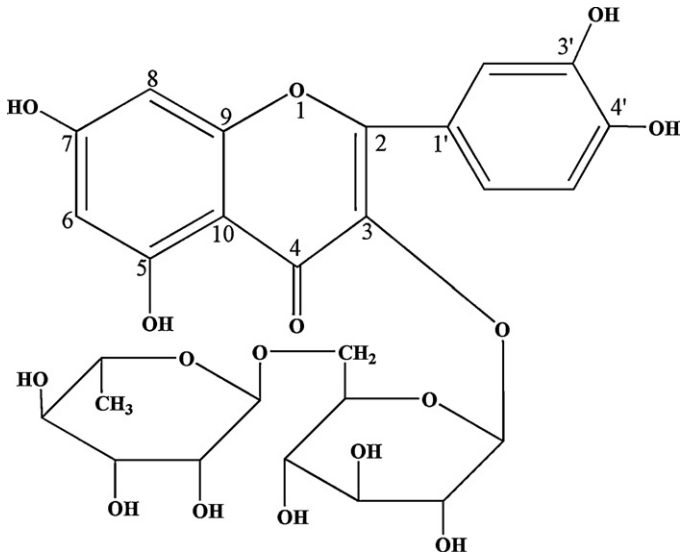


Fig. 1. The chemical structure of rutin.

and flavonoids which include quercetin, quercitin, iso-quercitrin, hyperin, rutin and avicularin [13].

Flos Sophorae is dry flower and flower bud of leguminous plant "Sophora japonica L." [14], which is mainly produced in many provinces of China, such as Hebei, Shandong, Henan and Jiangsu [15]. It has been used to cure the diseases including hemafecia, hemorrhoids blood, bloody flux, uterine bleeding, hematemesis, liver heat and red eyes, headache and dizziness, and so on [16]. Recent years, it is commonly used for curing vascular hypertension [17]. Flos Sophorae mainly contains rutin whose content may reach as high as 23.5% [18].

Rutin (3′,4′,5,7-tetrahydroxyflavone-3-β-D-rutinoside), whose structure was shown in Fig. 1, is an effective compound for curing hypertension, angiocardiopathy, gastropathy, dermatosis, diabetes. It has been primarily applied to treat the fragility of capillary and permeability bleeding and reduce the blood-fat and cholesterol of human body. Besides, it exhibits activities of radio-resistance, anti-inflammatory and anti-virus. With the improvement of the living standards, the incidence rate of the cardiac and cerebral vascular diseases, whose mortality rate have the maximum value of all diseases, is increasing. Therefore, it is significant to develop medicines for curing and preventing these diseases. Rutin is just one of the right substances of the medicines [19].

For extracting *rutin* from medicinal herbs, heat-reflux extraction (HRE) is the most widely used traditional technique. Whereas HRE is laborious, time consuming, and requires abundant, volatile and hazardous organic solvents [20]. The benefit of using microwave-assisted extraction (MAE) to extract organic compounds directly from solid matrixes has already been demonstrated in recent years [21–22]. Compared with traditional and other modern extraction techniques, MAE is proposed as an efficient and alternative procedure for sample pretreatment. Furthermore, ILs can efficiently absorb and transfer microwave energy. As extraction solvents, they are very attractive in the microwave-assisted extraction of bioactive components from medicinal herbs.

The aim of the present study is to develop a rapid and effective ionic liquid-based microwave-assisted extraction (ILMAE) approach for extracting bioactive rutin from medicinal plants both "S. chinensis" and "Flos Sophorae". To our best knowledge, there is no report about ILMAE of rutin from the two kinds of herbs.

2. Experimental

2.1. Reagents and materials

S. chinensis was purchased from a wholesale web of Chinese medicine. Flos Sophorae was got from Yangtianhe Pharmacy (Hunan, China). Standard rutin and HPLC grade methanol used for mobile phase were bought from Sinopharm Chemical Reagent Co. (Shanghai, China). 1-Chlorobutane (≥98%) and 1-bromobutane (≥98%) were also got from Sinopharm Chemical Reagent Co. (Shanghai, China). 1-Methylimidazole (≥99%) was obtained from Aladdin Reagent (Shanghai, China). Sodium p-toluene sulfonate (≥98%) was purchased from WuXiYangShan Biochemical Co. (Jiangsu, China). Sodium tetrafluoroborate was got from Aladdin Reagent (Shanghai, China), and these chemical reagents are chemical grade. Other reagents are analytical grade and purchased from Sinopharm Chemical Reagent Co. (Shanghai, China). HPLC grade methanol, phosphoric acid and redistilled water were filtrated before used.

2.2. Apparatus

HPLC analysis was carried out on a Hitachi Series 2000 liquid chromatograph, equipped with a vacuum degasser, a quaternary pump (L2130), and a UV-Vis Detector system (L2420), connected to a reversed-phase column (ODS-2C_{18} 5 μm 250 mm × 4.6 mm i.d., Hitachi, Japan). Pressure self-control microwave decomposition system (MDS-2002AT, Shanghai, China) and Auto Science AS-3120 Ultrasonic Cleaner (Tianjin, China) were used for extraction. High-speed centrifugation (TGL-16C, Shanghai, China) was employed to accelerate the phase separation process. A versatile plant pulverizer (FW-100, Beijing, China) was used to make the plant materials into powder. A rotary evaporator (RE52CS, Shanghai, China) was used to remove ethyl acetate, acetone and dichloromethane. HH-SD and Magnetic Stirrer were applied to synthesize ionic liquids and heat for extraction. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian-INOVA 400 NMR spectrometry (USA). FTIR spectra were registered on Spectrum One NTS (Perkin Elmer, USA) equipped with a DTGS detector. The mass-spectrograms of rutin in different matrixes were registered on LCQ-Advantage LC/MS^n (Thermo-Finnigan, USA) using the ionization source of ESI.

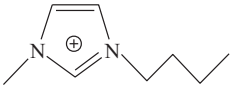
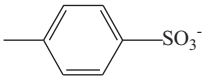
2.3. Preparation of ILs and standard solutions

2.3.1. Preparation of ILs

4 kinds of ILs (as shown in Table 1) were prepared according to the literature procedures with minor modification [23–27]. Flow charts of synthesis process were described in Supplementary Information Fig. S1. To ensure the ILs synthesized with high yield and good quality, reaction conditions, including amount of reactants, synthetic method, reaction temperature and time, were optimized systematically.

All ionic liquids synthesized were dried at 70 °C for 24 h under vacuum and then checked by ^{1}H NMR and ^{13}C NMR spectra which were shown in Supplementary Information Fig. S2 and Table S (A

Table 1
The chemical structures of studied ILs.

ILs	Cations	Anions
[bmim]Br		Br^-
[bmim]Cl		Cl^-
[bmim]BF_4		BF_4^-
[bmim]TsO		

and B). And the characterization of NMR spectroscopy showed that there were no organic impurities in ILs.

2.3.2. Preparation of standard solutions

A certain volume of 1.5 M [bmim]Br aqueous solution, [bmim]Cl aqueous solution, [bmim]BF_4 aqueous solution and [bmim]TsO aqueous solution were prepared with deionized water, respectively. The preparation steps of standard solution of rutin (0.21 mg mL^{-1}) were as follows: rutin (0.0053 g) was accurately weighed and dissolved in 1.5 M [bmim]Br aqueous solution, and then diluted the solution to 25 mL in a volumetric flask (25 mL) by 1.5 M [bmim]Br aqueous solution. 2.0, 4.0, 6.0, and 8.0 mL of rutin standard solutions (0.21 mg mL^{-1}) were diluted to 10 mL by adding 1.5 M [bmim]Br aqueous solution respectively. And then stored at 1–4 °C in darkness. Deionized water was used throughout the work.

2.4. Sample preparation

All medicinal herb samples were cleaned with water, and dried at 60 °C, after that, they were triturated by a pulverizer, and passed through a stainless steel sieve. And then the milled samples of S. chinensis were degreased by Soxhlet extractor with petroleum ether until the eluate became colorless. It was unnecessary to degrease the samples of Flos Sophorae, as they have little lipid which would not influence the detection of rutin. Then stored them in a closed desiccator.

2.5. Extraction methods

2.5.1. Ionic liquid-based microwave-assisted extraction (ILMAE)

A certain amount of accurately weighed samples were put into sealed vessels by adding certain volume of different ILs aqueous solutions. Then the vessels were placed into the pressure self-control microwave decomposition system followed by microwave extraction one bye one. The optimum ionic liquid and its concentration, solid–liquid ratio, radiating time of microwave, extracting temperature were studied systematically through a series of single factor tests and orthogonal design L_9 (3^4) experiment in this work.

2.5.2. Ionic liquid-based ultrasonic-assisted extraction (ILUAE)

A certain amount of accurately weighed samples (0.4 g of S. chinensis; 0.2 g of Flos Sophorae) were put into a conical flask by adding a certain volume of 2.5 M [bmim]Br aqueous solution (10 mL for S. chinensis; 8 mL for Flos Sophorae). Then the conical flask was placed into the ultrasonic cleaning bath, followed by sonication (S. chinensis for 80 min; Flos Sophorae for 60 min) at room temperature.

2.5.3. Ionic liquid-based heating extraction (ILHE)

A certain amount of accurately weighed samples (0.4 g of S. chinensis; 0.2 g of Flos Sophorae) were put into a round-bottomed flask by adding a certain volume of 2.5 M [bmim]Br aqueous solution (20 mL for S. chinensis; 12 mL for Flos Sophorae), then the flask was placed into oil bath with a reflux device, followed by extracting 12 h at 100 °C.

2.5.4. Ionic liquid-based marinated extraction (ILME)

A certain amount of accurately weighed samples (0.4 g of S. chinensis; 0.2 g of Flos Sophorae) were put into a round-bottomed flask by adding a certain volume of 2.0 M [bmim]Br aqueous solution (24 mL for S. chinensis; 16 mL for Flos Sophorae), then the round-bottomed flask was equipped with a magnetic stirrer at ambient temperature for 48 h with consecutively stirring.

The extraction conditions of the ILUAE, ILHE and ILME methods had been optimized through a series of single factor tests and orthogonal design L_9 (3^4) experiment, respectively.

After extraction, all obtained extracts were cooled to ambient temperature, centrifuged at 10,000 rpm for 30 min, diluted to 25 mL in a volumetric flask (25 mL) using corresponding ILs aqueous solutions. Besides, fetched 1 mL Flos Sophorae extract from 25 mL volumetric flask and diluted to 10 mL by adding deionized water (Because rutin content in Flos Sophorae is quite high, dilutes ten times before HPLC analysis).

The extraction yield of rutin was determined as follows:

$$\text{yield}\ \ (\text{mg/g}) = \frac{\text{mean mass of rutin in herb samples}\ \ (\text{mg})}{\text{mean mass of the herb samples}\ \ (\text{g})}$$

The mean mass of rutin in herb samples was determined by HPLC–UV analysis of five samples extracted for one step by [bmin]Br aqueous solution under the optimized ILMAE conditions. The mean mass of the herb samples was the average mass of five samples before extracted.

2.6. HPLC analysis

Before HPLC analysis, all standard solutions of rutin and extracts were filtrated through 0.45 μm microporous membranes. The conditions of HPLC analysis were as follows: Column temperature was ambient and injection volume was 10 μL. The mobile phase was composed of methanol (solvent A) and 0.05% (v/v) phosphoric acid aqueous solution (solvent B) with 0.8 mL min^{-1} of the flow rate. The gradient elution program used for S. chinensis here was as follows: 30% of solvent A from 0 to 11 min, 30–40% from 11 to 14 min, 40% from 14 to 25 min, 40–50% from 25 to 27 min, 50% from 27 to 33 min, and 50–40% from 33 to 40 min. While the mobile phase of isocratic elution applied for Flos Sophorae was composed of 43% A and 57% B. The UV detection wavelength applied was 356 nm for the two herbs. Peak area was used for quantification. And external standard method was used for the determination of rutin content in herbs.

3. Results and discussion

3.1. Selection of extraction solvent

As the structures of ILs determine their physical and chemical properties, ILs have great impact on extracting analytes. In order to investigate ILs with different anions on extraction efficiency, 1-butyl-3-methylimidazolium ionic liquids with four kinds of anions were studied by ILMAE. The structures of ILs used in this work were shown in Table 1 and the experimental results were shown in Fig. 2.

As seen from Fig. 2, compared the results of four ILs with that of water, the addition of ILs to water could greatly enhance the extraction yields of rutin from the two plants. These can be explained as follows: On one hand, ILs had strong solvation power and multiple interactions [28–30] with rutin, especially hydrogen bonding, polarity, π–π, π–n and ionic/charge–charge, all of which contribute to raising the solubility of rutin in ILs aqueous solutions [31]. On the other hand, ILs, which consist of organic cations and inorganic or organic anions, can efficiently absorb and transfer microwave energy and consequently make the solvent and the sample warming rapidly. Both of the above causes immensely improved the extraction efficiency of rutin from the two researched herbs. Besides, compared with the extraction results using four different ILs aqueous solutions with same cations but different anions, the extraction yields of rutin from S. chinensis and Flos Sophorae were of great disparity, respectively. And the obtained extraction yields indicated that [bmim]Br and [bmim]TsO were more efficient than other two ILs in the ILMAE of rutin, which was likely related to the subacidity that was better for the extraction of rutin. The acidities of [bmim]Br or [bmim]TsO aqueous solutions were higher than those of the others. And the results obtained by [bmim]Br solution

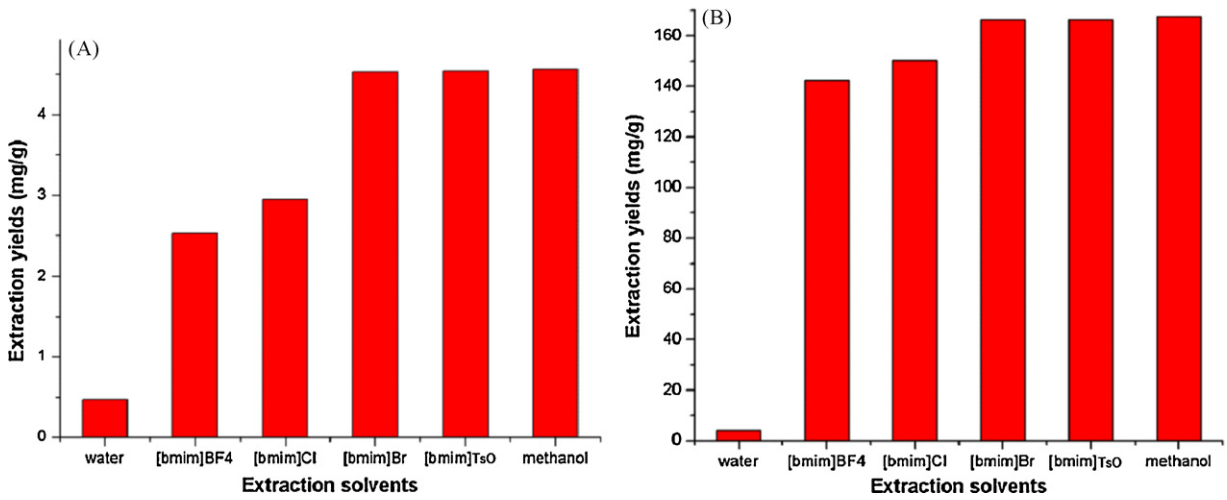


Fig. 2. The effect of different extraction solvents on extracting rutin from S. chinensis (A) and Flos Sophorae (B), respectively.

and [bmim]TsO solution were quite approach to that obtained by methanol. Comprehensively considering that methanol is volatile, flammable and harmful to human and environment, ionic liquid aqueous solutions were selected as a class of green and alternative solvents to displace volatile organic solvents like methanol in the ILMAE process.

Although the extraction yields achieved by [bmim]TsO and [bmim]Br were almost the same, the synthesis process of [bmim]TsO which needs more time and expense was more complicated than that of [bmim]Br which just needs one step to synthesize. Therefore, [bmim]Br aqueous solution was the first option as extraction solvent for rutin both from S. chinensis and Flos Sophorae.

3.2. Optimization of ILMAE conditions

There are many factors affecting the extraction of rutin from medicinal herbs. We worked out the optimum levels of each factor by the single factor test. However, because of the interaction among the factors, the combination effects of the optimum levels of each factor may not be the optimum extraction conditions. The orthogonal test is a scientific method of arranging multi-factors and multi-factors. Based on single factor tests, the optimal extraction conditions can be worked out by orthogonal test.

3.2.1. Single factor experiments

There are many factors affecting the extraction yields, among which the concentration of [bmim]Br aqueous solution (A), solid–liquid ratio (B), extracting temperature (C) and radiating time of microwave (D) are the main factors. Single factor test was performed by one factor varied with different levels while other factors fixed. All results of single factor experiments were shown in Fig. 3.

In the light of Fig. 3, it can be observed that when the concentration of [bmim]Br aqueous solution was 2.5 M for S. chinensis and 1.5 M for Flos Sophorae, respectively, the extraction yield reached the top. Below 2.5 M and 1.5 M of [bmim]Br aqueous solution respectively, the extraction yields of rutin increased rapidly. That is because with the addition of [bmim]Br, both the solubility and the extracting capacity of the solvent were enhanced. At the same time, the capabilities of microwave absorption and microwave conversion were both increased. Furthermore, the interactions between [bmim]Br and rutin were strengthened. While when the concentration of [bmim]Br aqueous solution exceeded 2.5 M and 1.5 M respectively, the extraction yields both were decreasing instead. The major cause was that the greater the [bmim]Br concentration is, the greater the viscosity and the poorer the diffusion capacity of [bmim]Br solution are. As a result, it was more difficult for [bmim]Br to penetrate into the interior of sample matrixes. According to the above results, 2.5 M and 1.5 M of [bmim]Br aqueous solution were selected for extracting rutin from S. chinensis and Flos Sophorae, respectively.

It can be seen that the extraction yield of *rutin* from S. chinensis increased quickly with the decrease of solid–liquid ratio under 1:20. When the ratio of solid–liquid varied from 1:20 to 1:25, the extraction yield increased a little. While that of rutin from Flos Sophorae had similar increase path under 1:35 of solid–liquid ratio to S. chinensis. Because the smaller the ratio of solid–liquid is, the more sufficient the contact between sample matrixes and [bmim]Br aqueous solution is, as a result the more rutin we got. But it is not conducive to extract *rutin* when the solid–liquid ratio was too small. And when the ratio of solid–liquid changed from 1:25 to 1:40 for S. chinensis and from 1:35 to 1:45 for Flos Sophorae, respectively, there were slight decreases of the extraction yield of rutin. Thus, 1:25 for S. chinensis and 1:35 for Flos Sophorae were adopted in next work.

As shown in Fig. 3, it can be seen that both the extraction yields of rutin from S. chinensis and Flos Sophorae increased with the increase of the extracting temperature. Because the increasing of the extracting temperature contributes to reducing the viscosity of ILs and enhancing the spread ability and solubility of ILs, which was beneficial to dissolve and extract rutin. When the temperature reached at 80 °C, the samples of S. chinensis were burnt black. While when the temperature was higher than 60 °C, the extraction yield of rutin from Flos Sophorae decreased a little, which may due to the decomposition of some rutin at high temperature. Thus, 70 °C and 60 °C were selected for further study of S. chinensis and Flos Sophorae, respectively.

In the light of Fig. 3, it can be seen that with the radiating time increasing, the extraction yields of rutin were increasing rapidly, and after 12 min for S. chinensis and 8 min for Flos Sophorae respectively, they kept slightly increasing. Therefore, 12 min for S. chinensis and 8 min for Flos Sophorae were enough to extract bulk rutin from the sample powders, respectively.

3.2.2. Orthogonal design $L_9(3^4)$ experiment

Orthogonal experiment of four factors and three levels was adopted to optimize the ILMAE conditions for the extraction of rutin from the two herbs. The levels setting values of four factors (A, B, C and D) used in the orthogonal arrayed design were shown in Table 2a. Nine experimental trials performed with different con-

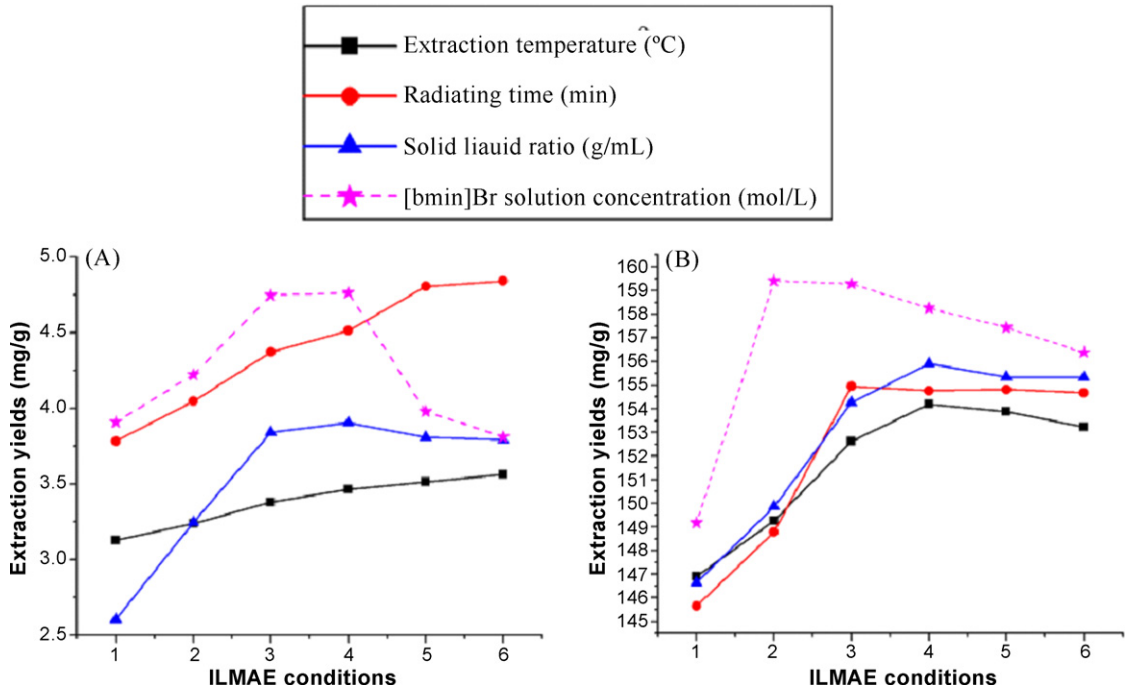


Fig. 3. The effect of the concentrations of selected ILs ([bmim]Br) (1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mol/L), extraction temperature (30, 40, 50, 60, 70, and 80 °C), extraction time (4, 6, 8, 10, 12 and 14 min) and solid–liquid ratio (1:10, 1:15, 1:20, 1:25, 1:30 and 1:40 g/mL for S. chinensis; 1:20, 1:25, 1:30, 1:35, 1:40 and 1:45 g/mL for Flos Sophorae) on the extraction efficiency of rutin from S. chinensis (A) and Flos Sophorae (B), respectively.

Table 2a
Factors and levels of orthogonal test.

Factors	S. chinensis			Flos Sophorae		
	1	2	3	1	2	3
(A) [bmim]Br solution concentration (mol/L)	2.0	2.5	3.0	1.0	1.5	2.0
(B) Solid/liquid ratio (g mL^{-1})	1:20	1:25	1:30	1:30	1:35	1:40
(C) Radiating time (min)	10	12	14	6	8	10
(D) Extraction temperature (°C)	60	70	80	50	60	70

Table 2b
Arrangement and results of L_9 (3^4) orthogonal test.

No.	Factors				Extraction yields of rutin (mg/g)[a]	
	A	B	C	D	S (mean ± S.D.)	F (mean ± S.D.)
1	1	1	1	1	4.229 ± 0.057	159.23 ± 2.04
2	1	2	2	2	4.521 ± 0.063	145.53 ± 1.77
3	1	3	3	3	4.608 ± 0.069	157.39 ± 1.98
4	2	1	2	3	3.999 ± 0.053	154.55 ± 1.84
5	2	2	3	1	4.262 ± 0.060	161.40 ± 2.16
6	2	3	1	2	4.784 ± 0.076	151.21 ± 1.80
7	3	1	3	2	3.811 ± 0.047	165.45 ± 2.35
8	3	2	1	3	3.990 ± 0.052	167.4 ± 2.68
9	3	3	2	1	4.669 ± 0.072	153.61 ± 1.89

	Factors							
	A		B		C		D	
	S	F	S	F	S	F	S	F
K_{1j}[b]	4.453	154.05	4.013	159.74	4.334	159.28	4.387	158.08
K_{2j}	4.348	155.72	4.258	158.11	4.396	151.23	4.372	154.06
K_{3j}	4.157	162.15	4.687	154.07	4.227	161.41	4.119	159.78
R[c]	0.296	8.103	0.674	5.673	0.169	10.183	0.188	5.717
O[d]	A_1	A_3	B_3	B_1	C_2	C_3	D_1	D_3

S: S. chinensis; F: Flos Sophorae.

[a] Extraction yield values of the two herbs were comprised of mean and standard deviation (S.D.) of three independent experiments.

[b] Logesh $K_{ij} = (1/3)\sum$ mean extraction yield of rutin at factor j ($j = A, B, C, D$).

[c] Logesh $R_{ij} = \max\{\overline{K_{ij}}\} - \min\{K_{ij}\}$, j and i mean extraction factor and setting level here, respectively.

[d] O means the optimum condition. The optimum combination of conditions for S. Chinensis, is $A_1B_3C_2D_1$, while for Flos Sophorae, is $A_3B_1C_3D_3$.

Table 2c
Analysis of variance (ANOVA) for orthogonal test.

Source	Sum of squares		Degrees of freedom		*F*-value		*P*-value	
	S	F	S	F	S	F	S	F
A	0.135	109.841	2		0.573	1.139		
B	0.698	57.176	2		2.964	0.561	*	
C	0.044	173.054	2		0.187	1.794		*
D	0.065	51.704	2		0.276	0.536		
Error	0.94	385.77	8					

S: S. chinensis; F: Flos Sophorae.
* Significant ($P > 0.05$).

dition arrays and results of them were shown in Table 2b. And the analysis of variance (ANOVA) for orthogonal test was shown in Table 2c.

The *K* and *R* values were calculated and listed in Table 2b. *K* value is the average extraction efficiency of every factor under each level. According to the largest donating rule, the largest value of *K* is the optimized value. Therefore, in the light of Table 2b, K_1–K_3 were the extraction yield of rutin under every level of an investigating factor, respectively the largest value was the optimized value. *R* value is the range of *K* value. Consequently the optimum experimental conditions of ILMAE were as follows: for S. chinensis, the concentration of [bmim]Br aqueous solution was 2.0 mol/L, the solid–liquid ratio was 1:30, the extracting temperature was 60 °C and the radiating time was 12 min; and for Flos Sophorae, the concentration of [bmim]Br aqueous solution and the solid–liquid ratio were as same as those of S. Chinensis, the extracting temperature was 70 °C and the radiating time was 10 min. Three samples of S. chinensis and Flos Sophorae extracted under optimum conditions respectively were determined to validate the optimized conditions of ILMAE, and the mean extraction yields of them were higher than any group of the orthogonal experiment respectively, which indicated that the extraction yields of rutin from the two herbs could be increased using the combination of four optimized factors under ILMAE, respectively. According to the R values in Table 2b and the analysis of variance in Table 2c, it showed that the solid–liquid ratio had much more obvious effects on the extraction of rutin from S. chinensis than other factors. While for Flos Sophorae, the radiating time was the predominant factor among the four factors, followed by the concentration of [bmim]Br aqueous solution.

3.3. Comparison of different extraction procedures

In order to find a better extraction method for rutin from the investigated plants, ILMAE was compared with the optimized ILUAE, ILHE and ILME methods. The results were shown in Table 3. Compared to other methods, the extraction efficiency of rutin in the two herbs with ILME which consumed most solvent and extracting time was the worst. The cause was that ILME was based on the concentration difference of the target between the sample and the extraction solvent for extraction. Ultrasound can break plant tissue and accelerate the solvent penetrating through plant tissue, sequentially ILUAE can improve extraction yields of active ingredients from herbs and save extracting time. MAE has so unique extraction mechanism including superheating, mass heating and fast heating, which is very different from ILHE just having conventional heating, that it can availably break cytoderm and heat cells to accelerate dissolution of intracellular effective components. Compared ILMAE with ILUAE, the extraction efficiency of the former was higher than that of the latter, while the extracting time of the former was much shorter than that of the latter. In conclusion, compared with other three extraction methods, ILMAE had the highest extraction yield of rutin from the two plants with the least solvent consumption and the shortest extracting time.

3.4. Method validation

Rutin was identified by its corresponding chromatograms and retention time in comparison to that of the authentic standard rutin. Examples of chromatograms of standard rutin and extracts of S. chinensis and Flos Sophorae from different places are shown in Fig. 4(A)–(E)) and Fig. 5(A)–(D)), respectively. Figs. 4A and 5(A) showed that [bmim]Br aqueous solution did not show any interference for the analysis of rutin using different elution methods, because there was little absorbance to [bmim]Br at 356 nm. Because of different elution ways, the retention time was 31.23 min and 13.31 min for rutin from S. chinensis and Flos Sophorae, respectively.

3.4.1. Linearity studies

A series of standard solutions of rutin at six levels in the concentration range from 30 to 300 mg L^{-1} were prepared to determine the linearity of this method. Each of them was analyzed in triplicate. The linearity monitored at 356 nm was $Y = 17675.9X + 190450.4$ ($r = 0.99917$) over the concentration range from 42 to 252 mg L^{-1}, where *X* was rutin concentration as mg L^{-1} and *Y* was the peak area. The limit of detection was 5.3 μg L^{-1} which was evaluated on the basis of a signal-to-noise ratio of 3. Good linearity was observed with the regression coefficient of 0.99917.

3.4.2. Recovery studies

0.5 g of S. chinensis powders added in 5 mg of rutin and 0.2 g of Flos Sophorae powders added in 10 mg of rutin were extracted by

Table 3
Comparative study of extraction efficiency of rutin under different extraction methods using [bmim]Br aqueous solution as extraction solvent ($n = 3$).

Sample	ILMAE[b]				ILUAE				ILHE				ILME			
	Observed values[a](mg/g)		Recovery(%)		Observed values(mg/g)		Recovery(%)		Observed values(mg/g)		Recovery(%)		Observed values(mg/g)		Recovery(%)	
	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
S. chinensis	4.879	0.065	101.2	1.0	4.861	0.073	99.3	2.4	4.853	0.057	98.6	2.2	4.561	0.049	97.5	2.9
Flos Sophorae	171.82	2.53	99.6	1.1	171.08	2.77	98.2	3.6	170.21	2.31	99.7	4.1	163.28	2.47	94.8	4.3

[a] Each value was the mean and standard deviation (S.D.) of three independent experiments using the optimal conditions of corresponding methods. Observed value (mg/g) = mass of rutin in extraction solution (mg) / mass of sample (g).
[b] The four extraction methods were compared under their optimum combination of conditions.

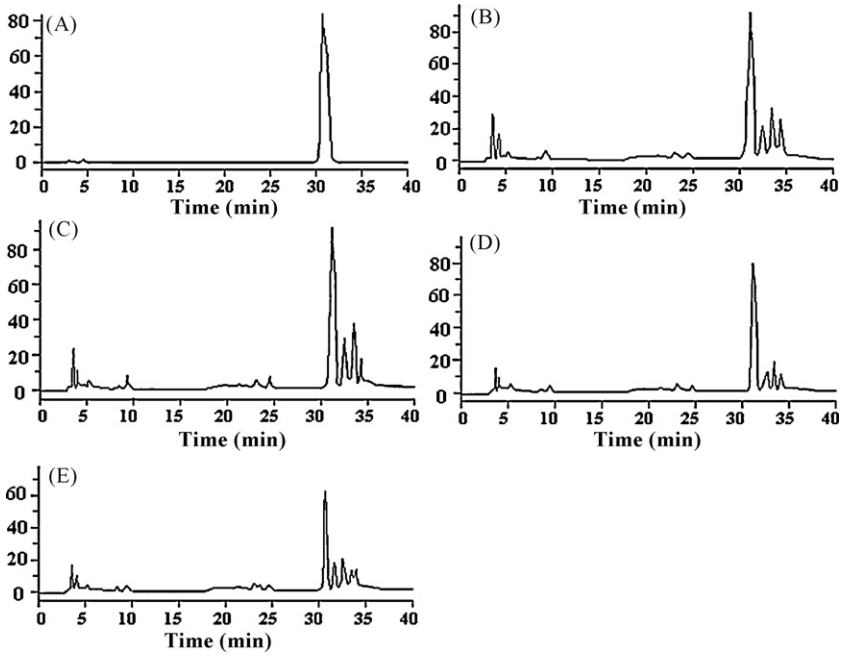


Fig. 4. The chromatograms of standard rutin solution of 0.21 mg/mL (A) and the extracts of S. chinensis from different places extracted by [bmim]Br aqueous solution (B, from Suzhou City of Jiangsu Province; C, from Ningbo City of Jiangsu Province; D, from Changsha city of Hunan Province; E, from Zhangshu city of Jiangxi Province, respectively).

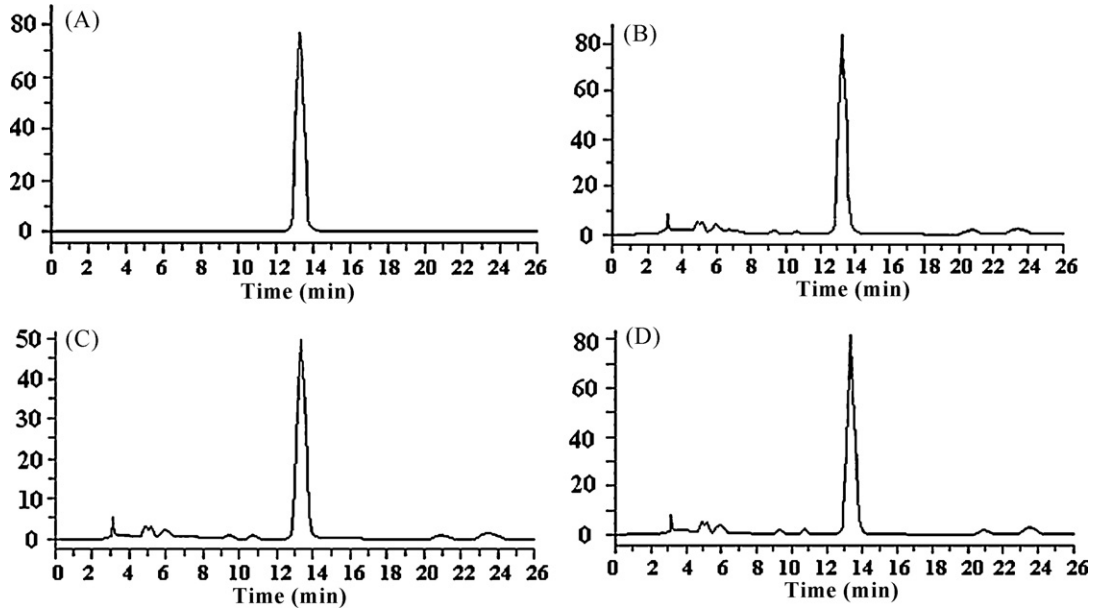


Fig. 5. The chromatograms of standard rutin solution of 0.21 mg/mL (A) and the extracts of Flos Sophorae from different places extracted by [bmim]Br aqueous solution (B, from Suzhou City of Jiangsu Province; C, from Ningbo City of Jiangsu Province; D, from Changsha city of Hunan Province, respectively).

Table 4
Recovery of rutin from S ("S. chinensis") and F (Flos Sophorae) ($n = 3$).

Herbs	No.	Sample (g)	Content (mg)	Added (mg)	Found (mg)	Recovery (%)	Mean recovery (%)	RSD (%)
S	1	0.5001	4.8604	5.1	10.0913	101.31	101.23	1.02
	2	0.5002	4.8611	5.1	10.1822	102.22		
	3	0.5001	4.8596	5.0	9.8747	100.15		
F	1	0.2001	168.54	10.1	177.69	99.19	99.62	1.12
	2	0.2000	168.40	10.2	176.83	98.79		
	3	0.2002	168.86	10.1	180.25	100.89		

Table 5
The analytical results of rutin from S ("S. chinensis") and F (Flos Sophorae) from different batches ($n = 5$).

Herbs	No.	Herbal origin	Degreased or not	Mean contents of rutin (mg/g)	RSD (%)
S	1	Suzhou City of Jiangsu Province	Yes	4.8794	0.83
	2	Ningbo City of Jiangsu Province	Yes	4.8151	1.08
	3	Changsha City of Hunan Province	Yes	4.4494	1.05
	4	Jiangxi Province	Yes	3.3023	1.10
	5	Suzhou City of Jiangsu Province	No	4.4145	1.19
F	1	Suzhou City of Jiangsu Province	No	166.877	1.07
	2	Ningbo City of Jiangsu Province	No	167.134	0.97
	3	Changsha City of Hunan Province	No	162.902	0.79

ILMAE under optimized conditions respectively, and then determined by HPLC–UV detection to examine the recovery of the promoted method. The results which were shown in Table 4 indicated that the mean recoveries for rutin from S. chinensis and Flos Sophorae were 101.23% (RSD = 1.02%) and 99.62% (RSD = 1.12%), respectively.

3.4.3. Reproducibility of analytical method

To assess the reproducibility of the promoted method, five extraction solutions of the two herb samples which were made by optimum ILMAE method, were determined with 0.46% and 0.57% of RSD respectively, which showed accredited reproducibility.

3.4.4. Precision test of the apparatus

The precision of the chromatographic determination was evaluated by the analysis of 0.21 mg mL^{-1} of rutin standard solution for ten consecutive times by HPLC–UV detection. RSD obtained was 0.37%.

3.4.5. Stability test of the solution

The stability of rutin in [bmim]Br aqueous solution and extracts was evaluated by determining extraction solutions of the two samples and standard solution of rutin in the interval of every 2 h for 5 times. RSDs obtained were 0.57%, 0.65%, 0.43%, respectively, which indicated that rutin was stable in ILs solutions and extracts for 10 h.

3.5. Sample analysis

Rutin in various S. chinensis and Flos Sophorae from different places extracted by the optimized ILMAE conditions respectively were determined by means of HPLC–UV detection, and the results were shown in Table 5. It can be seen that the extraction efficiency of rutin in S. chinensis from different places extracted by the optimum ILMAE method were different, which is identical with that in Flos Sophorae from different places. A comparison of rutin contents in S. chinensis between degreased and non-degreased samples was also investigated, which showed that lipid material had much influence on the detection results of rutin. Thus, all samples of S. chinensis used in this study were degreased before extraction.

3.6. Mechanism of ILMAE

3.6.1. Study of kinetic mechanism

The ILMAE kinetic mechanism was researched in this study to investigate the changes of the extraction yields of rutin from the two herbs in ILMAE process using 2.0 M [bmim]Br aqueous solution according to the time. The results of kinetic curve were shown in Fig. 6(A) and (B). We can see that the extraction yields of rutin from the two herbs increased rapidly at first and then reached an equilibrium yield after 12 min for S. chinensis and 8 min for Flos Sophorae, respectively. The above results indicated that the kinetic mechanism of ILMAE was correlated with sample structures [32]. Besides, the obtained results indicated that about 12 min and 8 min of MAE time were much enough to obtain high extraction yields of *rutin* from S. chinensis and Flos Sophorae respectively when ILs aqueous solutions were used as extraction solvents.

3.6.2. Structural changes after extraction

In order to further study the extraction mechanism, the identification of microstructures and chemical structures of S. chinensis samples and Flos Sophorae samples before and after extraction procedures including ILHE, ILUAE and ILMAE with [bmim]Br aqueous solution was carried out by SEM and FTIR spectroscopy, respectively. The results were shown in Supplementary information Figs. S3 and S4.

Compared Figs. S3(C), S3(D) with S3(A), the microstructures of S. chinensis both changed visibly after ILMAE and ILUAE. It can be seen that the surface of S. chinensis was greatly damaged and the structure of cell walls was fractured after the process of ILMAE or ILUAE, which led the target to bare in extraction solution that would dissolve it. The structure of Flos Sophorae sample was changed as that of S. chinensis, which was obtained from the comparison of Figs. S3(C′) and S3(D′) with S3(A′). Both the results of the two herbs implied that the mechanisms of ILMAE and ILUAE were both based on an explosion at the cell level and the former was in accord with the hypothesis investigated by Paré et al. [33,34]. However, compared Figs. S3(B) with S3(A), Figs. S3(B′) with S3(A′), the microstructures of S. chinensis and Flos Sophorae were both not appreciably changed and fractured after ILHE, which indicated that rutin was extracted by exuding from the two samples under ILHE.

As FTIR spectra can provide useful information for identifying the presence of certain function groups or chemical bonds in a molecule or an interaction system, it was applied here to investigate the changes in chemical structures of S. chinensis and Flos

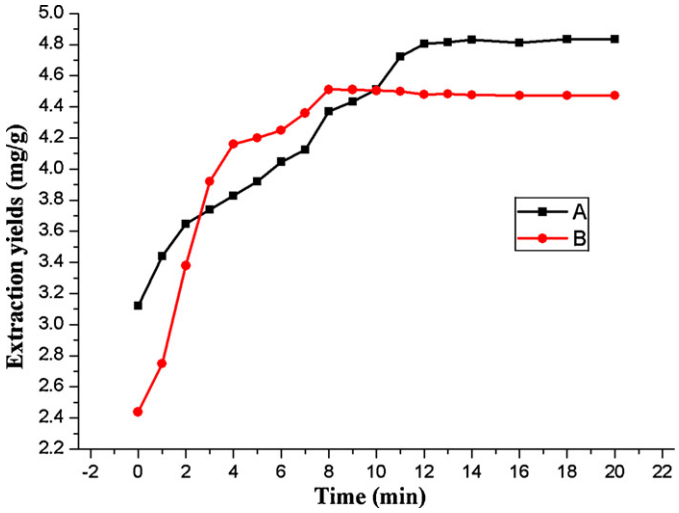


Fig. 6. The kinetic curves of rutin extracted from S. chinensis (A) and Flos Sophorae (B) respectively, using 2.0 mol/L [bmim]Br aqueous solution by ILMAE procedure. The extraction yields of Flos Sophorae were all divided by 35 in order to make the two curves in the same coordinate.

Sophorae before and after extraction by various methods, respectively. The results of FTIR spectra (see Fig. S4) showed that the signal situations and intensity of absorption bands at 1631, 1456, 1338, 1031 and 755 cm^{-1} for S. chinensis and 1638, 1400, 1168, 1086 and 622 cm^{-1} for Flos Sophorae were not apparently changed after ILMAE, ILUAE or ILHE with [bmim]Br aqueous solution, which indicated that the chemical structures of carbohydrate compounds, including cellulose, hemicellulose, lignin and insoluble starch, were unbroken after extracted by the three methods. The cause of the results was probably related to that water segregated the strong interactions between ILs and carbohydrate compounds of matrixes [35–37]. Therefore, the chemical bonding interactions between ILs and sample matrixes were not obvious, which coincides with the study of Fu-you Du et al. [1].

3.6.3. Characterisation of rutin by LC–MS

To further determine that the two Chinese medicinal herbs do contain rutin, and rutin was not changed after extracted by ionic liquids, the extracts obtained by ILMAE were characterised by LC–MS. The results were shown in Supplementary information Fig. S5. According to negative ion mass spectrum of rutin standard solution and two herb extracts, MS (ESI) m/z calcd. for $C_{27}H_{30}O_{16}$ $[MH]^+$: 609.1, found 609.1, which indicated that there is rutin which was not changed after extraction in the two herbs.

4. Conclusion

In this work, as a kind of relative green solvent, ILs were successfully used in the MAE procedure of the extracting of rutin from S. chinensis and Flos Sophorae instead of traditional volatile organic solvents. With the addition of ILs, the extraction yield of rutin was improved greatly. The structure of ILs, especially the anions have a significant impact on the extraction efficiencies of the target. The optimal conditions of this approach were successively identified by single-factor and orthogonal experiments. Compared to optimal ILHE, ILME and ILUAE procedures, the optimal ILMAE approach could provide higher extraction yields of rutin which is 4.879 mg/g in S. chinensis with RSD 1.33% and 171.82 mg/g in Flos Sophorae with RSD 1.47% within the shortest extracting time. And the enhanced extraction yields by ILMAE were primarily based on the damage of sample microstructures in the course of ILMAE. The kinetic mechanism curves of ILs-MAE method were probably related to the components and the interactions among the components of herb medicines, and longer time in MAE was not good for the extraction of rutin. It was future demonstrated through LC–MS that the two researched herbs do contain rutin and ionic liquids wouldn't influence the structure of rutin. Due to its good reproducibility and precision, the proposed green and effective ILs-MAE method shows a great promising prospect in the extraction and the separation of natural products.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2010.10.006.

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