Talanta 78 (2009) 1177–1184

Contents lists available at ScienceDirect

Talanta

journal homepage: www.elsevier.com/locate/talanta

Application of ionic liquids in the microwave-assisted extraction of polyphenolic compounds from medicinal plants

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ARTICLE INFO

Article history:
Received 12 October 2008
Received in revised form 16 January 2009
Accepted 20 January 2009
Available online 30 January 2009

Keywords:
Ionic liquids
Microwave-assisted extraction
Polyphenolic compounds
Medicinal plants

ABSTRACT

Ionic liquids (ILs) solutions as solvents were successfully applied in the microwave-assisted extraction (MAE) of polyphenolic compounds from medicinal plants. ILs, its concentration and MAE conditions were investigated in order to extract polyphenolic compounds effectively from *Psidium guajava* Linn. (*P. guajava*) leaves and *Smilax china* (*S. china*) tubers. The results obtained indicated that the anions and cations of ILs had influences on the extraction of polyphenolic compounds as well as the ILs with electron-rich aromatic π-system enhanced extraction ability. Under the optimized conditions, the extraction yields of the polyphenolic compounds were in the range of 79.5–93.8% with one-step extraction, and meanwhile the recoveries were in the range of 85.2–103% with relative standard deviations (R.S.D.s) lower than 5.6%. Compared to conventional extraction procedures, the results suggested that the proposed method was effective and alternative for the extraction of polyphenolic compounds from medicinal plants. In addition, the extraction mechanisms and the structures of samples before and after extraction were also investigated. ILs solutions as green solvents in the MAE of polyphenolic compounds from medicinal plant samples showed a great promising prospect.

1. Introduction

Ionic liquids (ILs) are composed of organic cations and inorganic or organic anions and are liquid near room temperature (or by convention below 100 °C). They have been proposed as greener alternatives to volatile organic solvents thanks to their unique characteristics: negligible vapor pressure, good thermal stability, very wide liquidus range, good dissolving and extracting ability, excellent microwave-absorbing ability, designable structures and among others [1–4]. In recent years, ILs have been received much attention as neoteric solvents in various applications including catalysis, synthesis, industrial cleaning, extraction and separation [4–8].

Applications of ILs in sample preparation have shown great promising prospect [7–10], which includes liquid–liquid extraction, liquid-phase microextraction, solid-phase microextraction and aqueous two-phase systems extraction, for that ILs could alleviate environmental pollution and improve the selectivity and the extraction yields of interesting compounds in sample pretreatment processes in comparison to conventional organic solvents. Considered that ILs as solvents and co-solvents can efficiently absorb microwave energy [2] and at the same time microwave-assisted extraction (MAE) is an attractive and rapid sample preparation technique [11], it is a rather interesting project that ILs solution as solvent is applied in the MAE of various useful substances from solid samples. Recently, 1-*n*-butyl-3-methylimidazolium-based ionic liquids aqueous solutions as solvents were investigated in the extraction of *trans*-resveratrol from *Rhizma Polygoni Cuspidati* [9] and alkaloids from medicinal plants [10,12], indicating that ILs had potential applications in the MAE of useful substances from medicinal plants.

Many medicinal plants contains various bioactive compounds, such as polyphenolic compounds, nitrogen compounds, vitamins, terpenoids and some other endogenous metabolites, and have been used for pharmaceutical and dietary therapy for several millennia [13]. *Psidium Guajava* Linn. (*P. guajava*) leaves and *Smilax china* (*S. china*) tubers are two popular and important herbal medicines, the extracts have multiple therapeutic effects and pharmacological activities, such as anti-oxidant, anti-inflammatory, anti-microbial, and anti-tumor effects, which were related with polyphenolic compounds including gallic acid, ellagic acid, quercetin and *trans*-resveratrol (see Fig. 1) [13–17]. They could be extracted with volatile organic solvents [17,18], and these polyphenolic compounds in samples proved to be stable under direct heating extraction (HE) and microwave-assisted heating extraction at temperatures even up to 100 °C for 20 min without degradation [9,19]. However, to our best knowledge, there are no reports on the extraction and determination of polyphenolic compounds in the two medicinal plants using ILs solution as solvent.

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doi:10.1016/j.talanta.2009.01.040

In order to study the potentiality of ILs solutions as alternative solvents in the MAE of polyphenolic compounds, including gallic acid, ellagic acid, quercetin and *trans*-resveratrol (see Fig. 1), from *P. guajava* leaves and *S. china* tubers, 11 ILs with different cations and anions were investigated (for chemical structures of ILs see Fig. S1, Supplementary Data). The influential parameters of the MAE procedure were optimized systematically. The ionic liquids-based microwave-assisted extraction (ILs-MAE) approach proposed here was compared with conventional extraction approaches. The extraction results were evaluated by the determination of the polyphenolic compounds in the extracts using high-performance liquid chromatography (HPLC) with UV detection. The extraction mechanisms and the microstructures and chemical structures of samples before and after extractions were also investigated.

2. Experimental

2.1. Reagents and samples

Gallic acid (with purity >99%) was obtained from Kemiou Chemical Reagent Company (Tianjin, China). Quercetin was purchased from Acros (Geel, Belgium). *trans*-Resveratrol and ellagic acid were purchased from Sigma (St. Louis, MO, USA) and of minimum 95% purity. HPLC grade acetonitrile used for mobile phase was purchased from Merck (Darmstadt, Germany). Sodium dicyanamide was purchased from Sigma–Aldrich Co. (St. Louis, USA). 1-Methylimidazole was received from Kaile Chemical Plant (Zhejiang, China) and of approximately 99% purity, 1-bromoethane, 1-chlorobutane, 1-bromobutane and 1-bromohexane were obtained from Sinopharm Chemical Reagent Company and of >98% purity, tetramethylammonium chloride was purchased from Shanghai Chemical Reagent Company (Shanghai, China), sodium tetrafluoroborate (≥98%) was purchased from Xiangyang Chemical Factory (Zhejiang, China), pyridine and other reagents used were supplied by Guangzhou Chemical Reagent Factory (Guangzhou, China).

P. guajava leaves (Guangxi, China) and *S. china* tubers (Hunan, China) were dried and then triturated to various particle sizes, respectively. The same batch of sample was used here in the experiments.

2.2. Apparatus

MAE experiments were performed with an MAS-I microwave oven (Sineo Microwave Chemistry Technology Company, Shanghai, China). The scheme and its illustration of the MAS-I microwave oven were described in previous work [9]. The LC-10AT (Shimadzu, Japan) HPLC system equipped with a SPD-10A UV–visible dual wavelength detector was used for analysis. A Luna C_{18} column (250 mm × 4.6 mm I.D., 5 μm, Phenomenex, USA) was used as LC analytical column. ^{1}H NMR and ^{13}C NMR spectra were recorded on Mercury-Plus 300 and Varian-INOVA 500 NMR spectrometry (Varian, USA). A XL-30 scanning electron microscope (Philips, Eindhoven, Netherlands) was used to observe pictures of sample before and after extraction. FTIR spectra were recorded in the region of 400–4000 cm^{-1} on a Nicolet Avatar 300 model FTIR spectrometer (USA). The pH values were measured with an E-201 pH-meter (Shanghai Precision & Scientific Instrument Company, China).

2.3. Preparation of ILs and standard solutions

1-Butyl-3-methylimidazolium chloride ([bmim]Cl), 1-butyl-3-methylimidazolium bromide ([bmim]Br) and 1-butyl-3-methylimidazolium tetrafluoroborate ([bmim][BF_4]) were prepared as previously described [9], the same procedure was used as for 1-ethyl-3-methylimidazolium bromide ([emim]Br), 1-hexyl-3-methylimidazolium bromide ([hmim]Br) and 1-ethyl-3-methylimidazolium tetrafluoroborate. 1-butyl-3-methylimidazolium dicyanamide ([bmim][$N(CN)_2$]), 1-butyl-3-methylimidazolium sulfate ([bmim]$_2$[SO_4]), *N*-butylpyridinium chloride (bPyCl) and 1-butyl-3-methylimidazolium dihydrogen phosphate ([bmim][H_2PO_4]) were synthesized according to the experimental procedures described in the literatures [20–22]. All ILs obtained were dried at 80 °C for 12 h under vacuum and then checked by ^{1}H NMR and ^{13}C NMR spectra on Mercury-Plus 300 or INOVA 500 NMR spectrometry at room temperature in D_2O and d_6-acetone. The NMR spectra data obtained were described in detail in the supplementary data (see Tables S1 and S2) and the characterization of NMR spectroscopy showed that there were no organic impurities in ILs.

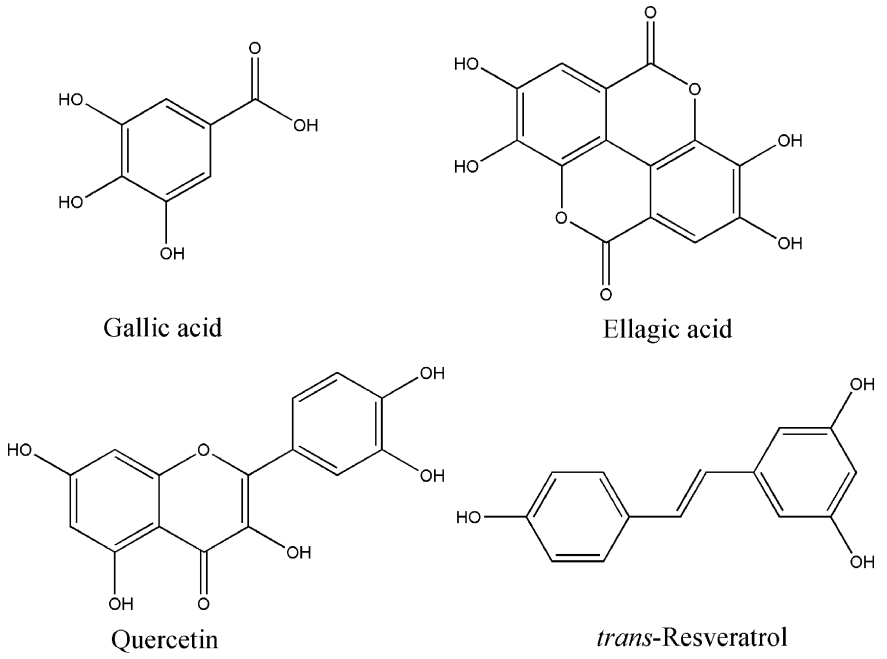

Fig. 1. Chemical structures of four polyphenolic compounds investigated.

Stock solution of gallic acid, ellagic acid and quercetin (each 200 mg/L) were prepared in 1.67 mol/L [bmim]Br acid solution (pH 2.5, adjusted with 6.0 mol/L hydrochloric acid). Stock solution of *trans*-resveratrol and quercetin (each 100 mg/L) were prepared in 1.67 mol/L [bmim]Br solution. Working standard solutions were prepared by serial dilution of the two stock solutions in the corresponding [bmim]Br solution, respectively, and then stored at 1–4 °C in darkness. Deionized water was used throughout the work.

2.4. Ionic liquids-based microwave-assisted extraction (ILs-MAE)

1.0 g of accurately weighed sample was extracted with 20 mL of different ILs solution. 0.75 mol/L $[bmim]_2[SO_4]$ and 1.50 mol/L other ILs aqueous solutions were used here, respectively. *P. guajava* leaves were extracted for 10 min at 70 °C. *S. china* tubers were extracted for 10 min at 60 °C.

The influences of the MAE conditions in the extraction of polyphenolic compounds were investigated by an orthogonal design $L_9(3^4)$ and subsequently a factorial design experiments. All extraction experiments were repeated for three times. The extracts obtained were filtrated and then diluted to 30.0 mL with deionized water. The pH value of the extract of *P. guajava* leaves was adjusted to 2.5 with 6.0 mol/L hydrochloric acid solutions.

The extraction yield of polyphenolic compound was defined as follows:

$$\text{Yield}\ (\%) = \frac{\text{Mass of polyphenolic compound in one-step extraction solution}}{\text{Sum of the mass of polyphenolic compound in sample}} \times 100$$

The total mass of gallic acid, ellagic acid, quercetin or *trans*-resveratrol in sample was determined by analysis of the total extraction solutions after five consecutive extraction with fresh [bmim]Br solution under the optimized MAE conditions. In this work, our experimental results showed that the mean of total mass of gallic acid, ellagic acid and quercetin in *P. guajava* leaves was 0.608, 2.947 and 0.679 mg/g, respectively, and the mean of total mass of *trans*-resveratrol and quercetin in *S. china* tubers was 0.581 and 0.235 mg/g, respectively.

2.5. Conventional reference extraction procedure

Methanol was selected as the reference solvent in the MAE of polyphenolic compounds in *P. guajava* leaves and *S. china* tubers. The extraction experiments were operated under the optimized conditions except for solvent type and extraction temperature. 65 °C (the best extraction temperature according to our preliminary experiments) was selected for *P. guajava* leaves. After extraction, the obtained extracts were cooled to ambient temperature and then diluted to 30 mL with methanol.

Heating extraction was selected as the reference method for extraction of the four polyphenolic compounds. A water-bath extraction was performed with a 1.0 g sample and 40 mL 2.50 mol/L [bmim]Br in a flask (100 mL) and the suspensions were heated for 4 h at the optimized temperature under mechanical stirring. After that, the extracts were filtrated and then diluted to 60 mL with deionized water.

2.6. HPLC analysis

The filtrated extracts were collected in a graduate cuvette and then diluted to 30 mL with water. The pH of diluted solution of the extracts of *P. guajava* leaves was adjusted to 2.5. An aliquot was filtrated through a 0.45 μm microporous membrane for subsequent HPLC analysis. Injection volume was 10 μL and column temperature was ambient. The mobile phase consisted of acetonitrile (solvent A) and 0.6% (v/v) acetic acid aqueous solution (solvent B) with a flow rate of 1 mL/min. For analysis of the extracts of *P. guajava* leaves, the gradient elution program was the following: 5–9% of solvent A from 0 to 8 min, 9–20% from 8 to 10 min, 20–30% from 10 to 25 min, 30–60% from 25 to 40 min and then held for 5 min; the UV detection wavelengths were 254 and 273 nm. The extract of *S. china* tubers was monitored at 254 and 306 nm, the corresponding gradient elution program was as follows: 28% of solvent A from 0 to 10 min, 28–50% from 10 to 20 min, 50–70% from 20 to 25 min and then held for 5 min. Registering of the detector signal and operating of the system were accomplished by the software Shimadzu Class-VP.

Gallic acid, ellagic acid, quercetin and *trans*-resveratrol in each extracts were identified by comparing their retention time and UV spectra with those of the reference standards. The external standard method was set up for quantitative determination of the analytes.

3. Results and discussion

3.1. Optimization of ILs-MAE

3.1.1. Selection of ILs

ILs have strong solvent dissolving power and can efficiently absorb microwave energy, thus they were employed as solvents and co-solvents in the MAE of analytes [9,10,12]. Moreover, the structures of ILs have significant influences on the extraction yields of analytes, owing greatly to their distinct multiple interactions with analytes [23] and their dissolving ability for polyphenolic compounds [24]. To find out the optimal ILs and evaluate its performance in the MAE of polyphenolic compounds from *P. guajava* leaves and *S. china* tubers, ILs with different cations and anions were tested in the present work.

From Table 1, it can be seen that the addition of ILs to the extraction solvent obviously improved the extraction yields of polyphenolic compounds, especially ellagic acid, quercetin and *trans*-resveratrol, from *P. guajava* leaves and *S. china* tubers compared with the extraction using water as solvent in MAE. The results have been indicated by the similar results of Du et al. [9]. On the other hand, the results of Table 1 suggested that the cations and anions of ILs influenced the extraction yields of polyphenolic compounds. For the 1-*n*-butyl-3-methylimidazolium based ionic liquids with Br^-, Cl^-, BF_4^-, SO_4^{2-}, $N(CN)_2^-$ and $H_2PO_4^-$, the obtained extraction yields indicated that Br^- and $H_2PO_4^-$ were more efficient than other four anions in the MAE of polyphenolic compounds from *P. guajava* leaves, and Br^- and BF_4^- were more efficient than other four anions in the MAE of *trans*-resveratrol and quercetin from *S. china* tubers. With the same anion of Br^-, [emim]Br, [bmim]Br and [hmim]Br were used to investigate the effects of the alkyl chain length on the MAE of polyphenolic compounds. The results indicated that the increasing alkyl chain length has influence on the extraction, and the [bmim]Br was more efficient than the other two ILs in the MAE of polyphenolic compounds from the two medicinal plants. Although the hydrogen bond acidity for the three cations increased from ethyl to hexyl of 1-position of the 1-alkyl-3-methylimidalizium ring [24], the hydrophobicity increased with the increasing of alkyl chain length. Both the right hydrogen bonding and hydrophobic interactions of [bmim]Br resulted in the stronger solvation interactions for polyphenolic compounds and then the higher extraction yields in comparison with these of [emim]Br and [hmim]Br. For the extraction efficiency of [emim][BF_4] and [bmim][BF_4], there was no obvious differentia in the MAE of polyphenolic compounds except for quercetin in *S. china* tubers. The above results suggested that the extraction yields of polyphenolic compounds were largely anion-dependent for the same class ILs, owing to the anion-dependency of the solubilities of analytes in ILs [24].

Table 1
Results of different ILs effects on extraction yields of polyphenolic compounds[a] ($n = 3$).

Ionic liquids	*P. guajava* leaf			*S. china* tuber	
	Gallic acid (mean ± S.D., %)	Ellagic acid (mean ± S.D., %)	Quercetin (mean ± S.D., %)	*trans*-Resveratrol (mean ± S.D., %)	Quercetin (mean ± S.D., %)
[bmim]Br	88.7 (±2.2)	65.9 (±3.3)	69.5 (±3.7)	57.0 (±3.3)	59.6 (±3.7)
[bmim]Cl	91.0 (±2.3)	59.4 (±1.7)	41.5 (±1.1)	34.6 (±1.7)	21.3 (±1.1)
[bmim][BF_4]	65.6 (±2.8)	74.8 (±3.0)	59.4 (±2.7)	51.7 (±3.0)	51.5 (±2.7)
[emim][BF_4]	67.3 (±1.7)	71.5 (±2.9)	55.8 (±2.1)	47.5 (±2.9)	39.6 (±2.1)
[bmim][$N(CN)_2$]	76.8 (±2.1)	59.7 (±2.2)	49.2 (±1.4)	56.5 (±2.2)	30.2 (±1.4)
[bmim]$_2$[SO_4]	75.2 (±2.4)	42.8 (±2.5)	29.9 (±2.4)	50.7 (±2.5)	47.7 (±2.4)
[bmim][H_2PO_4]	90.6 (±3.8)	65.9 (±3.7)	74.5 (±3.0)	58.3 (±3.7)	45.5 (±3.0)
$(CH_3)_4NCl$	78.0 (±2.8)	26.5 (±0.9)	21.4 (±0.3)	10.3 (±0.9)	4.3 (±0.3)
bPyCl	80.3 (±3.4)	62.2 (±2.9)	50.7 (±1.5)	52.4 (±3.8)	26.4 (±1.9)
[emim]Br	74.3 (±1.1)	59.1 (±1.9)	47.6 (±1.3)	40.3 (±1.9)	28.5 (±1.3)
[hmim]Br	77.0 (±2.6)	61.3 (±2.0)	49.7 (±1.4)	52.7 (±2.0)	46.0 (±1.4)
Water	63.3 (±0.5)	35.1 (±0.3)	15.2 (±0.2)	3.0 (±0.3)	1.8 (±0.2)

[a] Extraction yield values are expressed as mean and standard deviation (S.D.) calculated from three independent experiments.

For $(CH_3)_4NCl$, [bmim]Cl and bPyCl, the obtained results indicated that the extraction yields of polyphenolic compounds were greatly influenced by the cations species. ILs which have cationic moieties with an electron-rich aromatic π-system produced stronger interactions with solute molecules capable of undergoing polarity, π–π and n–π interactions [23,25]. ILs containing the *N*-butylpyridinium cation have a more aromatic character than the imidazolium based ionic liquids, and they had stronger solvation interactions and better dissolving ability [24,26], leading to the higher extraction yields of polyphenolic compounds. In lack of π–π and n–π interactions between ammonium cation and polyphenolic compounds, the extraction ability of $(CH_3)_4NCl$ for polyphenolic compounds was lower than the other two ILs, contributed to the lower extraction yields.

Considering both the extraction yields of polyphenolic compounds and the simple synthesis of ILs, [bmim]Br was selected to simultaneously extract polyphenolic compounds from *P. guajava* leaves and *S. china* tubers in the present work.

3.1.2. Optimization of ILs concentration

Our previous work [9] indicated that [bmim]Br concentration had significant influence on the extraction of *trans*-resveratrol. Therefore, the concentration effect of [bmim]Br on extraction of polyphenolic compounds from *P. guajava* leaves and *S. china* tubers was studied and the results are shown in Fig. 2.

In the light of Fig. 2, it can be observed that the extraction yields of polyphenolic compounds from *P. guajava* leaves and *S. china* tubers were increased with the increase of [bmim]Br concentration.

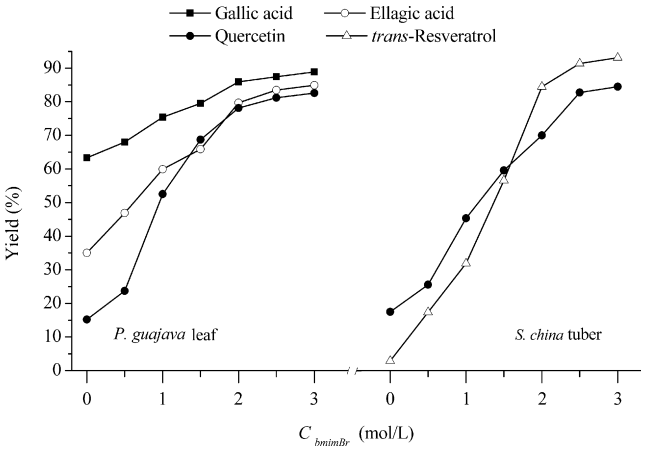

Fig. 2. Effect of [bmim]Br concentration on extraction yields of polyphenolic compounds from *P. guajava* leaves and *S. china* tubers.

The increasing tendency of the extraction yields of quercetin from *P. guajava* leaves and *S. china* tubers was the similar, which indicated that the influence by the nature of sample in the extraction of quercetin was not obvious. Below 2.0 mol/L [bmim]Br solution, the extraction yields of quercetin and *trans*-resveratrol increased more rapidly than that of ellagic acid, while that of gallic acid increased slowly. The reasons were related to the solubility of the polyphenolic compounds in extraction solvents. Gallic acid is soluble in water, but ellagic acid is poorly soluble, and quercetin and *trans*-resveratrol are insoluble, leading to the obvious difference in their extraction yields when water was used as extraction solvent (see Table 1). The addition of [bmim]Br improved the extraction yields of the polyphenolic compounds, especially quercetin and *trans*-resveratrol, due to the solvation power and multiple interactions of [bmim]Br. The strong interactions between imidazolium cation and phenolic compounds [23–26], especially hydrogen bonding, π–π, π–n, ionic/charge–charge and dipolarity, contributed greatly to this increase. On the other hand, [bmim]Br changed the dissipation factor of solution and improved the transfer efficiency of microwave energy, and thus also improved the extraction yields of the targets.

When [bmim]Br concentration was between 2.5 and 3.0 mol/L, the extraction yields of the four polyphenolic compounds were nearly stable, due to that the diffusion and transfer capacity of the solutions changed a little, even though the solvation ability of the solution was slightly increased. Given the similar extraction yields, 2.5 mol/L of [bmim]Br was selected for the subsequent experiments.

3.1.3. Optimization of MAE conditions

In this work, the influential factors of MAE procedure, including sample particle size (A), liquid/solid ratio (B), extraction temperature (C) and time (D), were optimized by means of an orthogonal design L_9 (3^4). The factors and the corresponding levels for the extraction of polyphenolic compounds from the two medicinal plants are shown in Table 2. Nine experimental trials were carried out according to the orthogonal array designs and the results are also shown in Table 2. The *K* and *R* values are calculated and listed in Table 3.

The differences of the extraction yields of polyphenolic compounds under different MAE conditions were obvious (see Table 2), illustrating that each of MAE factors had different influences on extraction. According to the largest donating rule, the largest value of *K* under every level of an investigating variable is the optimized value, however, the optimal MAE conditions of each polyphenolic compound presented in the same sample were not identical (see Table 3). The *R* values shown in Table 3 indicated that the influences of the investigated factors to the mean extraction yields were also not identical, and the influence of extraction time was minor

Table 2
Extraction yields[a] extracted with the orthogonal design L_9 (3^4) (n = 3).

Design ID number	Factor[b]						*P. guajava* leaf			*S. china* tuber	
	A	B		C		D	Gallic acid (%)	Ellagic acid (%)	Quercetin (%)	*trans*-Resveratrol (%)	Quercetin (%)
	Particle size (mm)	Liquid/solid ratio (mL:g)		Temperature (°C)		Time (min)					
1	A_1 (0.90–0.45)	B_1 (10:1)	(15:1)	C_1 (60)	(50)	D_1 (5)	50.3 ± 1.0	37.1 ± 1.2	32.1 ± 1.0	71.8 ± 3.4	48.8 ± 1.6
2	A_1	B_2 (15:1)	(20:1)	C_2 (60)	(60)	D_2 (10)	61.8 ± 3.6	64.5 ± 1.5	45.4 ± 2.3	91.4 ± 3.7	80.7 ± 3.1
3	A_1	B_3 (20:1)	(25:1)	C_3 (80)	(70)	D_3 (15)	82.1 ± 3.5	67.0 ± 2.8	53.0 ± 2.6	85.8 ± 3.0	89.2 ± 4.3
4	A_2 (0.45–0.30)	B_1		C_2		D3	68.4 ± 1.5	42.9 ± 0.7	34.2 ± 0.9	70.8 ± 1.6	51.9 ± 3.7
5	A_2	B_2		C_3		D_1	77.3 ± 2.4	65.2 ± 1.7	56.3 ± 1.5	77.9 ± 3.9	65.0 ± 2.6
6	A_2	B_3		C_1		D_2	56.4 ± 2.6	79.1 ± 3.1	76.7 ± 2.9	73.5 ± 3.4	66.9 ± 3.3
7	A_3 (0.30–0.12)	B_1		C_3		D_2	77.0 ± 2.8	38.2 ± 0.8	35.3 ± 1.5	55.6 ± 3.7	42.7 ± 2.3
8	A_3	B_2		C_1		D_3	58.2 ± 1.7	58.4 ± 1.6	52.7 ± 1.7	63.1 ± 2.3	56.5 ± 4.2
9	A_3	B_3		C_2		D_1	71.1 ± 2.9	77.4 ± 3.0	66.0 ± 2.0	63.9 ± 2.0	67.0 ± 3.5

[a] Each extraction yield value was the mean and standard deviation (S.D.) of three independent experiments.
[b] The levels of the other two factors (A and D) set were the same for *P. guajava* leaves and *S. china* tubers.

in comparison with the other three factors. The optimal extraction time was 10 min, while sample particle size, extraction temperature and liquid/solid ratio were further optimized by a factorial design experiments, respectively.

For sample particle size, Fig. 3A shows that it had remarkable effects on the extraction of polyphenolic compounds from *S. china* tubers, while it had slight effects on the extraction of polyphenolic compounds from *P. guajava* leaves contributing to their soft material which were permeated easily by solvent and ruptured easily under microwave irradiate. A particle size of 0.45–0.30 mm for *P. guajava* leaves and 0.90–0.45 mm for *S. china* tubers was adopted in the present work.

Fig. 3B shows that the extraction yields of the four polyphenolic compounds increased with the increase of temperature below 60 °C. From 60 to 80 °C, the extraction yields of gallic acid and ellagic acid kept slightly increasing. The extraction yield of quercetin extracted from *P. guajava* leaves or *S. china* tubers slightly decreased when the temperature was higher than 70 °C, while that of *trans*-resveratrol reached its maximum value at 60 °C, though our preliminary experimental results showed that the four polyphenolic compounds were not degraded below 100 °C in MAE process, and the similar results also have been indicated by Liazid et al. [19]. Thus, 70 °C for *P. guajava* leaves and 60 °C for *S. china* tubers were used as the optimum extraction temperature.

As for liquid/solid ratio, Fig. 3C shows that the extraction yields of the four polyphenolic compounds increased rapidly with the increase of the ratio of liquid/solid below 20:1. When the liquid/solid ratio varied from 20:1 to 30:1, the extraction yields changed a little. Thus, a liquid/solid ratio of 20:1 was selected in this study.

Under the optimized conditions, the polyphenolic compounds present in the two medicinal plants were successfully extracted out (Table 4). Namely, the extraction yields were in the range of 79.5–93.8% with one-step extraction.

3.2. Comparison of different extraction procedures

Methanol was used to extract polyphenolic compounds from *P. guajava* leaves and *S. china* tubers in order to compare the extraction efficiency by ILs solution with volatile organic solvents in MAE process. The results shown in Table 4 indicated that, as extraction solvent, the [bmim]Br solution and methanol had not remark-

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

Sample	Polyphenolic compound	Factor	K_1[a]	K_2	K_3	R[b]	Optimal level[c]
P. guajava leaf	Gallic acid (%)	A	64.7	67.4	68.8	4.1	A_3
		B	65.2	65.8	69.9	4.7	B_3
		C	55.0	67.1	78.8	23.8	C_3
		D	66.2	65.1	69.6	4.5	D_3
	Ellagic acid (%)	A	56.2	62.4	58.0	6.2	A_2
		B	39.4	62.7	74.5	35.1	B_3
		C	58.2	61.6	56.8	4.8	C_2
		D	59.9	60.6	56.1	4.5	D_2
	Quercetin (%)	A	43.5	55.7	51.3	12.2	A_2
		B	33.9	51.5	65.2	31.3	B_3
		C	53.8	48.5	48.2	5.6	C_1
		D	51.5	52.5	46.6	5.9	D_2
S. china tuber	*trans*-Resveratrol (%)	A	83	74.1	60.9	22.1	A_1
		B	66.1	77.5	74.4	11.4	B_2
		C	69.5	75.4	73.1	5.9	C_2
		D	71.2	73.5	73.2	2.3	D_2
	Quercetin (%)	A	72.9	61.3	55.4	17.5	A_1
		B	47.8	67.4	74.4	26.6	B_3
		C	57.4	66.5	65.6	9.1	C_2
		D	60.3	63.4	65.9	5.6	D_3

[a] $K_i^F = (1/3)\sum$ the extraction yield of target compounds at F_i.
[b] $R_i^F = \max\{K_i^F\} - \min\{K_i^F\}$, here F and i means extraction factor and setting level, respectively.
[c] For key to factors, see Table 2.

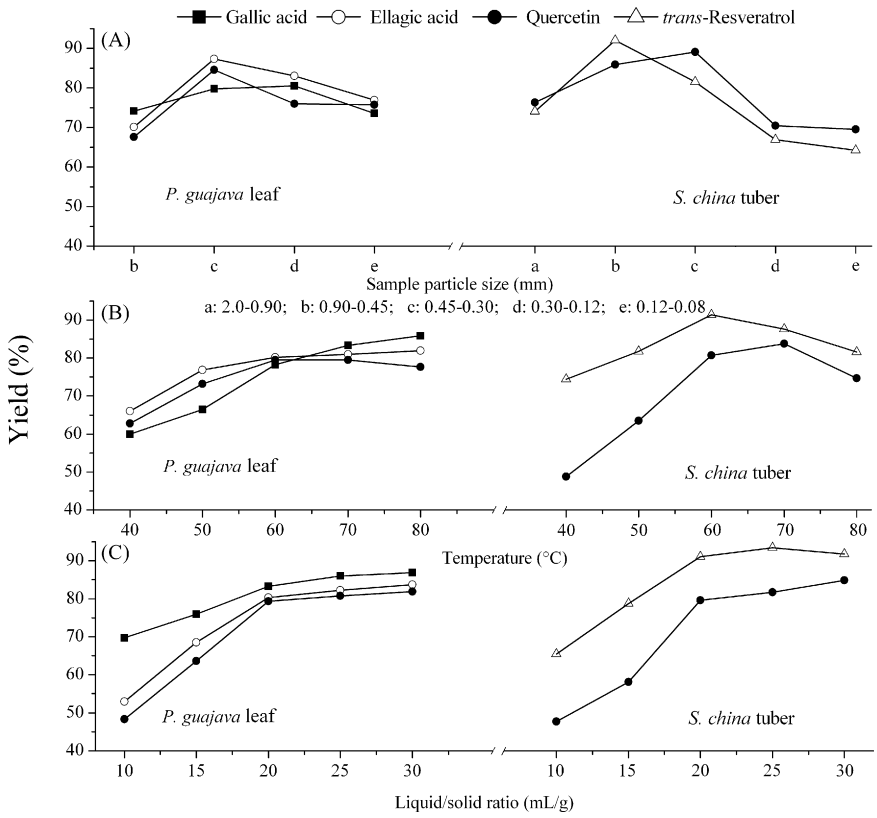

Fig. 3. Effect of sample particle size (A), extraction temperature (B) and liquid/solid ratio (C) on extraction yields of polyphenolic compounds from *P. guajava* leaves and *S. china* tubers.

able difference in the extraction of polyphenolic compounds from *P. guajava* leaves and *S. china* tubers, which suggested that the [bmim]Br solution was an alternative solvent to replace organic solvent in the MAE of polyphenolic compounds from the two Chinese herbs.

To evaluate the extraction efficiency of MAE with conventional extraction techniques, HE was carried out. The results in Table 4 suggested that higher ratio of liquid/solid (40:1) and longer time (4 h) in HE process were preferred in order to obtain high extraction yields compared to MAE. The diverse results between MAE and HE were mainly due to the unique extraction mechanism of MAE. Superheating, mass heating and fast heating were obtained by microwave heating but not conventional heating, so, MAE could obtain higher extraction yields with less solvent consumption and shorter time compared with conventional extraction techniques [11].

3.3. Method validation

The four polyphenolic compounds were identified by their corresponding chromatograms and retention time in comparison to those of the authentic standard compounds. Examples of chromatograms of standards and extracts are shown in Figs. 4 and 5.

A series of standard solutions of gallic acid, ellagic acid and quercetin at eight levels in the concentration range from 0.5 to 200 mg/L were prepared to determine the linearity of this method for analyzing polyphenolic compounds in *P. guajava* leaves. The similar process was performed for the analysis of *trans*-resveratrol and quercetin in *S. china* tubers. Each of them was analyzed in triplicate. Table 5 summarized the linear ranges and limits of detection (LODs, S/N = 3). Good linearity was observed with the regression coefficients (r) between 0.9990 and 0.9998. The LODs obtained were between 0.012 and 0.029 mg/L for the polyphenolic compounds.

Table 4
Comparative study of extraction efficiency using different extraction methods.

Sample	Analytes	Proposed method[a]		Conventional method[b]			
		ILs-MAE ([bmim]Br)		Regular MAE (methanol)		Heating extraction ([bmim]Br)	
		Observed values (mean ± S.D., mg/g)	Recovery (mean ± S.D., %)	Observed values (mean ± S.D., mg/g)	Recovery (mean ± S.D., %)	Observed values (mean ± S.D., mg/g)	Recovery (mean ± S.D., %)
P. guajava leaf	Gallic acid	0.507 ± 0.024[c]	103.0 ± 3.9	0.446 ± 0.017	90.5 ± 2.8	0.529 ± 0.030	107.4 ± 5.0
	Ellagic acid	2.387 ± 0.118	93.7 ± 5.6	1.930 ± 0.068	75.8 ± 2.3	2.599 ± 0.159	102.0 ± 5.4
	Quercetin	0.540 ± 0.027	98.5 ± 4.8	0.618 ± 0.032	107.2 ± 6.9	0.572 ± 0.031	104.0 ± 4.6
S. china tuber	*trans*-Resveratrol	0.531 ± 0.030	100.5 ± 3.7	0.545 ± 0.027	103.1 ± 4.6	0.445 ± 0.016	84.2 ± 2.7
	Quercetin	0.189 ± 0.012	85.2 ± 3.1	0.188 ± 0.010	84.5 ± 3.8	0.169 ± 0.010	75.9 ± 3.1

[a] Operation under optimized conditions.
[b] See experimental section for operation conditions.
[c] Each value was the mean and standard deviation (S.D.) of three independent experiments, Observed vaule (mg/g) = mass of polyphenolic compound in extraction solution (mg)/mass of sample (g).

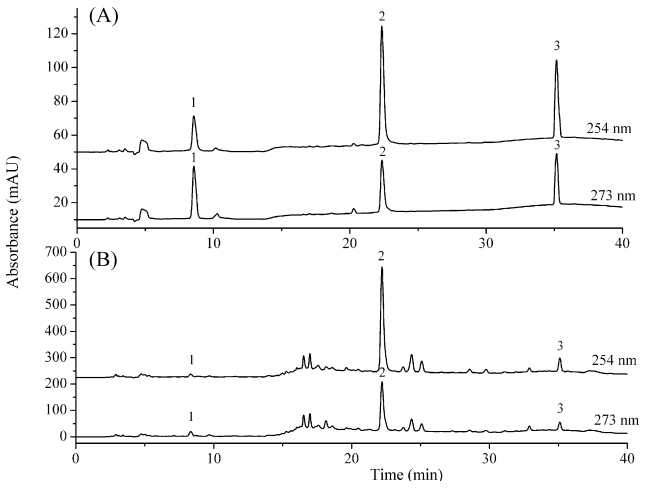

Fig. 4. Chromatograms of standard solution of 20 mg/L (A) and extract of *P. guajava* leaves by [bmim]Br solution (B). Peaks: (1) gallic acid, (2) ellagic acid and (3) quercetin.

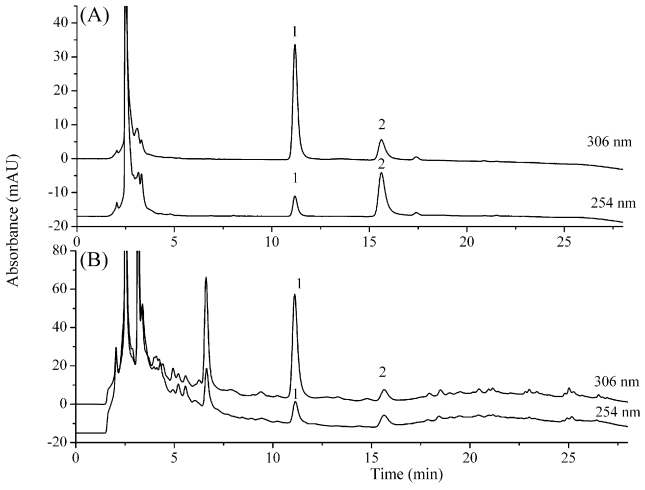

Fig. 5. Chromatograms of standard solution of 10 mg/L (A) and extract of *S. china* tubers by [bmim]Br solution (B). Peaks: (1) *trans*-resveratrol and (2) quercetin.

The precision of the chromatographic determination was evaluated by standard solution of 10 mg/L of the polyphenolic compounds. The reproducibility of the peak area and retention time of each polyphenolic compounds were estimated for 15 days. The relative standard deviations (R.S.D.s) of intra-day precisions for polyphenolic compounds ranged from 1.4 to 2.9%, and the corresponding R.S.D.s of inter-day precisions between 4.3% and 6.8%. Recoveries were evaluated by standard-addition method. Each polyphenolic compound was added at concentrations *ca.* 0.5, 1 and 1.5 times the observed concentration in the two original samples. Results showed that the recoveries were in the range of 85.2–103%

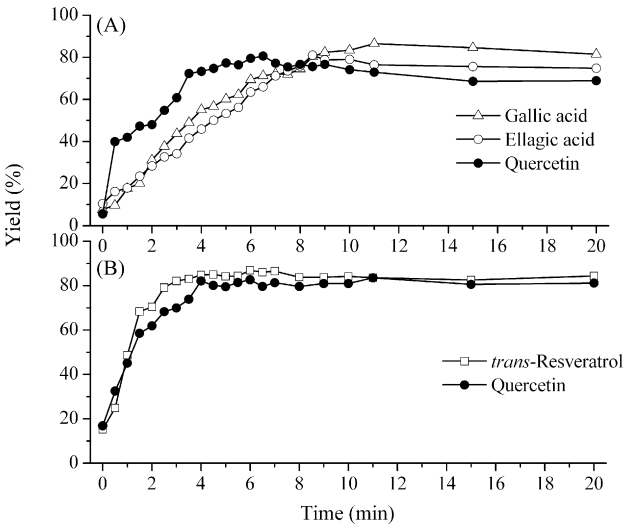

Fig. 6. Kinetic curves of polyphenolic compounds extracted from *P. guajava* leaves (A) and *S. china* tubers (B) using 2.50 mol/L [bmim]Br.

for the polyphenolic compounds with R.S.D.s lower than 5.6%. The reproducibility and recovery proved that the present method was credible.

The stability was investigated by determining the varieties of the polyphenolic compounds in [bmim]Br solution and extracts on 15 separate days. All the R.S.D.s of intra-day and inter-day were less than 3.7% and 8.5%, respectively. The results suggested that the polyphenolic compounds were stable in the IL solution and in the extracts.

3.4. Mechanism of ILs-MAE

3.4.1. Study of kinetic mechanism

To investigate the changes of the extraction yields in MAE process, the ILs-MAE kinetic mechanism was studied in this study. The results of kinetic curves for the MAE of polyphenolic compounds, according to time, from *P. guajava* leaves and *S. china* tubers were shown in Fig. 6(A and B), respectively. For the polyphenolic compounds from the same sample, the extraction yields increased rapidly at first and then reached an equilibrium concentration, and all the kinetic curves obtained were similar. For *S. china* tubers, the kinetic curves of *trans*-resveratrol and quercetin were similar, the extraction yields of *trans*-resveratrol and quercetin reached the corresponding maximum values (about 90%) before 6 min, which were different from the kinetic curves of polyphenolic compounds from leaf samples. The extraction yields of polyphenolic compounds in leaves arrived to the maximum after 6 min. The above results indicated that the kinetic mechanisms were correlated with the sample structures [27]. The extraction yields of gallic acid, ellagic acid and quercetin from *P. guajava* leaves reached the maximum at 10, 11 and 6.5 min, respectively, suggesting that the structures and character-

Table 5
The calibration curves, correlation coefficients (r), limits of detection (LODs) of the proposed analytical procedures.

Sample	Polyphenolic compound[a]	Calibration curve[b]	r	Linear range (mg/L)	LODs (mg/L)	RSD[c] ($n = 6$)
P. guajava leaf	Gallic acid	$Y = 26794X + 20150$	0.9993	0.50–200	0.029	6.8
	Ellagic acid	$Y = 77004X - 25168.3$	0.9993	0.50–200	0.012	4.7
	Quercetin	$Y = 37446X - 10646.6$	0.9990	0.50–200	0.027	5.1
S. china tuber	*trans*-Resveratrol	$Y = 60985X - 16314$	0.9998	0.50–100	0.022	4.3
	Quercetin	$Y = 32625X - 3651$	0.9994	0.50–100	0.024	4.5

[a] Gallic acid was monitored at 273 nm, ellagic acid and quercetin were monitored at 254 nm, *trans*-resveratrol was monitored at 306 nm.
[b] X was compound concentration as mg/L and Y was peak area.
[c] R.S.D. was monitored with 10 mg/L polyphenolic compounds mixed standard solution in 15 separate days.

istics of polyphenolic compounds influenced kinetic mechanisms. Moreover, the results of the MAE kinetic mechanism indicated that about 10 min of MAE time was enough to obtain high extraction yields of polyphenolic compounds from medicinal plants when ILs solutions were used as solvents.

3.4.2. Structural changes after extraction

In order to further elucidate the extraction mechanism, the identification of microstructure and chemical structure of sample was carried out by SEM and FTIR spectroscopy, respectively. Compared Fig. S2A with Fig. S2B (see Supplementary Data), the microstructures of *P. guajava* leaves changed obviously after MAE. The surface of the *P. guajava* leaves was greatly destroyed and the structure of cells walls was ruptured after MAE using [bmim]Br solution as solvent, which resulted in exposing gallic acid, ellagic acid and quercetin to [bmim]Br solution which could trap them and dissolve them. The results suggested that the mechanism of MAE was based on an explosion at the cell level, which was in accord with the hypothesis investigated by Paré et al. [28,29]. The structure of *S. china* tuber sample also was severely ruptured after MAE (see Fig.S3B), which suggested that the MAE mechanism of *trans*-resveratrol and quercetin from *S. china* tubers was similar to that of polyphenolic compounds from *P. guajava* leaves. However, the microstructures of *P. guajava* leaves and *S. china* tubers were not considerably changed and ruptured after HE (Figs. S2C and S3C in Supplementary Data), indicating that the polyphenolic compounds were extracted by exuding from the two samples.

The changes in chemical structures of *P. guajava* leaves and *S. china* tubers were investigated by FTIR spectra, since FTIR spectra can provide useful information for identifying the presence of certain function groups or chemical bonds in a molecule or an interaction system, attributable to the unique energy absorption bands for specific bonding environments or interactions, it has been applied in the rapid and nondestructive identification and quantification of medicinal plants [30] and the analysis of interactions between ILs and proteins or DNA [31,32]. Generally, reflectance peaks between 1800 and 700 cm^{-1} were selected as representative peaks for known carbohydrate compounds [33]. The results of FTIR spectra showed that the signal situations and intensity of absorption bands at 1622, 1447, 1318, 1035 and 780 cm^{-1} for *P. guajava* leaf sample and at 1733, 1624, 1426, 1374, 1247, 1160 and 1038 cm^{-1} were not obviously changed after MAE or HE with the [bmim]Br solution, which suggested that the chemical structures of carbohydrate compounds, including lignin, cellulose, hemicellulose and insoluble starch, were not destroyed in extraction process, the reasons probably were that water segregated the strong interactions between ILs and carbohydrate compounds of matrix [34–36]. The results indicated that the chemical bonding interactions between IL and sample matrix were not obvious.

S. china tubers samples are stiff lignocellulosic materials, *P. guajava* leaves are soft, however, the changes of microstructures and chemical structures of *P. guajava* leaves and *S. china* tubers after MAE were similar, which indicated the MAE mechanism of polyphenolic compounds from *P. guajava* leaves and *S. china* tubers was not related with the characteristics of sample.

4. Conclusion

In this work, ILs aqueous solution was proved to be a possible alternative solvent in the MAE of polyphenolic compounds from medicinal plants. The cations and especially anions of ILs had influences on the extraction, and the ILs with electron-rich aromatic π-system enhanced extraction ability. Under the optimized extraction conditions, the extraction yields of polyphenolic compounds from *P. guajava* leaves and *S. china* tubers were higher than 79.5% with 2.5 mol/L [bmim]Br solution. Compared to conventional extraction procedures, the proposed method could provide higher extraction yields and take a much shorter extraction time. The MAE kinetic mechanisms were related with the structures and characteristics of polyphenolic compounds, and the enhanced extraction was mainly based on the destruction of sample microstructures in MAE process. No obvious chemical interactions between ILs and sample matrix were identified. To alleviate environmental pressure and to develop green sample preparation techniques, ILs solutions as solvents in the MAE of polyphenolic compounds from medicinal plants showed a great promising prospect.

Acknowledgements

This work was supported by the National Natural Science Foundation of China (No. 20375050), by the project of the National Key Technologies R&D Programme of the 11th-five-year Plan (No. 2006BAK03A08), and by the Natural Science Foundation of Guangdong Province (No. 7300594), respectively. The authors thank Sineo Microwave Chemistry Technology Company for the support of MAS-I microwave oven.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.talanta.2009.01.040.

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