Short communication

Enhanced extraction of patchouli alcohol from *Pogostemon cablin* by microwave radiation-accelerated ionic liquid pretreatment

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ABSTRACT

A microwave radiation-accelerated ionic liquid pretreatment (MRAILP) was developed to enhance extraction of patchouli alcohol from *Pogostemon cablin*. 1-*N*-butyl-3-methylimidazolium chloride ([C$_4$mim]Cl) was selected as microwave absorbing and cellulose dissolution medium and microwave was applied to accelerate sample dissolution. The conditions of MRAILP including particle size, solvent, microwave pretreatment time and power and the ratio of ionic liquid (IL) to sample were optimized. Under the optimized conditions, the extraction yield of patchouli alcohol by the MRAILP was 1.94%, which has increased by 166% compared with microwave-assisted extraction. The recovery was in the range of 95.71–103.7% with relative standard deviation lower than 3.0%. It was a novel alternative extraction method for the fast extraction and determination of patchouli alcohol from *Pogostemon cablin*.

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1. Introduction

Patchouli (*Pogostemon cablin*) is widely used in the traditional Chinese medicine as it offers various types of pharmacological activities including removing dampness, relieving summer-heat, exterior syndrome, stopping vomiting and stimulating appetite [1]. The composition of patchouli essential oil is unique and complex because it consists of over 24 different sesquiterpenes [2], rather than a blend of different mono-, sesqui- and di-terpene compounds [3]. The sesquiterpene patchouli alcohol (CAS number: 5986-55-0, Fig. 1) is the major constituent and the primary component responsible for the typical patchouli aroma [4].

Patchouli essential oil is normally obtained by steam distillation [5], supercritical fluid extraction [4], soxhlet extraction [5], and pressurized fluid extraction [6]. However, some limitations exist in these protocols, such as degradation of thermally labile compounds [7], long extraction time [8], and labor-intensive step of sample processing [9] and high-pressure. As an alternative, extraction of essential oils using ionic liquid pretreatment will be the subject of considerable interest. The first case of ionic liquid pretreatment of medicinal plants in oil bath was reported [10], but it took 8 h

to pretreat sample in the [C$_4$mim]Cl. In 2002, Swatloski et al. [11] reported that the best conditions for the dissolution of cellulose were under microwave heating and the [C$_4$mim]Cl. So microwave heating and [C$_4$mim]Cl were used to pretreat medicinal plants. IL was used to loosen the structural integrity of botanical materials thereby increasing the extraction yield of the desired components. Compared with conventional extraction methods, IL pretreatment of cellulose sample has many advantages including more selective extractant, simple separation and shorter pretreatment time thus providing an oil of superior quality.

The aim of the current work was to explore the feasibility of MRAILP for fast extraction of effective constituents in medicinal plants and *Pogostemon cablin* was chosen as the representative target analyte. Factors such as solvents, the ratio of sample to IL, particle size and microwave pretreatment time were investigated to assess the effect of experimental conditions on the performance of MRAILP.

2. Materials and methods

2.1. Materials and chemicals

Dried leaf and stem of *Pogostemon cablin* was purchased from Tongrentang Medicine Cooperation in Guangzhou, China. All samples were pulverised and passed through stainless steel sieves.

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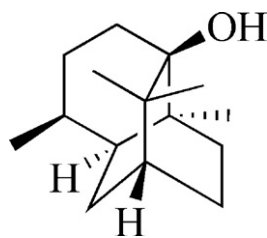E-mail address: LL_fan@hotmail.com (S. Chen).

Fig. 1. Molecular structure of patchouli alcohol.

Different sample sizes were obtained and stored in a desiccator at room temperature.

Ethanol, methanol, acetone, ethyl acetate, isopropanol and ethyl ether used in the experimental work were all of analytical grade. Patchouli alcohol standard was purchased from the Shanghai Jing Chun Reagent Co. Ltd. (Shanghai, China). [C_4mim]Cl, mp 63 °C, was purchased from Shanghai Cheng Jie Chemical Co. Ltd. (Shanghai, China) and used after drying under vacuum at 70 °C for 24 h.

2.2. Apparatus

A domestic microwave-assisted extraction unit (Media, Shunde, China) was modified in our laboratory with the addition of a mechanical stirrer. The whole system was run at atmospheric pressure and could be employed at the maximum power of 800 W.

2.3. Microwave radiation-accelerated ionic liquid pretreatment

A domestic microwave-assisted extraction unit was used for optimization of the extraction conditions. The influential factors of MRAILP procedure, including solvent (A), particle size (B), extraction time (C) and the ratio of sample to IL (D) were optimized though an orthogonal design L_9 (3^4). The experimental factors and their corresponding levels are shown in Table 1. Nine experimental trials were carried out according to the orthogonal array designs and the results are also shown in Table 1.

In the MRAILP, 0.5 g of sample (80-mesh) was placed in a 50 mL round bottom flask and 4.5 g of the [C_4mim]Cl was added as a liquid at 70 °C (i.e., above the melting point). The flask was then placed in the extraction unit, irradiated for 60 s with continuous stirring, and cooled to room temperature inside the extraction unit. The operation was repeated ten times until the dissolution reached maximum.

After the above pretreatment, diethyl ether (10 mL) was added into the mixture with continuous stirring for two minutes and then supernatant was transferred into a 50 mL volumetric flask. The process was repeated three times and the flask was filled up to the

mark with diethyl ether. An aliquot was filtrated through a 0.45 μm microporous membrane prior to GC analysis.

2.4. Comparison with other extraction methods

In this study, five different extraction methods were evaluated for their effectiveness in extraction of patchouli alcohol. Quantification of patchouli alcohol in the extracts was analyzed by GC. All extraction methods were carried out in triplicate under optimized conditions.

Extraction at room temperature (ERT): 0.5 g of sample (80-mesh) was extracted with 30 mL of ethanol for 24 h.

Heat reflux extraction (HRE): 0.5 g of sample (80-mesh) was extracted with 30 mL of ethanol. The suspension was refluxed for 60 min in a 95 °C water bath.

Ultrasonic extraction (UE): 0.5 g of sample (80-mesh) was extracted at with 30 mL of ethanol. The suspension was sonicated at 300 W for 60 min in a water bath.

Soxhlet extraction (SE): 0.5 g of sample (80-mesh) was extracted with 30 mL of ethanol for 60 min. The suspension was refluxed for 60 min in a 95 °C water bath.

Microwave-assisted extraction (MAE): 0.5 g of sample (80-mesh) was extracted with 30 mL of ethanol. The suspension was placed in a 136 W of microwave oven, continuously irradiated for 10 min with stirring.
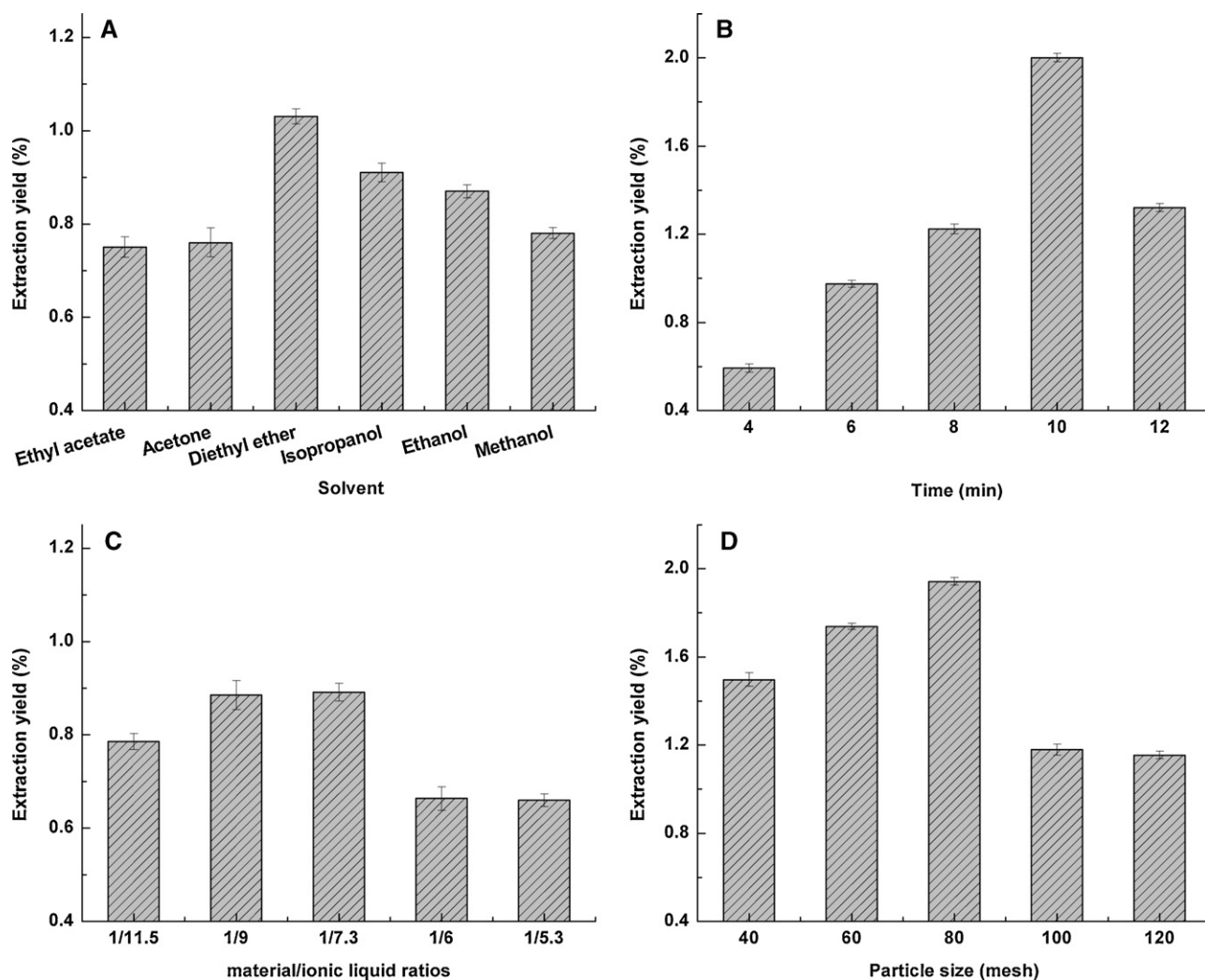
2.5. GC analysis and quantification

An Agilent 6890 gas chromatography with a hydrogen flame ionization detector (FID) was used for chemical analysis.

Patchouli alcohol was separated on a HP-5 (30m × 0.25 mm i.d.) capillary column coated with 0.25 μm film of 5% phenyl methyl siloxane. The column temperature started at 100 °C for 1 min, rose at 6 °C min^{-1} to 170 °C and held for 1 min, then at 1 °C min^{-1} to 180 °C. Split injection (10 μl) was employed with a split ratio of 1:20 and nitrogen was used as a carrier gas at 0.9 ml min^{-1} flow rate. The analytical operation was completed in 28 min. The inlet and detector temperature were 250 °C and 280 °C, respectively.

Patchouli alcohol standard solutions were prepared from a stock solution and diluted into concentrations of 10.4, 20.7, 31, 41.4, 62, 104 and 312 μg mL^{-1} for GC analysis. Each solution was measured three times from a start of the most diluted concentration. Patchouli alcohol content in *Pogostemon cablin* extracts was determined by standard curve method after demonstrating linearity in the detector response to patchouli alcohol concentrations between 10.4 and 312 μg mL^{-1}.

Table 1
Extraction yields extracted with the orthogonal design L_9 (3^4) ($n=3$).

Design ID number	Factor				*Pogostemon cablin*
	A Solvent	BParticle size (mesh)	CTime (min)	DSample/ionic liquid ratio (g/g)	Extraction yield (%)
1	A_1 (Ethanol)	B_1 (60)	C_1 (6)	D_1 (1/11.5)	0.69
2	A_1	B_2 (80)	C_2 (8)	D_2 (1/9.0)	0.52
3	A_1	B_3 (100)	C_3 (10)	D_3 (1/7.3)	0.92
4	A_2 (Ethyl acetate)	B_1	C_2	D_3	0.39
5	A_2	B_2	C_3	D_1	0.37
6	A_2	B_3	C_1	D_2	0.65
7	A_3 (Methanol)	B_1	C_3	D_2	0.66
8	A_3	B_2	C_1	D_3	0.58
9	A_3	B_3	C_2	D_1	0.71
K_1	0.71	0.58	0.64	0.59	
K_2	0.47	0.49	0.52	0.61	
K_3	0.65	0.76	0.65	0.63	
R	0.24	0.27	0.13	0.04	
Optimal level	A_1	B_3	C_3	D_3	

Fig. 2. Effects of different solvents, microwave times, ionic liquid and sample ratios, particle sizes on patchouli alcohol extraction yield. Experimental conditions: (A) 30 mL of solvent, microwave time 6 min, 0.5 g of sample, microwave power 136 W, ionic liquid and sample ratio: 7.3:1; (B) 30 mL of diethyl ether, 0.5 g of sample, microwave power 136 W, ionic liquid and sample ratio: 7.3:1; (C) 30 mL of diethyl ether, 0.5 g of sample, microwave time 10 min, microwave power 136 W; (D) 30 mL of diethyl ether, 0.5 g of sample, microwave time 10 min, microwave power 136 W, ionic liquid and sample ratio: 9:1.

Extraction yield of patchouli alcohol was defined as follows:

$$\text{Extraction yield (\%)} = \frac{\text{Quantity of patchouli alcohol in extract}}{\text{Quantity of patchouli alcohol in raw material}}\%$$

3. Results and discussion

3.1. Determination of optimal parameters for the MRAILP

The results shown in Table 1 indicated that there were great yield differences among each set of MRAILP conditions. The influence to the mean extraction yields of patchouli alcohol decreased in the order: B > A > C > D according to the R values. MRAILP condition of $B_3A_1C_3D_3$ achieved the highest extraction yield. Single-factor test was conducted to further optimize those factors.

A correct choice of solvent is fundamental for obtaining an optimal extraction of effective constitutes in medicinal plants. Initial extractions performed to determine the best solvent choice were carried out on solvents including methanol, ethanol, acetone, isopropanol, diethyl ether and ethyl acetate. The solvent that produced the highest extraction yield was diethyl ether (Fig. 2A). It was probable that diethyl ether was a good penetrating solvent and easy to

separate by dumpage. So diethyl ether was chosen as the extraction solvent.

Microwave pretreatment time has both positive and negative influence on extraction yield. As reported by Barthel et al. [12], the mixture of cellulose/IL was stirred up to 12 h to guarantee the complete dissolution. However, dissolution rates could be significantly improved by heating in a microwave oven [11]. It could be observed in Fig. 2B that extraction yield increased with the increasing of microwave pretreatment time and reached maximum at 10 min. However, extraction yield was significantly decreased with prolonged microwave pretreatment time. This may be due to low-melting point of patchouli alcohol (55–56.5 °C)[13]. Thus, microwave time was set at 10 min.

The amount of ionic liquid has an indirect effect on the extraction yield. As shown in Fig. 2C, the extraction yield increased with increasing ratio of sample to IL and reached the highest yield at a ratio of 1:9 (g/g). On the one hand, the amount of the dissolved sample increased with an increasing amount of ionic liquid, which might have increased the permeability of the cell wall thus resulting in higher yield. On the other hand, most room temperature ionic liquids were vicious liquid [14] and the viscosity of the cellulose/IL increased with an increasing amount of ionic liquid, which negatively affected mass transfer [15] and barricaded the
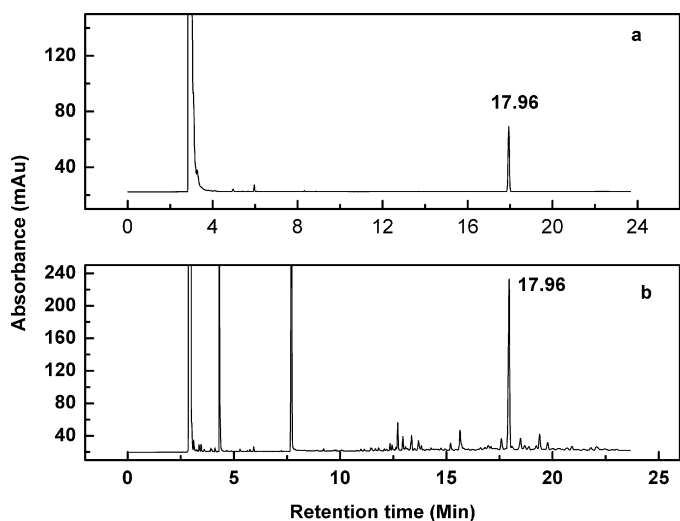
Fig. 3. GC chromatograms of *Pogostemon cablin* extracts.

Table 2
Comparison of extraction yields using different extraction methods (averages of the three replicates).

Extraction methods	Extraction time	Extraction yield (%)	RSD (%)
MRAILP	10 min	1.94	1.5
ERT	24 h	0.58	0.9
HRE	60 min	0.87	1.3
UE	60 min	1.07	2.1
SE	60 min	0.84	3.1
MAE	10 min	0.73	0.8

release of patchouli alcohol into an extraction solvent. Therefore, the sample and IL ratio of 1:9 was selected in the following experiments. Particle size has significant influence on the extraction yield. Fig. 2D showed that extraction yield increased with the particle size being decreased from 40 to 80-mesh. On the one hand, the higher amount of patchouli alcohol was released as the plant cells were destroyed by milling and this amount of patchouli alcohol was extracted easily for direct exposure to the extraction solvent. On the other hand, it was difficult for ionic liquid to dissolve larger size of plant sample, because the amount of dissolved cellulose decreases with increasing polymerization degree for sample [16]. Moreover, small particle size can increase mass transfer areas and decrease mass transfer resistance [17]. However, the particle size should not be too small, because the sample was easily conglomerated by the viscous ionic liquid. The residua and extract did not easily separate each other so that some patchouli alcohol extracted-out might remain in the residue. Thus, 80-mesh was preferred for the extraction of patchouli alcohol.

The effect of microwave pretreatment power on the extraction yield was studied. Three levels of microwave power, i.e., 136, 264 and 440 W, were selected, while other parameters such as solvent (30 mL of diethyl ether), amount of sample (0.5 g), microwave pretreatment time (10 min) and sample/ionic liquid ratio (1:9.0), were kept constant. The results indicated that the extraction yield decreased with an increasing microwave power. It is because that ILs with high polarity, which have good absorption of microwave radiation are appropriate microwave heating media. But its shortcomings are that, microwave heating conditions can be too harsh and must be carefully controlled to avoid the pyrolysis of cellulose in plant samples [18]. Sample was slightly carbonized when the microwave power was 264 W. Therefore, microwave power was set at 136 W in subsequent experiments.

3.2. Microwave radiation-accelerated ionic liquid pretreatment

In the MRAILP, 0.5 g of sample (80-mesh) was extracted under such optimized conditions, i.e., 30 mL of diethyl ether, 10 min of microwave pretreatment time, 1:9 of the ratio of sample to ionic liquid and 136 W of microwave power. Identification and quantification of patchouli alcohol in the MRAILP extract were analyzed by GC. As can be seen in Fig. 3, the patchouli alcohol showed a single peak at the retention time of 17.96 ± 0.03 min and was completely separated from other compounds.

To validate the effectiveness of the MRAILP approach, GC analysis parameters such as linearity, reproducibility, and recovery were determined under the optimized conditions. Calibration curve was obtained by dissolving the standard patchouli alcohol to ethanol at seven concentrations in the range of 10.4–312 $\mu g\,mL^{-1}$ under the same GC conditions. Linear regression equation and correlation coefficient were $y = 4964x + 7.732$ and 0.9990, respectively. The limit of detection (LOD) and the limit of quantification (LOQ) were $0.156\,mg\,L^{-1}$ and $1.04\,mg\,L^{-1}$, respectively. As can be seen in Table 2, the relative standard deviation (RSD) obtained by the proposed approach was 1.5%. To evaluate the accuracy of the present method, standard solution of patchouli alcohol was added to *Pogostemon cablin* sample at five levels of 0.062, 0.124, 0.186, 0.248 and 0.31 $mg\,mL^{-1}$ and satisfactory results were found with recovery values between 95.71% and 103.7%.

3.3. Comparison of MRAILP with other extraction methods

The results in Table 2 indicated that the extraction yield of patchouli alcohol was significantly different among the methods. In terms of extraction yield, MRAILP produced the highest extraction yield of 1.94%, which has increased by 166% compared with MAE, 123% with UE and SE, 234% with ERT and 81% with HRE. Such high effectiveness of MRAILP extraction resulted from the physiochemical properties of ILs. The primary cell wall of medicinal plants is made primarily of cellulose. The major action of the ILs is on cell walls. They act on cell wall components, dissolve them in turn, increase the permeability of the cell wall thus resulting in higher yield of the effective constituents [10].

4. Conclusions

Significant enhancement of the release of patchouli alcohol in both rate and yield was achieved. This was mainly due to the destruction of the structural integrity of medicinal plants. Several parameters affecting the extraction yield were optimized, e.g., solvent, microwave pretreatment time, ionic liquid and sample ratio and particle size. Under the optimized conditions, the extraction yield by the MRAILP was higher than that of other extraction methods. So it was a novel alternative extraction method for the fast extraction and determination of patchouli alcohol. The results in this study may serve as a reference for the extraction of active constitutes from medicinal plants.

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