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Research Article

Ultrasonic-Assisted Extraction of Procyanidins Using Ionic Liquid Solution from Larix gmelinii Bark

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An ionic liquid-based ultrasonic-assisted extraction method has been developed for the effective extraction of procyanidins from *Larix gmelinii* bark. So as to evaluate the performance of ionic liquids in ultrasonic-assisted extraction process, the effects caused by changes in the anion and the alkyl chain length of the cation on the extraction efficiency were investigated in this paper. The results indicated that the characteristics of anions had remarkable effects on the extraction efficiency of procyanidins, and 1-butyl-3-methylimidazolium bromide ([Bmim]Br) aqueous solution was the best among the investigated ionic liquids. The optimum conditions for the extraction were as follows: [Bmim]Br concentration 1.25 M, soak time 3 h, solid-liquid ratio 1 : 10, ultrasonic power 150 W, and ultrasonic time 30 min. This work not only introduces a simple, green, and highly efficient sample preparation method for extraction of procyanidins from *L. gmelinii* bark, but also reveals the tremendous application potential of ionic liquids.

1. Introduction

The procyanidins, one subclass of proanthocyanidins, consisting of (+)-catechin and/or (−)-epicatechin units are linked mainly through C4–C8 and/or C4–C6 bonds [1]. Procyanidins are important bioresources, which are the most abundant polyphenols in plants after lignins [2, 3]. Procyanidins have attracted attention in the fields of pharmacology and food chemistry because of their beneficial pharmacological effects such as radical scavenging [4], antioxidative [5], antiviral [6], antimicrobial [7], anticarcinogenic [8], and anti-inflammatory effects [9], as well as cardiotonic and antiarteriosclerotic activities [10, 11]. Because of their wide spectrum of pharmacological action, they are considered as functional ingredients in botanical and nutritional supplements [12]. Procyanidins are extracted from various natural sources, most notably apples, maritime pine bark, cinnamon, grape seed, and grape skin [13].

Larix gmelinii is a deciduous tree primarily distributed in northeast, China, north Sakhalin, and east Siberia. *L. gmelinii* bark containing numerous procyanidins are extremely useful natural products. In recent years, procyanidins have been found in large quantities in *L. gmelinii* bark and have been recognized as a multipurpose natural component with great economic potential and environmental value, attracting the increasing attention of people [14–17].

The extraction of procyanidins from *L. gmelinii* bark has been accomplished by several extraction methods in the past. These include heating reflux extraction [15, 16] and homogenated extraction [17] with water, methanol, ethanol, acetone, acetic ether, and some mixtures as solvents [15–17]. However, the main disadvantage of traditional extraction lies in the complicated working procedure which increases the cost; repeated distillations prolong the heating time and accelerate oxidation of the extract. Moreover, these organic solvents used are problematic in the extraction of procyanidins because of their toxicity, volatility, and flammability. To overcome the above-mentioned problems, environment friendly techniques become attractive following the development of the "Green Chemistry." Much wider attention has

been given to applications of ultrasound-assisted extraction (UAE) [18] and microwave-assisted extraction (MAE) [19, 20]. Among the two methods, UAE can more easily be scaled up for commercial production [18]. And the UAE is one of the promising extraction techniques that can offer high reproducibility in a shorter time, simplified manipulation, reduced solvent consumption and temperature, and lower-energy input, which has been widely used to extract analytes from many matrixes [21, 22]. Ultrasound enhancement of extraction is attributed to the disruption of cell walls, particle-size reduction, and the enhancement on the mass transfer of the cell content to the solvent caused by the collapse of the bubbles produced by cavitations [23, 24]. The UAE is expeditious, inexpensive, efficient, and an environmental protection alternative to conventional extraction techniques, which is also a well-established method in the processing of plant material and in the extraction of analytes from different parts of plants.

Ionic liquids, also known as molten salts, which are composed of organic cations and inorganic or organic anions, are liquid near room temperature (or by convention below 100°C) [25]. They have been proposed as greener alternatives to traditional organic solvents due to their unique characteristics such as good stability, negligible vapor pressure, wide liquidus range, good dissolving, and extracting ability, which have been attributed mainly to their nonmolecular nature [26–28]. In comparison with conventional organic solvents, ionic liquids could alleviate environmental pollution and improve the selectivity and the extraction efficiencies of compounds in separation technologies and sample pretreatment processes [29–33]. Ionic liquids as solvents are of promising potential in the application of the preparation of various useful substances from plant samples such as alkaloids [18, 19, 34, 35], stilbene [36], quinines [37], lignans [38, 39], and coumarins [40, 41]. As alternative solvents, the experimental results have indicated that ILs are promising solvents which are available in a simple and efficient technique for sample preparation and separation added in the introduction.

The aim of this work is to develop an effective, rapid, and environment friendly ionic liquid-based ultrasonic-assisted approach for the extraction of procyanidins from *L. gmelinii* bark and to compare the results with conventional extraction methods. Herein, we describe our investigations on the performance of various ionic liquids with different cations and anions in an ionic liquid-based ultrasonic-assisted extraction (ILUAE) method. It was found that parameters including the ionic liquid concentration, soak time, solid-liquid ratio, and ultrasonic power and time were influential on the extraction efficiency, and these parameters were optimized systematically.

2. Experimental

2.1. Chemicals and Materials. (+)-Catechin and (−)-epicatechin (with purity >98%) standards were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (Beijing, China), procyanidin dimers B2 [(−)-epicatechin-(4β → 8)-(−)-epicatechin] (with purity ≥90%) and trimer C1 [(−)-epicatechin-(4β → 8)-(−)-epicatechin-(4β → 8)-(−)-epicatechin] (with purity ≥75%) were purchased from Sigma-Aldrich Inc. (St. Louis, USA), and B4 [(+)-catechin-(4α →8)-(−)-epicatechin] (with purity ≥97%) was obtained from Tianjin Jianfeng Natural Product R&D Co., Ltd. All ionic liquids ([Emim]Br, [Bmim]Br, [Hmim]Br, [Omim]Br, [Dmim]Br, [Bmim]BF_4, [Bmim]Cl, [Bmim]NO_3, [Bmim]HSO_4, [Bmim]OH, and [Bmim]Ac, where Ac = acetate; Emim = 1-ethyl-3-methylimidazolium, Bmim = 1-butyl-3-methylimidazolium, Hmim = 1-hexyl-3-methylimidazolium, Omim = 1-octyl-3-methylimidazolium, and Dmim = 1-decyl-3-methylimidazolium) were obtained from Chengjie Chemical Co. LTD. (Shanghai, China) and used without further purification. Acetonitrile used for HPLC analysis was of chromatographic grade and purchased from J&K Chemical Ltd. (Beijing, China) and used as received. Methanol, hydrochloric acid, vanillin and other reagents were all analytical grade and were obtained from Beijing Chemical Reagents Co. (Beijing, China). Reverse osmosis Milli-Q water (Millipore, Bedford, MA, USA) was used for all solutions and dilutions. All solutions and samples prepared for analysis were filtered through a 0.45 μm nylon membrane (Guangfu Chemical Reagents Co., Tianjin, China).

L. gmelinii bark was provided by Mohe Forestry (Heilongjiang, China) and authenticated by Professor Shaoquan Nie from the State Engineering Laboratory for Bioresource Eco-Utilization, Northeast Forestry University, China. The bark was dried at room temperature for a month and then was powdered into a homogeneous size and then sieved (60–80 mesh). The same batch of samples was used here in the experiments.

2.2. Ultrasonic-Assisted Extraction Apparatus. For the ultrasonic-assisted extraction experiments, an ultrasonic bath was used as an ultrasonic source. KQ-250DB ultrasonic bath (Kunshan, Jiangsu, China) was used in the extraction step. The bath was a rectangular container (23.5 × 13.3 × 10.2 cm), to which 50 kHz transducers were annealed at the bottom. The bath power rating was 250 W on the scale of 40%–100%. The temperature control achieved by the replacement of inlet and outlet water to avoid water temperature rises.

2.3. Ionic Liquid-Based Ultrasonic-Assisted Extraction. 0.5 g of dried sample powder was mixed with 5 mL of the various ionic liquid aqueous solutions in a 25 mL flask. The flask was then partially immersed in the ultrasonic bath, which contained 2.5 L of water. The suspension was extracted by UAE. The cation and anion of the ionic liquid, concentration of selected ionic liquid, soak time, solid-liquid ratio, and ultrasonic power and time were systematically optimized in this work to obtain the best extraction efficiency. After each extraction, the extract was filtered through a 0.45 μm nylon membrane (Guangfu Chemical Reagents Co., Tianjin, China) prior to the analysis. The extraction efficiency was expressed as the observed values of procyanidins, and the maximum amount in each curve was taken to be 100%.

2.4. Reference and Conventional Extraction Method. Pure water, 1.25 M sodium chloride, and 80% ethanol were selected for use as reference solvents in the UAE of procyanidins from *L. gmelinii* bark. The extraction experiments were operated under the optimized conditions except for solvent type. 0.5 g of sample powder was mixed with 5 mL of the above solvents and soaked for 3 h. The suspension was extracted for 30 min by UAE. Ultrasonic power and the solid-liquid ratio were 150 W and 1 : 10, respectively. The extract was filtered through a 0.45 μm microporous membrane for analysis.

80% ethanol was selected as solvent in conventional heat reflux extraction (HRE) and maceration extraction (ME). The main technical parameters used were the same as above except extraction time and temperature 4 h and 85°C for HRE and 24 h and 25°C for ME, respectively.

2.5. Vanillin-HCl Method Quantitative Analysis. Procyanidins in the extract solution were determined by the standard vanillin-HCl method [42] using (+)-catechin as standard. Briefly, to 1.0 mL of the extract solution in a brown tube, 9.0 mL of 2% vanillin/HCl-methanol reagent (2 g vanillin dissolved in 12 N HCl-methanol (1 : 2) solution to get final volume of 100 mL) was added, immediately capped, mixed for 10 seconds, and incubated at 19–21°C for 15 min. Absorbance of this solution was measured by spectrophotometer (UV-2550, Shimadzu, Japan) at 500 nm (reference: water) (A_{SOLUTION}). Procyanidins content was calculated from the value of (A_{SOLUTION})-(A_{BLANK}) by using working curve.

Working curve was obtained as follows: 1, 2, and 3 mg of (+)-catechin was dissolved in water to a final volume of 10 mL (the standard solution). 1.0 mL of each standard solution was taken in a brown tube and 9.0 mL of 2% vanillin/HCl-methanol reagent was added, immediately capped, mixed for 10 seconds and incubated at 19–21°C for 15 min. Absorbance of this solution was measured at 500 nm by spectrophotometer (reference: water) (A_{CAL}). In case of blank, water was used instead of standard solution (A_{BLANk}). Working curve was obtained with correcting values: (A_{CAL})-(A_{BLANK}). The working curve was constructed for procyanidins: $Y = 0.0052x + 0.0164$, ($R^2 = 0.9974$), where Y = Absorbance (Abs) and x = Concentration of reference substance (μg mL^{-1}). A good linearity was found for absorbance in the range of 0.107 Abs–1.034 Abs.

2.6. HPLC Qualitative Analysis. The way ANOVA test was used to calculate the significance of the differences of extraction efficiency for the procyanidins. The results of spectrophotometric analysis were expressed as means of extraction efficiency ± SD.

3. Results and Discussion

3.1. Screening of the Ionic Liquid-Based Extracting Solvent. The structure of ionic liquids had a significant influence on their physicochemical properties, which might have greatly affected the extraction efficiency of target analytes [43]. The optimal ionic liquid for extraction was sought and the general trends observed are described below.

3.2. Anion Effect. Some papers indicate the important influence of the cation part in different properties. For the series of ionic liquids studied here, the water miscibility of the ionic liquid was important to the extraction efficiency. *N*-Methylimidazolium based ionic liquids with seven different anions (Cl^-, Br^-, BF_4^-, NO_3^-, HSO_4^-, Ac^-, and OH^-) were studied and differences in their extraction efficiency were readily apparent, as shown in Figure 1(a). All of the ionic liquids tested were sufficiently miscible in any proportion with water. The results showed that the ionic liquids based on Br^- and HSO_4^- were the more efficient of the liquids tested, with Br^- showing the best results. The hydrogen bonding and hydrophobic interactions of [Bmim]Br and [Bmim]HSO_4 caused the stronger solvation interactions with procyanidins. With the addition of ionic liquids, the extraction yields of procyanidins were improved greatly. This result indicates that extraction efficiency of procyanidins is anion dependent, which is similar to previous studies [43, 44].

3.3. Effect of the Alkyl Chain Length of the Ionic Liquid Cation. Using the same anion of Br^- a series of 1-alkyl-3-methylimidazolium cations including $Emim^+$, $Bmim^+$, $Hmim^+$, $Omim^+$, and $Dmim^+$ were evaluated, and the results are shown in Figure 1(b); the results implied that, for procyanidins, extraction efficiency increased slightly with the increasing alkyl chain length from ethyl to butyl. The alkyl chain length of cation was increased from butyl to dodecyl while the extraction efficiency decreased rather than increased. It could be attributed to the increase of the alkyl chain length in the cation moiety leading to larger steric clash. Having optimized both the anion and cation of the ionic liquid, [Bmim]Br was selected for subsequent extraction parameter optimization studies.

3.4. Concentration Effect. The optimum [Bmim]Br concentration in aqueous solution for UAE of procyanidins extraction was sought by carrying out extractions with [Bmin]Br solutions of different concentrations (from 0.25 to 1.25 M). Based on the results shown in Figure 1(c), it can be seen that the extraction efficiency increased in the [Bmin]Br concentration range of 0.25–1.25 M. We propose that the high viscosity of the solvent at high ionic liquid concentrations may lead to poor penetration of the solvent into the plant tissue and high ionic liquids consumption. 1.25 M [Bmin]Br solution was therefore selected as the optimal ionic liquid concentration.

3.5. Optimization of the UAE Parameters. The univariate method was used to optimize the following parameters: soak time, solid-liquid ratio, and ultrasonic power and time.

3.5.1. Soak Time and Solid-Liquid Ratio. Experiments were conducted by soaking the dry bark powder in the ionic liquid solution for 1, 2, 3, 4, or 8 h before UAE. Figure 2(a) shows the effect of soaking the sample powder in 1.25 M

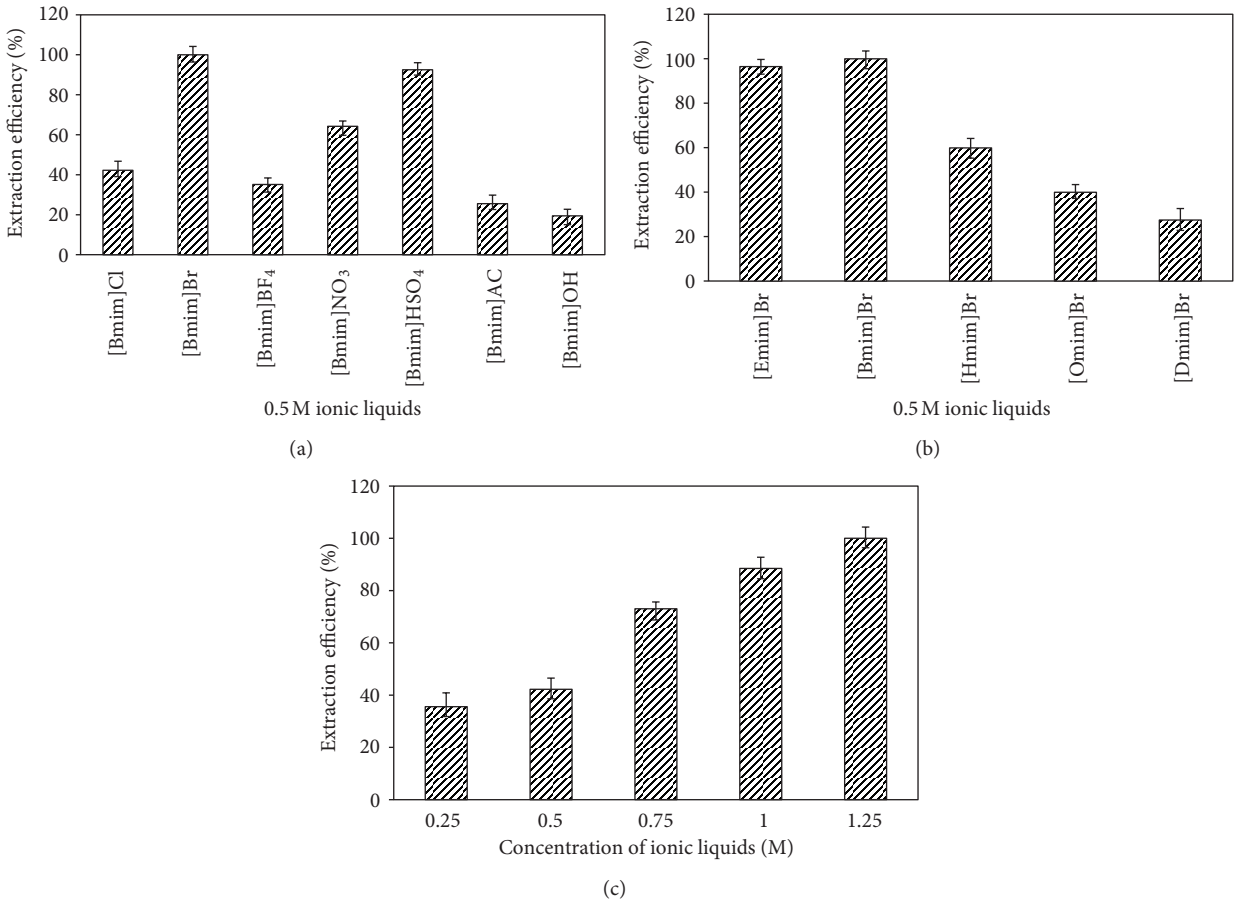

Figure 1: Effects of ionic liquids anions (a), cations (b), and concentration (c) on the extraction efficiency of target analytes. Sample: 0.5 g; extractant volume: 5 mL; soak time: 2 h; ultrasound power: 250 W; ultrasound time: 30 min; ionic liquid concentration: 0.5 M for (a) and (b). The extraction efficiency is expressed as the observed values of target analytes, and the maximum amount in each curve was taken to be 100%.

[Bmim]Br on the extraction of procyanidins from *L. gmelinii* bark at room temperature. It demonstrated the substantial increase in extraction efficiency obtained after soaking the bark. To extract procyanidins from the cellular structure, the solvent must have access to the cellular compartments, where the procyanidins are located. An intact cell structure restricts accessibility of the solvent to the procyanidins, while ultrasound treated cells have a more open, fragmented structure, which facilitates efficient extraction. The increase in extraction efficiency of the procyanidins after soaking with the solvent is probably because of increased diffusion of the solvent into the cellular structure allowing improved solubilization of the procyanidins. The extraction efficiency of procyanidins increased significantly when the soak time was 0–3 h, however; longer soak times did not lead to further increases in efficiency. Hence 3 h was chosen as the optimal soak time.

The solid-liquid ratio is a crucial factor and was also studied to optimize extraction efficiency. Large solvent volumes could make the procedure difficult and lead to unnecessary waste, while small volumes may lead to incomplete extraction. A series of experiments were carried out with different solid-liquid ratios (1 : 6, 1 : 8, 1 : 10, 1 : 12, and 1 : 14 g mL^{-1}) to evaluate the effect of the solid-liquid ratio. As shown in Figure 2(b), the extraction efficiency increased evidently with the increase of the solvent volume for solid-liquid ratio of up to 1 : 10. Higher solvent volumes, however, did not significantly improve the extraction efficiency. Thus, a solid-liquid ratio of 1 : 10 was adopted as the optimal solid-liquid ratio in this study.

3.5.2. Ultrasonic Power and Time. Ultrasonic power is believed to be the driving force for the complete dispersion of [Bmim]Br into the solid sample. To examine the effect of the ultrasonic power on the extraction efficiency, experiments were carried out at 100, 150, 200, and 250 W, respectively. The UAE time was maintained constant throughout this experiment at 30 min. Figure 2(c) shows the effect of ultrasonic power on extraction efficiency. With the ultrasonic power increasing from 100 to 150 W, the extraction efficiency of the procyanidins increased. However, when the ultrasonic power increased above 150 W, no obvious change could be observed at higher ultrasonic power. It meant that the ultrasonic power of 150 W was large enough to ensure the dispersion of [Bmim]Br. Thus, the ultrasonic power of UAE was set at 150 W in the following experiments.

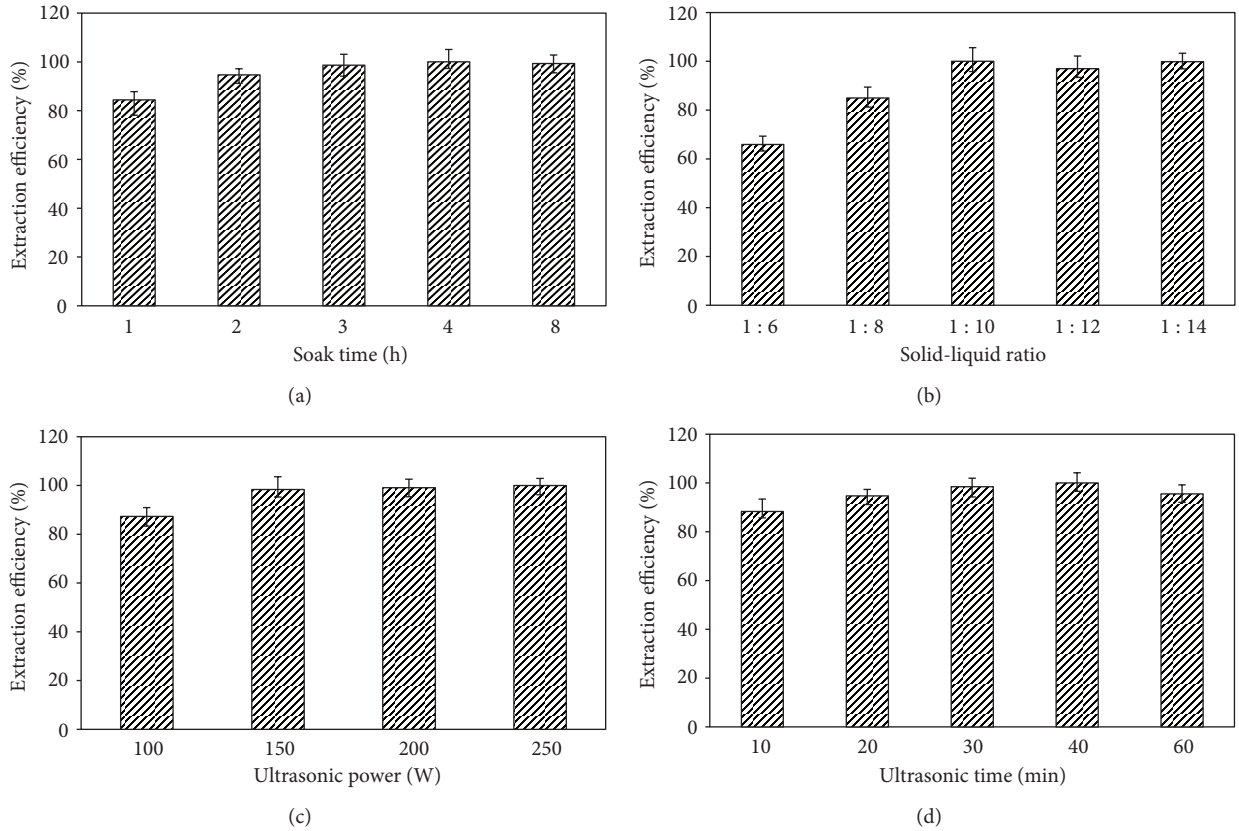

Figure 2: Optimization of extraction conditions: (a) 0.5 g of dried sample was mixed with 5 mL 1.25 M [Bmim]Br and then soaked for different times (1, 2, 3, 4, and 8 h) before the suspension was extracted for 30 min by UAE (250 W); (b) 0.5 g of dried sample was mixed with 1.25 M [Bmim]Br with different solid-liquid ratios (1 : 6, 1 : 8, 1 : 10, 1 : 12, and 1 : 14 w/v) and then soaked for 3 h before the suspension was extracted for 30 min by UAE (250 W); (c) 0.5 g of dried sample was mixed with 5 mL 1.25 M [Bmim]Br and then soaked for 3 h before the suspension was extracted for 30 min by UAE at different ultrasound powers (100, 150, 200, and 250 W); (d) 0.5 g of dried sample was mixed with 5 mL 1.25 M [Bmim]Br and then soaked for 3 h before the suspension was extracted for different times (10, 20, 30, 40, and 60 min) by UAE (150 W). The extraction efficiency is expressed as the observed values of proanthocyanidins, and the maximum amount in each curve was taken to be 100%.

The influence that the time ultrasonic was applied to the sample on the extraction efficiency of the alkaloids was examined over a range of 10 to 60 min, and the results are shown in Figure 2(d). They show that the extraction efficiencyiy of procyanidins increased when the ultrasonic time was increased from 10 to 30 min. When the variable was changed from 30 to 60 min, slight improvements were observed. The extraction efficiency was low during the first 20 min of ultrasonication, indicating that more time was needed for ultrasound to disrupt the cell walls and aid the release of the procyanidins into the solvent. Prolonged application of ultrasound, of more than 30 min, did not result in any further significant improvement in extraction efficiency. It was found that more than 98% of the procyanidins content extracted during the first 30 min of UAE. The application of ultrasonic for 30 min was therefore selected for all subsequent experiments.

Reverse-phase HPLC was also used to analyze the composition of procyanidins. The HPLC apparatus was a Waters 717 automatic sample handling system series HPLC system (Waters Corporation, Milford, USA), consisting of a Waters 1525 bin pump with a steel column heater module controlling the column temperature, and a Waters 2487 UV-detector monitored by a Waters Millenium 32 chromatography manager software. Chromatographic separation was performed on a HiQ sil-C18 reversed-phase column (4.6 mm × 250 mm, 5 μm, KYA TECH). The elution conditions were as follows: flow rate 1.0 mL min^{-1}, column temperature 24°C, injection volume 10 μL, and solvent A: methanol, solvent B: 0.5% (v/v) phosphoric acid in water. The elution profile was: 0 min 18% A in B, 0–10 min 18% to 24% A in B, 10–15 min 24% A in B, 15–65 min 24% to 64% A in B, 65–75 min 100% A (wash-out), and 75–90 min 18% A in B (reconditioning). The detection wavelengths were set at 280 nm.

3.6. Statistical Analysis. Based on the above experiments, the optimum ultrasonic-assisted conditions were found to be: 1.25 M [Bmin]Br as extraction solvent, soak time of 3 h, solid-liquid ratio of 1 : 10 (w/v), ultrasonic power of 150 W, and extraction time of 30 min.

3.7. Comparison of ILUAE Approach with the Reference and Conventional Methods. The reference methods tested

Table 1: Comparison of ILUAE with other extraction methods, mean ± S.D. (n = 3).

Number	Solvent	Extraction method	Time (h)	Solvent consumption (mL g^{-1})	Temperature (°C)	Extraction efficiency[a] ± SD (%)
1	Pure water	UAE	0.5	10	25	19.62 ± 0.76
2	1.25 M NaCl	UAE	0.5	10	25	17.47 ± 0.85
3	80% ethanol	UAE	0.5	10	25	67.10 ± 3.21
4	80% ethanol	ME	24.0	10	25	49.92 ± 2.51
5	80% ethanol	HRE	4.0	10	85	54.89 ± 2.66
6	1.25 M [Bmim] Br	UAE	0.5	10	25	100.00 ± 4.89

[a]The extraction efficiency is expressed as the observed values of procyanidins, and the maximum amount in each curve was taken to be 100%.

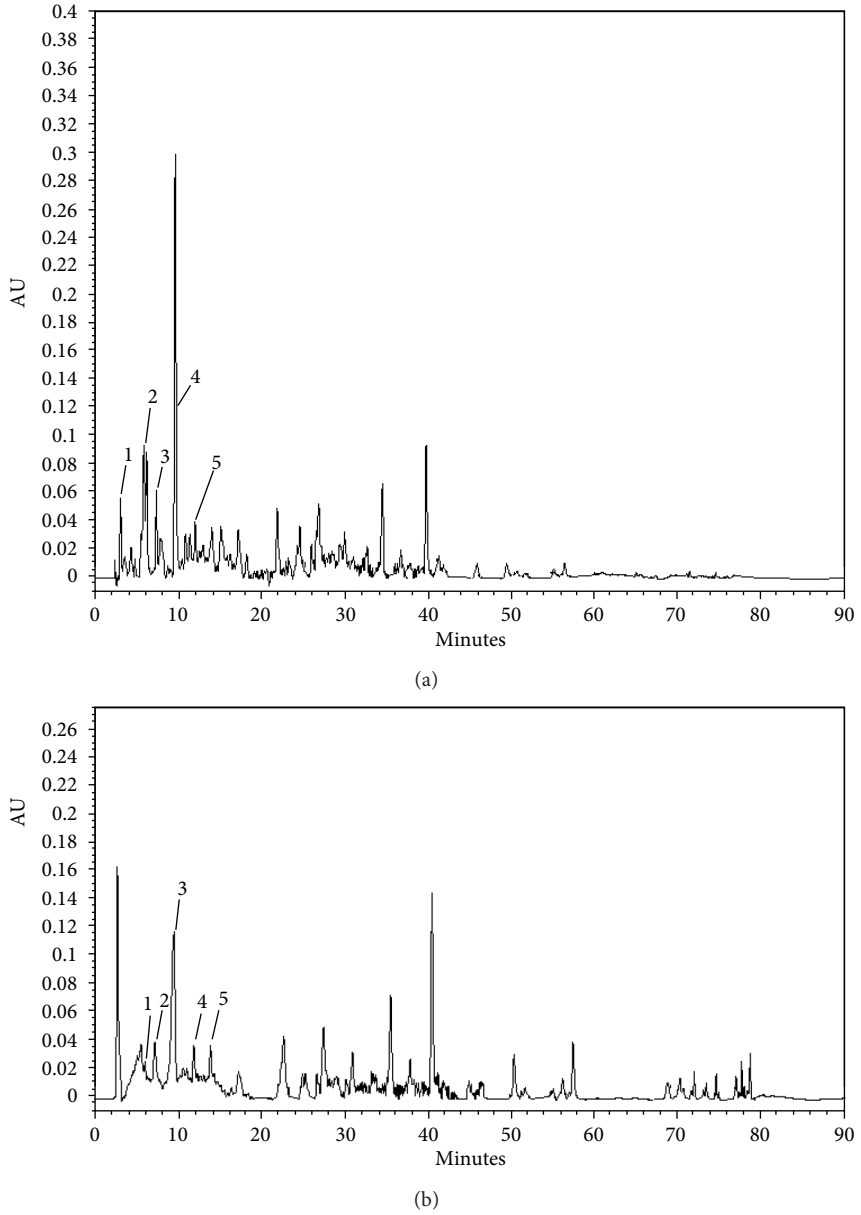

Figure 3: HPLC chromatogram recorded at 280 nm of procyanidins in an extract obtained using 1.25 M [Bmim]Br (a) and 80% methanol (b) as extraction solvent. Retention times (min): 1: B4; 2: B2; 3: (+)-catechin; 4: C1; 5: (−)-epicatechin.

included pure water extraction and sodium chloride solution extraction. Water is the most common and inexpensive solvent and is therefore often selected as a cosolvent in various extraction process. We compared the extraction capacities of ionic liquid solutions with pure water. As can be seen from Table 1, the extraction efficiency of the procyanidins was only 19.62 ± 0.76% with water, while that obtained when using 1.25 M [Bmim]Br was 100.00 ± 4.89%. The main contributor to procyanidins extraction efficiency was therefore the ionic liquid rather than water in the ionic liquid-water system. The procyanidins extraction efficiency achieved using 1.25 M NaCl solution was only 17.47±0.85%. The solvent effect of the ionic liquid was therefore more important in achieving high extraction efficiencies than the salt effect derived from NaCl. Hence, salt effects do not play a major role in improving the extraction of procyanidins.

In the present study, UAE, HRE, and ME techniques were compared for their efficiency in the extraction of procyanidins from *L. gmelinii* bark. The extraction efficiency of the procyanidins obtained under six different extraction methods using optimal conditions is summarized in Table 1. The extraction times used for UAE, HRE, and ME were 0.5, 4, and, 24 h, respectively. The extraction temperature of HRE was 85°C, while the extraction temperature used for UAE and ME was room temperature (25°C). The procyanidins extraction efficiency obtained using ILUAE methods was higher than those achieved using 80% ethanol HRE or ME methods.

3.8. HPLC Qualitative Analysis. The amount of the procyanidins studies presented in *L. gmelinii* bark extracts was qualitatively analyzed by using the chromatographic methodology. The chromatograms of HPLC of samples extracted with [Bmim]Br and 80% ethanol were shown in Figure 3. As can be seen from Figure 3, the relative contents of these characteristic procyanidin fraction ((+)-catechin, (−)-epicatechin, procyanidin dimers B2 and B4, and procyanidin trimer C1) in the [Bmim]Br extraction solution had improved distinctly, and [Bmim]Br had good effect on the extraction of procyanidins. It is clear that ILUAE represents an efficient method for the extraction of procyanidins from *L. gmelinii* bark.

4. Conclusions

In this work, we propose a novel extracting method for procyanidins from *L. gmelinii* bark based on the use of ionic liquids in UAE followed by Vanillin-HCl method analysis and quantification. The UAE conditions were optimized in detail. Considering the effect of both anion and cation, [Bmim]Br was selected for the subsequent evaluation. The optimum conditions for the extraction were as follows: [Bmim]Br concentration 1.25 M, soak time 3 h, solid-liquid ratio 1 : 10, ultrasonic power 150 W, and ultrasonic time 30 min. Under this condition, satisfactory extraction efficiency of the procyanidins was obtained. Relative to other methods, the proposed approach provided higher extraction efficiency and obviously reduced energy consumption time. The method may also prove useful in the development of energy saving and environment friendly extraction methods for procyanidins from other plant materials.

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References

[1] L. Gu, M. Kelm, J. F. Hammerstone et al., "Fractionation of polymeric procyanidins from lowbush blueberry and quantification of procyanidins in selected foods with an optimized normal-phase HPLC-MS fluorescent detection method," *Journal of Agricultural and Food Chemistry*, vol. 50, no. 17, pp. 4852–4860, 2002.

[2] L. Yang, J. M. Huang, Y. G. Zu et al., "Preparation and radical scavenging activities of polymeric procyanidins nanoparticles by a supercritical antisolvent (SAS) process," *Food Chemistry*, vol. 128, no. 4, pp. 1152–1159, 2011.

[3] J. Yamakoshi, M. Saito, S. Kataoka, and M. Kikuchi, "Safety evaluation of proanthocyanidin-rich extract from grape seeds," *Food and Chemical Toxicology*, vol. 40, no. 5, pp. 599–607, 2002.

[4] J. Fan, X. Ding, and W. Gu, "Radical-scavenging proanthocyanidins from sea buckthorn seed," *Food Chemistry*, vol. 102, no. 1, pp. 168–177, 2007.

[5] M. Škerget, P. Kotnik, M. Hadolin, A. R. Hraš, M. Simonič, and Ž. Knez, "Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities," *Food Chemistry*, vol. 89, no. 2, pp. 191–198, 2005.

[6] X. Su, A. B. Howell, and D. H. D'Souza, "Antiviral effects of cranberry juice and cranberry proanthocyanidins on foodborne viral surrogates—a time dependence study in vitro," *Food Microbiology*, vol. 27, no. 8, pp. 985–991, 2010.

[7] S. Sivakumaran, A. L. Molan, L. P. Meagher et al., "Variation in antimicrobial action of proanthocyanidins from *Dorycnium rectum* against rumen bacteria," *Phytochemistry*, vol. 65, no. 17, pp. 2485–2497, 2004.

[8] V. Nandakumar, T. Singh, and S. K. Katiyar, "Multi-targeted prevention and therapy of cancer by proanthocyanidins," *Cancer Letters*, vol. 269, no. 2, pp. 378–387, 2008.

[9] C. Gonçalves, T. Dinis, and M. T. Batista, "Antioxidant properties of proanthocyanidins of *Uncaria tomentosa* bark decoction: a mechanism for anti-inflammatory activity," *Phytochemistry*, vol. 66, no. 1, pp. 89–98, 2005.

[10] D. Bagchi, C. K. Sen, S. D. Ray et al., "Molecular mechanisms of cardioprotection by a novel grape seed proanthocyanidin extract," *Mutation Research*, vol. 523-524, pp. 87–97, 2003.

[11] G. Aldini, M. Carini, A. Piccoli, G. Rossoni, and R. M. Facino, "Procyanidins from grape seeds protect endothelial cells from peroxynitrite damage and enhance endothelium-dependent relaxation in human artery: new evidences for

cardio-protection," *Life Sciences*, vol. 73, no. 22, pp. 2883–2898, 2003.

[12] R. L. Prior and L. Gu, "Occurrence and biological significance of proanthocyanidins in the American diet," *Phytochemistry*, vol. 66, no. 18, pp. 2264–2280, 2005.

[13] X. Xu, B. Xie, S. Pan et al., "A new technology for extraction and purification of proanthocyanidins derived from sea buckthorn bark," *Journal of the Science of Food and Agriculture*, vol. 86, no. 3, pp. 486–492, 2006.

[14] Z. Shen and E. Haslam, "Proanthocyanidins from *Larix gmelini* (Rupr.) Rupr. bark," *Chemistry and Industry of Forest Products*, vol. 5, no. 1, pp. 1–8, 1985.

[15] Z. Shen, E. Haslam, C. P. Falshaw, and M. J. Begley, "Procyanidins and polyphenols of *Larix gmelini* bark," *Phytochemistry*, vol. 25, no. 11, pp. 2629–2635, 1986.

[16] L. Yang, W. Su, Y. Li, S. Hao, and W. Liu, "Extraction technology of oligomeric proanthocyanidins," *Chemistry and Industry of Forest Products*, vol. 24, no. 2, pp. 57–60, 2004.

[17] J. Jia, L. Yang, and Y. Zu, "homogenated extraction of oligomeric proanthocyanidins from larch bark and its optimization by response surface methodology," *Chemistry and Industry of Forest Products*, vol. 29, no. 3, pp. 78–84, 2009.

[18] C. Ma, S. Wang, L. Yang, and Y. Zu, "Ionic liquid-aqueous solution ultrasonic-assisted extraction of camptothecin and 10-hydroxycamptothecin from *Camptotheca acuminata* samara," *Chemical Engineering and Processing*, vol. 57-58, pp. 59–64, 2012.

[19] S. Wang, L. Yang, Y. Zu et al., "Design and performance evaluation of ionic-liquids-based microwave-assisted environmentally friendly extraction technique for camptothecin and 10-hydroxycamptothecin from samara of *Camptotheca acuminata*," *Industrial & Engineering Chemistry Research*, vol. 50, no. 24, pp. 13620–13627, 2011.

[20] T. Liu, X. Sui, R. Zhang et al., "Application of ionic liquids based microwave-assisted simultaneous extraction of carnosic acid, rosmarinic acid and essential oil from *Rosmarinus officinalis*," *Journal of Chromatography A*, vol. 1218, no. 47, pp. 8480–8489, 2011.

[21] Y. Jiao and Y. Zuo, "Ultrasonic extraction and HPLC determination of anthraquinones, aloe-emodine, emodine, rheine, chrysophanol and physcione, in roots of *Polygoni multiflori*," *Phytochemical Analysis*, vol. 20, no. 4, pp. 272–278, 2009.

[22] Y. Zuo, L. Zhang, J. Wu, J. W. Fritz, S. Medeiros, and C. Rego, "Ultrasonic extraction and capillary gas chromatography determination of nicotine in pharmaceutical formulations," *Analytica Chimica Acta*, vol. 526, no. 1, pp. 35–39, 2004.

[23] L. Paniwnyk, E. Beaufoy, J. P. Lorimer, and T. J. Mason, "The extraction of rutin from flower buds of *Sophora japonica*," *Ultrasonics Sonochemistry*, vol. 8, no. 3, pp. 299–301, 2001.

[24] S. Rodrigues and G. A. S. Pinto, "Ultrasound extraction of phenolic compounds from coconut (*Cocos nucifera*) shell powder," *Journal of Food Engineering*, vol. 80, no. 3, pp. 869–872, 2007.

[25] W. Liu, L. Cheng, Y. Zhang, H. Wang, and M. Yu, "The physical properties of aqueous solution of room-temperature ionic liquids based on imidazolium: database and evaluation," *Journal of Molecular Liquids*, vol. 140, no. 1–3, pp. 68–72, 2008.

[26] C. F. Poole, "Chromatographic and spectroscopic methods for the determination of solvent properties of room temperature ionic liquids," *Journal of Chromatography A*, vol. 1037, no. 1-2, pp. 49–82, 2004.

[27] F. van Rantwijk and R. A. Sheldon, "Biocatalysis in ionic liquids," *Chemical Reviews*, vol. 107, no. 6, pp. 2757–2785, 2007.

[28] J. S. Wilkes, "A short history of ionic liquids—from molten salts to neoteric solvents," *Green Chemistry*, vol. 30, no. 2, pp. 73–80, 2002.

[29] J. F. Liu, G. B. Jiang, J. F. Liu, and J. A. Jönsson, "Application of ionic liquids in analytical chemistry," *Trends in Analytical Chemistry*, vol. 24, no. 1, pp. 20–27, 2005.

[30] A. Berthod, M. J. Ruiz-Ángel, and S. Carda-Broch, "Ionic liquids in separation techniques," *Journal of Chromatography A*, vol. 1184, no. 1-2, pp. 6–18, 2008.

[31] G. A. Baker, S. N. Baker, S. Pandey, and F. V. Bright, "An analytical view of ionic liquids," *Analyst*, vol. 130, no. 6, pp. 800–808, 2005.

[32] J. L. Anderson, D. W. Armstrong, and G. T. Wei, "Ionic liquids in analytical chemistry," *Analytical Chemistry*, vol. 78, no. 9, pp. 2892–2902, 2006.

[33] X. Han and D. W. Armstrong, "Ionic liquids in separations," *Accounts of Chemical Research*, vol. 40, no. 11, pp. 1079–1086, 2007.

[34] L. Yang, H. Wang, Y. Zu et al., "Ultrasound-assisted extraction of the three terpenoid indole alkaloids vindoline, catharanthine and vinblastine from *Catharanthus roseus* using ionic liquid aqueous solutions," *Chemical Engineering Journal*, vol. 172, no. 2-3, pp. 705–712, 2011.

[35] X. Cao, X. Ye, Y. Lu, Y. Yu, and W. Mo, "Ionic liquid-based ultrasonic-assisted extraction of piperine from white pepper," *Analytica Chimica Acta*, vol. 640, no. 1-2, pp. 47–51, 2009.

[36] F. Y. Du, X. H. Xiao, and G. K. Li, "Application of ionic liquids in the microwave-assisted extraction of *trans*-resveratrol from *Rhizma Polygoni Cuspidati*," *Journal of Chromatography A*, vol. 1140, no. 1-2, pp. 56–62, 2007.

[37] K. K. Wu, Q. L. Zhang, Q. Liu, F. Tang, Y. M. Long, and S. Z. Yao, "Ionic liquid surfactant-mediated ultrasonic-assisted extraction coupled to HPLC: application to analysis of tanshinones in *Salvia miltiorrhiza* bunge," *Journal of Separation Science*, vol. 32, no. 23-24, pp. 4220–4226, 2009.

[38] C. Ma, T. Liu, L. Yang, Y. Zu, S. Wang, and R. Zhang, "Study on ionic liquid-based ultrasonic-assisted extraction of biphenyl cyclooctene lignans from the fruit of *Schisandra chinensis* Baill," *Analytica Chimica Acta*, vol. 689, no. 1, pp. 110–116, 2011.

[39] C. Ma, T. Liu, L. Yang et al., "Ionic liquid-based microwave-assisted extraction of essential oil and biphenyl cyclooctene lignans from *Schisandra chinensis* Baill fruits," *Journal of Chromatography A*, vol. 1218, no. 48, pp. 8573–8580, 2011.

[40] L. Yang, Y. Liu, Y. Zu et al., "Optimize the process of ionic liquid-based ultrasonic-assisted extraction of aesculin and aesculetin from *Cortex fraxini* by response surface methodology," *Chemical Engineering Journal*, vol. 175, pp. 539–547, 2011.

[41] J. Albo, P. Luis, and A. Irabien, "Carbon dioxide capture from flue gases using a cross-flow membrane contactor and the ionic liquid 1-ethyl-3-methylimidazolium ethylsulfate," *Industrial and Engineering Chemistry Research*, vol. 49, no. 21, pp. 11045–11051, 2010.

[42] R. B. Broadhurst and W. T. Jones, "Analysis of condensed tannins using acidified vanillin," *Journal of the Science of Food and Agriculture*, vol. 29, no. 9, pp. 788–794, 1978.

[43] W. Ma, Y. Lu, R. Hu, J. Chen, Z. Zhang, and Y. Pan, "Application of ionic liquids based microwave-assisted extraction of three alkaloids N-nornuciferine, O-nornuciferine, and nuciferine from lotus leaf," *Talanta*, vol. 80, no. 3, pp. 1292–1297, 2010.

[44] Z. Guo, B. M. Lue, K. Thomasen, A. S. Meyer, and X. Xu, "Predictions of flavonoid solubility in ionic liquids by COSMO-RS: experimental verification, structural elucidation, and solvation characterization," *Green Chemistry*, vol. 9, no. 12, pp. 1362–1373, 2007.