Comparison of the Extraction Efficiency of Isoflavone Compounds from *Puerariae lobatae* by Ionic Liquids with 11 Anions and 8 Imidazolium-Based Cations

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**ABSTRACT:** Although the field of natural product extraction with ionic liquids (ILs) is already a crowded one, the guiding rules regarding the selection of suitable ILs for extraction are still lacking. In this study, the extraction capacity of 32 ILs was investigated using *Puerariae lobatae* as a testing material, namely, 11 [BMIM]-based, 9 [CMIM]-based, 8 [C₅MIM][Br] and 8 [C₆MIM][BF₄] ILs (n = 2, 4, 6, 8, 10, 12, 14, 16) were studied. Three series of concentrations (0.02, 0.50, 2.00 M) were investigated, and the results demonstrated that the extraction capacity increased with the rising IL concentrations except for 1-butyl-3-methyl-imidazolium tosylate ([BMIM][Tos]). Generally, all the 0.50 and 2.00 M ILs led to the best extraction results. At low concentrations, the IL extraction capacity is strongly dependent on the anions and cations. However, the relationship between extraction capacity and anions or cations gradually becomes weak as increasing IL concentrations. Finally, the solution of ILs was prepared in aqueous or 60% methanol depending on the solubility. The pH can vary from strong acid to weak alkaline. Extraction capacity of 32 ILs varying in concentrations has a good negative correlation with the pH, except for five [BMIM]-ILs.

1. INTRODUCTION

Ionic liquids (ILs) are salts with melting temperatures below 100 °C and usually composed of an unsymmetrical organic cation and an organic or inorganic anion.¹ In the past few years, much attention is given to the ILs due to unique properties such as chemical, thermal, and electrochemical stability, negligible vapor pressure, and nonflammability.²⁻⁵ Hence, it is hitherto successively applied in numerous processes, and extractions may be one of the widest application types, commonly including liquid−liquid extractions,⁶⁻⁸ solid−liquid extractions,⁹⁻¹⁰ and three-phase partitioning and crystallization.¹¹⁻¹³ Therefore, it is important to investigate the general rules of ILs in extraction.

A large number of ILs as solvents within the range of extraction of bioactive compounds from natural products has been investigated.¹⁴⁻¹⁷ Zhang et al. evaluated eight kinds of imidazolium-based IL on the molecular level by density functional theory and founded that [OMIM][Br] can be an excellent extraction solvent.¹⁸ Bogdanov and co-workers report the extraction of (+)-glaucine from *Glaucium avum* using aqueous solutions of ILs combined with [Cl], [Br], [Ace], and [Sac] anions.¹⁹ Lots of authors showed the superior performance of IL-based extraction (including microwave-assisted, ultrasound-assisted, ultrahigh pressure-assisted, negative-pressure cavitation-assisted, among others) when compared to traditional techniques and solvents.¹⁹,²⁰ It is obvious that only a limited matrix of ILs has been investigated, and the guiding rules regarding the selection of suitable ILs for extraction are still lacking. Thereby it is necessary to get a comprehensive judgment on ILs roles for extraction from natural products.

Extraction capacity of ILs mainly depends on the IL structure, that is, cation−anion type and cation modification. It is estimated that billions of different ILs can be synthesized by combining various kinds of cation−anion. As a result, the selection of anions and cations is an important issue. Ventura and co-workers have summarized the past, present, and future trends of IL-mediated extraction and separation process of small organic extractable compounds from biomass.³⁷ They addressed the use incidence of 11 cations and 31 anions. Thus, in terms of usage incidence and stability, 32 ILs composed of 8 imidazolium cations and 11 anions were chosen in this work. Cations investigated include 1-ethyl-3-methyl-imidazolium ([EMIM]), 1-butyl-3-methyl-imidazolium ([BMIM]), 1-hexyl-3-methyl-imidazolium ([HMIM]), 1-octyl-3-methyl-imidazolium ([OMIM]), 1-decyl-3-methyl-imidazolium ([DMIM]), 1-dodecyl-3-methylimidazolium ([DoMIM]), 1-tetradecyl-3-methylimidazolium ([TMIM]), and 1-cetyl-3-methylimidazolium ([CMIM]). Anions investigated are [Cl], [Br], [Ace], and [Sac] anions.¹⁹

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addition to this, some researches on the role of critical micellar concentration (cmc) of ILs in the extraction of bioactive compounds are limited, and the solution pH has not been considered by most authors. Taking into account the different sizes of micelles formed by ILs and limited data on cmc, a wider concentration range is thus studied, namely, 0.02, 0.20, 0.5, or 2.0 M, while attempting the correlation of the extraction efficiency from natural sources. On this basis, the effects of different concentrations of 10 [BMIM]-based ILs in aqueous solution ranging from 0.02 to 2.00 M and 6 [C\textsubscript{6}MIM][Br] ranging from 0.02 to 0.50 M in aqueous solution and 16 [CMIM]-based and [C\textsubscript{6}MIM][Br] ILs in 60% methanol ranging from 0.02 to 2.00 M on the extraction of flavonoid glycosides and aglycone from *P. lobatae* Radix were studied, as shown in Figure 2. Other conditions including extraction time, extraction temperature, matrix-to-solvent ratio, and the ultrasonic power were set as 10 min, 20 °C, 0.10:4 g/mL, and 240 W.

IL concentrations have been demonstrated to have an important influence on the extraction yield.23 The [BMIM]-ILs with 9 kinds of different anions ([Cl], [Br], [I], [BF\textsubscript{4}], [Tos], and [DCA], [HSO\textsubscript{4}], and [Ac]) and [C\textsubscript{6}MIM][Br] (n = 2, 4, 6, 8, 10, 12, 14, 16) are miscible in any proportion with water, whereas [BMIM]-ILs with [PF\textsubscript{6}] and [Tf\textsubscript{2}N] and [C\textsubscript{6}MIM][BF\textsubscript{4}] (n = 6, 8, 10, 12, 14, 16) have a poor solubility in water. For comparison, [BMIM]-ILs except for [PF\textsubscript{6}] and [Tf\textsubscript{2}N] and [C\textsubscript{6}MIM][Br] were dissolved in water; others were dissolved in 60% methanol at room temperature or by heating. Therefore, water, methanol, and 60% methanol were selected as the control group. As can be seen from Figure 2, total extraction yields of bioflavonoids in 0.2 and 0.5 M ILs were significantly higher than those obtained in the control group. The value of total bioflavonoids of 8.20% in 2.00 M [BMIM][DCA] is 1.5, 2.3, and 1.4 times higher than those obtained by water, methanol, and 60% methanol.

Usually, the total extraction yield follows the order y (0.02 M) < y (0.20 M) < y (0.50 or 2.00 M) for the [BMIM]-based ILs and [C\textsubscript{6}MIM][Br] ILs, that is to say, the extraction yield increases with the increase of IL concentration. This is consistent with most experimental results. However, the extraction efficiency was almost identical for three concentrations of [CMIM]-based ILs and [C\textsubscript{6}MIM][BF\textsubscript{4}]. This indicates that ILs studied in aqueous have a more positive effect on extraction than those in 60% methanol. It is reasonable because the concentration increases in aqueous solution, there are more anions and cations participating in extraction. When the concentration increased, the cmc may reach, to name just a few, [BMIM][Br] at 0.8 ± 0.1 M, and [OMIM][Br] at 0.15 ± 0.05 M, and [CMIM][Br] at 0.0005 M in aqueous solution at 298.15 K. Nevertheless, the form and number of ILs in 60% methanol may be different from those in water and showed a minor effect as an increase of IL concentrations. For example, as the concentrations increase from 0.02 to 0.50 M, [DMIM][Br] and [DoMIM][Br] enhance the extraction yields by up to 39 and 34%, respectively, while [BMIM][BF\textsubscript{4}] and [HMIM][BF\textsubscript{4}] only enhance the extraction yields by up to 2 and 6%, respectively. Unexpectedly, most of the 32 ILs have positive effects on extraction except for [BMIM] cation with [Tos] aromatic anion (the optimal concentration at 0.02 M). It is an interesting finding because most research only indicated that raising concentration of ILs is beneficial for extraction from natural products, whereas raising concentration of [BMIM]-[Tos] in aqueous solution cannot aid to extract, which may be attributed to [Tos] having an aromatic benzene ring and causing repulsion with isoflavones mother ring. This result needs further investigation. To sum up, it seems that

![Figure 1. Cation–anion matrix of the studied 32 ILs.](https://acsonline.org/journal/acsodf/article/2020/5/8963-8971)

2. RESULTS AND DISCUSSION

This work on the use of ILs as solvents in the extraction of bioactive isoflavone compounds from *P. lobatae* Radix mainly focused on IL concentrations, anions including hydrophilicity and lipophilicity, imidazolium-based cations bearing different lengths of their alkyl side chains, and solution pH of ILs. Five effective compounds are important for a comprehensive understanding of the concentrations of ILs, pH, and yields of five isoflavones which are listed in Table 1.

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2.1. Effects of IL Concentrations on the Extraction of Total Flavonoids. Various works have addressed that the concentration of ILs in solution has a significant impact on the extraction efficiency from natural sources. On this basis, the effects of different concentrations of 10 [BMIM]-based ILs in aqueous solution ranging from 0.02 to 2.00 M and 6 [C\textsubscript{6}MIM][Br] ranging from 0.02 to 0.50 M in aqueous solution and 16 [CMIM]-based and [C\textsubscript{6}MIM][Br] ILs in 60% methanol ranging from 0.02 to 2.00 M on the extraction of flavonoid glycosides and aglycone from *P. lobatae* Radix were studied, as shown in Figure 2. Other conditions including extraction time, extraction temperature, matrix-to-solvent ratio, and the ultrasonic power were set as 10 min, 20 °C, 0.10:4 g/mL, and 240 W.

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| no. | IL            | C/mol·L⁻¹ | pH   | yield⁺/% | yield⁻/% |
|-----|---------------|-----------|------|----------|----------|
| 1   | [EMIM][Br]    | 0.20      | 51   | 0.20     | 0.02     |
| 2   | [CMIM][HSO₄] | 0.20      | 45   | 0.20     | 0.02     |
| 3   | [CMIM][NO₃]  | 0.50      | 39   | 0.50     | 0.02     |
| 4   | [CMIM][Br]    | 0.02      | 38   | 0.02     | 0.02     |
| 5   | [CMIM][I]     | 0.20      | 37   | 0.02     | 0.02     |
| 6   | [BMIM][Ac]    | 0.02      | 34   | 0.02     | 0.02     |
| 7   | [BMIM][NO₃]  | 0.02      | 32   | 0.02     | 0.02     |
| 8   | [BMIM][I]     | 0.20      | 28   | 0.02     | 0.02     |
| 9   | [BMIM][Br]    | 0.02      | 25   | 0.02     | 0.02     |
| 10  | [BMIM][BF₄]  | 0.02      | 20   | 0.02     | 0.02     |
| 11  | [BMIM][NO₃]  | 0.02      | 10   | 2.00     | 2.00     |
| 12  | [BMIM][Br]    | 0.02      | 9    | 2.00     | 2.00     |
| 13  | [BMIM][I]     | 0.02      | 8    | 2.00     | 2.00     |
| 14  | [BMIM][BF₄]  | 0.02      | 7    | 2.00     | 2.00     |
| 15  | [BMIM][Br]    | 0.02      | 6    | 2.00     | 2.00     |
| 16  | [BMIM][BF₄]  | 0.02      | 5    | 2.00     | 2.00     |
| 17  | [BMIM][Br]    | 0.02      | 4    | 2.00     | 2.00     |
| 18  | [BMIM][Br]    | 0.02      | 3    | 2.00     | 2.00     |
| 19  | [BMIM][Br]    | 0.02      | 2    | 2.00     | 2.00     |
| 20  | [BMIM][NO₃]  | 0.02      | 1    | 2.00     | 2.00     |
| 21  | [BMIM][Br]    | 0.02      | 0    | 2.00     | 2.00     |
| 22  | [BMIM][Br]    | 0.02      | 9    | 0.02     | 0.02     |
| 23  | [BMIM][Br]    | 0.02      | 8    | 0.02     | 0.02     |
| 24  | [BMIM][Br]    | 0.02      | 7    | 0.02     | 0.02     |
| 25  | [BMIM][Br]    | 0.02      | 6    | 0.02     | 0.02     |
| 26  | [BMIM][Br]    | 0.02      | 5    | 0.02     | 0.02     |
| 27  | [BMIM][Br]    | 0.02      | 4    | 0.02     | 0.02     |
| 28  | [BMIM][Br]    | 0.02      | 3    | 0.02     | 0.02     |
| 29  | [BMIM][Br]    | 0.02      | 2    | 0.02     | 0.02     |
| 30  | [BMIM][Br]    | 0.02      | 1    | 0.02     | 0.02     |
| 31  | [BMIM][Br]    | 0.02      | 0    | 0.02     | 0.02     |
| 32  | [BMIM][Br]    | 0.02      | 9    | 0.02     | 0.02     |
| 33  | [BMIM][Br]    | 0.02      | 8    | 0.02     | 0.02     |
| 34  | [CMIM][Cl]    | 0.02      | 7    | 0.02     | 0.02     |
| 35  | [CMIM][Cl]    | 0.02      | 6    | 0.02     | 0.02     |
| 36  | [CMIM][Cl]    | 0.02      | 5    | 0.02     | 0.02     |
| 37  | [CMIM][Cl]    | 0.02      | 4    | 0.02     | 0.02     |
| 38  | [CMIM][Cl]    | 0.02      | 3    | 0.02     | 0.02     |
| 39  | [CMIM][Cl]    | 0.02      | 2    | 0.02     | 0.02     |
| 40  | [CMIM][Cl]    | 0.02      | 1    | 0.02     | 0.02     |
| 41  | [CMIM][Cl]    | 0.02      | 0    | 0.02     | 0.02     |
| 42  | [CMIM][Cl]    | 0.02      | 9    | 0.02     | 0.02     |
| 43  | [CMIM][Cl]    | 0.02      | 8    | 0.02     | 0.02     |
| 44  | [CMIM][Cl]    | 0.02      | 7    | 0.02     | 0.02     |
| 45  | [CMIM][Cl]    | 0.02      | 6    | 0.02     | 0.02     |
| 46  | [CMIM][Cl]    | 0.02      | 5    | 0.02     | 0.02     |
| 47  | [CMIM][Cl]    | 0.02      | 4    | 0.02     | 0.02     |
| 48  | [CMIM][Cl]    | 0.02      | 3    | 0.02     | 0.02     |
| 49  | [CMIM][Cl]    | 0.02      | 2    | 0.02     | 0.02     |
| 50  | [CMIM][Cl]    | 0.02      | 1    | 0.02     | 0.02     |
| 51  | [CMIM][Cl]    | 0.02      | 0    | 0.02     | 0.02     |
| 52  | [CMIM][Cl]    | 0.02      | 9    | 0.02     | 0.02     |
| 53  | [CMIM][Cl]    | 0.02      | 8    | 0.02     | 0.02     |
| 54  | [CMIM][Cl]    | 0.02      | 7    | 0.02     | 0.02     |
| 55  | [CMIM][Cl]    | 0.02      | 6    | 0.02     | 0.02     |
| 56  | [CMIM][Cl]    | 0.02      | 5    | 0.02     | 0.02     |
| 57  | [CMIM][Cl]    | 0.02      | 4    | 0.02     | 0.02     |
| 58  | [CMIM][Cl]    | 0.02      | 3    | 0.02     | 0.02     |
| 59  | [CMIM][Cl]    | 0.02      | 2    | 0.02     | 0.02     |

Table 1. pH of ILs and Ultrasound Extraction Yields from *P. lobatae*
concentrations of ILs are pivotal for extraction in aqueous solution and have a minor influence in 60% methanol.

Fan et al. compared the puerarin extraction efficiency using four imidazolium-based ILs and found that the extraction efficiency of puerarin was over 99%. A comparative analysis of isoflavone extraction and the inhibitory activity was evaluated. The authors found new innovative findings of their promising anti-cancer activity. In addition, Wang et al. successfully applied ILs for the hydrolysis of puerarin and daidzein derivatives from P. lobatae.

According to the total extraction yields, the IL extraction capacities could be classified into three levels: poorer extraction ($y \leq 6\%$), moderate extraction ($6\% < y < 7\%$), and higher extraction ($y \geq 7\%$) (Figure 2). Following this classification, the poorer results were achieved with 0.02 M [BMIM][BF$_4$], [CMIM][H$_2$SO$_4$], [DMIM][Br], and [DoMIM][Br], in which 0.02 M [CMIM][H$_2$SO$_4$] has the lowest extraction yields; 0.02 M [BMIM][Cl], [OMIM][Br], and [TMIM][Br], and 0.20 M [BMIM][PF$_6$] led to moderate results ($y < 7\%$) and [BMIM][Br], [BMIM][BF$_4$], [BMIM]-[DCA], [HMIM][Br], and [HMIM][BF$_4$] led to about 7% extraction yields; all of the 0.50 and 2.00 M ILs led to the best extraction results, especially 2.00 M [BMIM][DCA] with the highest extraction yield of 82.20%. This is similar to the results reported by Bogdanov et al. From the classification of the extraction yields, it can be seen that the extraction capacity varies from the poorest to moderate to the best just by altering the concentrations, regardless of cations or anions. Taking [BMIM][PF$_6$] for example, when the concentration increased from 0.02 to 0.20 M and then to 2.00 M, the extraction yields increased from 3.01% to 4.98% and then to 8.22%.

### Table 1. continued

| no. | IL         | C/mol-L$^{-1}$ | pH  | $y_1^1/%$ | $y_2^1/%$ | $y_3^1/%$ | $y_4^1/%$ | $y_5^1/%$ |
|-----|------------|----------------|-----|-----------|-----------|-----------|-----------|-----------|
| 60  | [BMIM][Br] | 0.20$^a$       | 6.30 | 0.51 ± 0.03 | 4.51 ± 0.22 | 0.91 ± 0.05 | 0.66 ± 0.04 | 0.16 ± 0.01 |
| 61  | [BMIM][Br] | 0.50$^a$       | 6.29 | 0.52 ± 0.02 | 4.68 ± 0.07 | 0.92 ± 0.02 | 0.68 ± 0.02 | 1.07 ± 0.01 |
| 62  | [BMIM][Br] | 0.02$^b$       | 5.04 | 0.55 ± 0.00 | 4.72 ± 0.02 | 0.96 ± 0.00 | 0.64 ± 0.00 | 0.10 ± 0.01 |
| 63  | [BMIM][Br] | 0.20$^b$       | 5.04 | 0.55 ± 0.00 | 4.72 ± 0.02 | 0.96 ± 0.00 | 0.64 ± 0.00 | 0.10 ± 0.01 |
| 64  | [BMIM][Br] | 0.50$^b$       | 5.37 | 0.63 ± 0.01 | 5.35 ± 0.10 | 1.07 ± 0.02 | 0.78 ± 0.01 | 0.18 ± 0.00 |
| 65  | [BMIM][Br] | 0.02$^b$       | 5.75 | 0.54 ± 0.00 | 4.66 ± 0.01 | 0.95 ± 0.01 | 0.62 ± 0.01 | 0.06 ± 0.00 |
| 66  | [BMIM][Br] | 0.20$^b$       | 5.04 | 0.55 ± 0.00 | 4.72 ± 0.02 | 0.96 ± 0.00 | 0.64 ± 0.00 | 0.10 ± 0.01 |
| 67  | [BMIM][Br] | 0.50$^b$       | 5.37 | 0.63 ± 0.01 | 5.35 ± 0.10 | 1.07 ± 0.02 | 0.78 ± 0.01 | 0.18 ± 0.00 |
| 68  | [BMIM][Br] | 0.02$^b$       | 5.75 | 0.54 ± 0.00 | 4.66 ± 0.01 | 0.95 ± 0.01 | 0.62 ± 0.01 | 0.06 ± 0.00 |

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increases from 5.88 to 6.84% and then to 7.34%. Moreover, extraction capacity is also governed by the IL cations and anions.

2.2. Effects of IL Structures on the Extraction of Total Flavonoids.

2.2.1. Anion Type. Effect of anions on extraction capacity of ILs is investigated with eleven types: [BMIM]-based ILs with anions [Cl], [Br], [I], [BF₄], [PF₆], [Tos], [NO₃], [Tf₂N], [DCA], [HSO₄], and [Ac]; [CMIM]-ILs with anions [Cl], [Br], [BF₄], [PF₆], [NO₃], [Tf₂N], [DCA], [HSO₄], and [Ac]. These anions can classify into two types: hydrophilic character such as [Cl], [Br], [I], [Tos], [NO₃], [Tf₂N], [DCA], [HSO₄], and [Ac] and hydrophobic nature such as [BF₄], [PF₆], and [Tf₂N].

The effect of anions on IL extraction capacity is a little complicated (Figure 3a−c) and summarized below:

(a) [PF₆] < [Cl], [Br], [BF₄], [I], [DCA] < [NO₃], [Tf₂N], [HSO₄], [Ac], [Tos] (0.02 M [BMIM])

(b) [PF₆] < [Cl], [Br], [BF₄], [I], [Tos], [Tf₂N] < [NO₃], [DCA], [HSO₄], [Ac] (0.20 M [BMIM])

(c) [PF₆], [Tos], [Tf₂N] < [Cl], [Br], [I], [Ac], [NO₃], [BF₄], [HSO₄] < [DCA] (2.00 M [BMIM])

(d) [HSO₄], [NO₃] < [Cl], [Br], [DCA], [Ac] (0.02 M [CMIM])

(e) [NO₃], [DCA], [Ac] < [Cl], [Br], [HSO₄] (0.20 M [CMIM])

(f) [NO₃], [DCA], [Ac], [Br] < [Cl], [HSO₄] (2.00 M [CMIM])

According to the above results, some rules regarding the use of limited anions could be concluded. First, anions have a great impact on extraction at a low concentration of ILs. Just taking [CMIM][HSO₄] and [CMIM][Br], for example, total extraction yields were 5.07 and 7.19%, respectively. Because the number of anions and its counterions is relatively few, each ion may need to participate in the extraction process. Each anion has its specific structure, size, water solubility, hydrogen bond acceptor ability, and so on, which determine the interactions on the disruption of the matrix structure, and the strength of the interactions between the anions and target molecules. Thus, the type and number of anions at low concentrations demonstrate a more important role on extraction. Second, the influence of anions at 0.20 and 2.00 M is not obvious. The extraction yields were very close; therefore, there were only 8.3 and 2.1% gap for 0.20 M [BMIM]- and [CMIM]-ILs. The cmc of [BMIM][Br] and [CMIM][Br] at 298.15 K are 0.8 ± 0.1 and 0.0005 M, respectively. The increase of IL concentrations may lead to an increase of micelles that displays similar structures. This may result in the minor role of anions at higher concentration. Third, [BMIM][PF₆] led to the poorest results at three concentrations. This is consistent with other conclusions that the [EMIM][PF₆] had poorer performance on the extraction
of shikimic acid from star anise than [EMIM][Ac]. They observed the hydrolysis of [PF6] anions at high temperatures and the production of hydrofluoric acid. Last, hydrophilic anions [NO3], [HSO4], and [Ac] of [BMIM]-ILs are the better choice for extraction of isoflavones, whereas hydrophilic anions [Cl] of [CMIM]-ILs is the better choice for extraction of isoflavones. This may be due to the main isoflavone components which possess glycoside bonds having better compatibility with hydrophilic anions.

2.2.2. Cation Type. The influence of the cation type has been investigated based on two anion types [Br] and [BF4]. As illustrated in Figure 3d−f, the use of ILs led either to an increase or to a decrease of the extraction yield of isoflavones, which demonstrated that the contribution of the IL cation has also a certain influence. It is interesting that with the increase of the alkyl chain length, the extraction efficiency shows a different trend due to different concentrations. From Figure 3d, the extraction yield at 0.02 M ILs declined gradually with the increase of chain length regardless of [Br] or [BF4] anion. With further increase from [DoMIM] to [CMIM] for [Br] or from [OMIM] to [TMIM] for [BF4], the extraction yields drastically showed an upward trend. The general tendency of extraction is to go down first and then go up at low concentration. This phenomenon mainly depends on the hydrophilic−lipophilic ratio of the target molecules 3′-hydroxy puerarin, puerarin, 3-methoxy puerarin, and daidzin possessing a lipophilic flavone group and hydrophilic glycoside group. With the increase of alkyl chain, hydrophobicity of ILs increased which is not beneficial for extraction. However, when the alkyl chain length exceeded hexyl with anion [BF4] or decyl with anion [Br], the micelles began to form. It is presumed that with the increase of the alkyl chain length, the extraction mechanism seems to be ruled from a hydrotropic effect to a micelle-mediated dominating. This is consisted of Lin et al.’s findings who studied the extraction of ginsenoside derivatives from Ginseng roots, finding [PMIM][Br] to be the best IL. With the increase of alkyl chain length from propyl to hexyl, the extraction efficiency of total ginsenosides reduced dramatically. Besides, a similar result was found by Pan and co-workers on the extraction of alkaloids from Nelumbo nucifera.

Effects of cations at higher concentration are significantly different from those at the lower concentration. Herein, it seems that the increase of alkyl chain length of IL-[Br] is advantageous from [EMIM][Br] to [HMIM][Br], then becomes disadvantageous from [OMIM][Br] to [CMIM][Br]. Puerarin is the main product in the extraction solution; thus, we use puerarin as the model molecule to calculate. The concentration range of the model molecule based on total extraction yields is between 0.014 and 0.020 M, and the ratio of cation of IL-[Br] is advantage from [EMIM][Br] to [HMIM][Br], then becomes disadvantage from [OMIM][Br] to [CMIM][Br].

It also has been proved by [CNMIM] with anion [BF4] because the total extraction yields remain stable at 0.20 and 0.50 M. Taking into account the general results reported, the cmc of 1-alkyl-3-methylimidazolium with anion [BF4] is lower than that of 1-alkyl-3-methylimidazolium with anion [Br]. In the case of [OMIM][BF4], the cmc is 0.028 M, which is only 1/18 of [OMIM][Br]. However, there are exceptions. For [DMIM]-
[BF₄] (0.709 M), the cmc is very close with [DMIM][Br] (0.7 ± 0.3 M), which are dependent of chain length. The mechanism of extraction seems to be ruled by a micelle-mediated phenomenon, as discussed by Ma et al. when dealing with the extraction of four dibenzocyclooctadiene-type compounds. They tested different anions and different alkyl side chain lengths (n = 2−12) and found [DoMIM][Br] providing the best extraction efficiency.

The influence of alkyl chain length (n = 2−16) on extraction efficiency with anions ([BF₄] or [Br]) could be interpreted as follows. On the one hand, at very low concentrations, a longer chain length results in greater hydrophobic nature. Meanwhile, it could also decrease the intramolecular interaction with the water-soluble glycoside group of target molecules, which leads to an overall decrease interaction and lower extraction efficiency. On the other hand, longer alkyl chain length easily to self-aggregate in aqueous media may result in surface-active ILs in the extraction of more hydrophobic bioactive compounds; thus, the total extraction yields have a slight change. Despite this explanation, there are more studies being addressed including the number, size, and type of IL aggregates.

2.3. Effects of IL pH on the Extraction of Total Flavonoids. The impact of pH has been ascertained by some authors in liquid−liquid extractions of pharmaceuticals with hydrophobic ILs. However, few studies evaluated the pH impact in solid−liquid extraction which remains unaddressed topic. In this section, we attempted to clarify the impact of pH on extraction efficiency.

First, the pH of IL-investigated was determined (Supporting Information Figure S2a−d). It is important to note that all of this pH is generated by the IL aqueous or 60% methanol solution, without no addition of acid or base or buffer solution. It may be seen as a descending trend for pH of IL solutions when raising the concentrations from 0.02 to 2.00 or 0.50 M with cations [BMIM] combined with the [Br], [I], [PF₆], [DCA], and [Ac] counterions being the exceptions. The anions with cations [BMIM] include Brønsted acids ([HSO₄]) and Lewis bases ([Cl], [Br], [BF₄], [PF₆], [Ac], [DCA], [Tos], [I], [Tf₂N], and [NO₃]). The results are presented in Supporting Information Figure S2a. Interestingly, pH of ILs solution is dependent of concentration. The results indicate that the [BMIM][HSO₄] has a very strong acidity, and acidity are summarized below [HSO₄] > [BF₄] > [Tos] > [Cl] > [Tf₂N] ≈ [Br] ≈ [PF₆] ≈ [Ac] ≈ [DCA] ≈ [I] ≈ [NO₃] at 0.02 M; [HSO₄] > [BF₄] > [Tos] > [Cl] > [Tf₂N] > [Br] > [PF₆] > [Ac] > [DCA] > [I] > [NO₃] at 2.00 M. It can be seen that IL anions have a pivotal role in pH which can be varied in a large gap from strong acidity 0.86 to weak alkalinity 7.73. Notably, pH of IL anions combined with cation [CMIM], different alkyl chain length coupled with anion [Br] or [BF₄], has a regular change rule, namely, pH gradually decreased with the increasing concentration.

Second, we focus on the impact of IL pH on the extraction efficiency of total flavonoids being depicted in Figure 4a−d. The variation of total extraction yields showed in Figure 4a is a little complex when compared to Figure 4b−d. A poor relationship between the total extraction yields and the pH is ascertained for [BMIM]-ILs, that is to say, increasing pH value
is advantageous for extraction if [Br], [PF₆⁻], [Ac], [DCA], [Tos], [I], or [Tf₂N] as anions, vice versa. However, this work only considered extraction of such as alkaloids, saponins, terpenes, steroids, and anthraquinone. Finally, the extraction capacity of 32 ILs varying in alkyl chain length coupled with anion [Br] or [BF₄⁻].

3. CONCLUSIONS

The impact of concentrations, cations, and anions, and pH of IL solutions on the extraction capacity of 32 ILs using P. lobatae Radix as a test material has been ascertained, and most IL solutions demonstrated the superior performance when compared to more traditional solvents.

There are positive correlations between concentrations and extraction efficiency except for [BMIM][Tos] possessing an aromatic benzene ring. Basically, all of the 0.50 and 2.00 M ILs led to the best extraction results. Based on extraction yields, the IL-studied can be classified into three levels (poor extraction, moderate extraction, and high extraction).

The effects of anions ([Br] and [BF₄⁻]) and cations ([BMIM] and [CMIM]) of ILs on the extraction capacity are analyzed based on our investigation, and two conclusions arise. (1) Anion and cation types play the utmost role in the extraction efficiency at low concentration (0.02 M); (2) anion and cation types play a little role in the extraction efficiency at high concentration (>0.20 M).

Finally, the extraction capacity of 32 ILs varying in concentrations has a good negative correlation with the pH, the exceptions being represented with five ILs, namely, cations [BMIM] combined with the [Br], [I], [PF₆⁻], [DCA], and [Ac] counterions.

The discussion on extraction capacity may guide ILs as promising media for the extraction of bioactive compounds, such as alkaloids, saponins, terpenes, steroids, and anthraquinone. However, this work only considered extraction of isoflavone compounds, thus a wide range of bioactive compounds should be further investigated.

4. EXPERIMENTAL SECTION

4.1. Reagents and Samples. The ILs tested in the present study are given in Table S1 in the Supporting Information and used without further purification. High-performance liquid chromatography (HPLC) grade acetoni-trile were obtained from Tedia Company Inc., USA. All other chemicals were of analytical grade (Tianjin Kermel Chemical Reagent Co., Ltd, China), and ultrapure water (Milli-Q system) was used throughout.

Analytical standards of 3'-hydroxypuerarin, puerarin, 3'-methoxypuerarin, daidzin, and daidzein (purity ≥98%) were supplied by Shanghai Yuanye Biotechnology Co., Ltd, China. P. lobatae Radix in dried bulk form was obtained from the local drugstore in Jinan, China. It was pulverized in a grinder (Zhongxing Weiye, Beijing, China), passed through 20–40 mesh sieves, and the sample was stored at room temperature.

4.2. Extraction of Sample Solution. Accurately weighed P. lobatae Radix powder (0.10 g) was extracted with 4.0 mL of various solvents (ILs, water, methanol or 60% methanol) by shaking vigorously and then ultrasonicitating for 10 min (KQ-600 KDE, Kunshan, China). The suspension was then filtered through a 0.22 μm nylon syringe (Jinteng, Tianjin) and diluted five times with 60% methanol for HPLC analysis.

4.3. Preparation of Standard Solution. Each accurately weighed standard was dissolved in 60% methanol as standard stock solutions. Concentrations of five standards in stock solutions were 0.108, 0.139, 0.166, 0148, and 0.172 mg/mL, respectively. Working standard solutions for HPLC were obtained by diluting the stock solution to a series of proper concentrations. All of the standard solutions were stored at 4 °C prior to use.

4.4. Characterization. High-performance liquid chromatography analysis of the samples was performed using an Agilent 1260 infinity II system (Agilent Technologies, Santa Clara, CA, USA) equipped with a quaternary pump, standard autosampler, ultraviolet detector, and thermostatized compartment. An Agilent 5 TC-C18 column (250 mm × 4.6 mm) was used for separation at a room temperature of 20 °C. The mobile phase was a mixture of water with 0.1% formic acid (A) and acetoni-trile (B) using a gradient elution of 85–70% A at 0–16 min, 70–0% A at 16–30 min, and 0–85% A at 30–31 min, with an equilibrium time of 10 min. The injection volume was 2 μL, and the detection wavelength was set at 260 nm, and the flow rate was set at 0.5 mL/min.

The pH value was measured using a pH meter (PB-10, Sartorius Group) with a pH-sensitive glass electrode and a reference electrode.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00724.

Structure and notation of 32 ILs and structural formulae of five isoflavone compounds, overview of 32 ILs used and selected physical properties, and relationship of IL concentration and pH (PDF)
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Notes

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