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Ultrasonic Extraction of Phenolic Compounds from *Laminaria japonica* Aresch Using Ionic Liquid as Extraction Solvent

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An ionic liquid-based ultrasonic-assisted extraction method has been successfully applied to the effective extraction of phenolic compounds from *Laminaria japonica* Aresch. Three kinds of 1-alkyl-3-methyl-imidazolium with different cations and anions were evaluated for extraction efficiency. The results showed that both the characteristics of anions and cations have remarkable effects on the extraction efficiency. In addition, the ionic liquid-based ultrasonic-assisted extraction procedure was also optimized on some extraction parameters, such as ultrasonic power, extraction time and solid-liquid ratio. Compared with the conventional solvent, the optimum approach gained the highest extraction efficiency within the shortest extraction time. Average recoveries of phenolic compounds were from 75.5% to 88.3% at three concentration levels.

Key Words : *Laminaria japonica* Aresch, Ionic-liquid ultrasonic-assisted extraction, Phenolic compounds

Introduction

The brown algae, *Laminaria japonica* Aresch (*L. japonica*), is a common seafood and important economic brown alga in many countries. It is considered effective in dispelling phlegm, promoting blood flow, suppressing cough, relieving dyspnea, removing obstruction and so on, which are attributable to the large amounts of bioactive compounds in it. Polysaccharides, such as alginate, fucoidan and laminarin, are important antioxidant extracted from *L. japonica*.[1-3] Besides, recent study also showed that phenolic compounds, such as 3,4-dihydroxybenzaldehyde (3,4-DHBD), 4-hydroxybenzoic acid (4-HBA), salicylic acid (SA), 2,3-dihydroxybenzoic acid (2,3-DHBA), and 4-hydroxybenzaldehyde (4-HBD) (the structures are shown in Figure 1) are widely exist in algae.[4] These phenolic compounds possess antioxidant, antimicrobial and antiviral activities that are important for against DNA damage and cell death.[5-7]

Ionic liquids (ILs) are classes of organic salts with melting points below 100 °C.[8] The unique properties of ILs, such as a negligible vapor pressure, good thermal stability, tunable viscosity and miscibility with water and organic solvents, as well as good extractability for various organic compounds and metal ions, mainly depend on their special structures. ILs have been successfully applied to determine the organic and metal compounds from environmental contaminant.[9-12] Liquid-liquid extraction (LLE), liquid-phase microextraction (LPME), solid-phase microextraction (SPME) and aqueous two-phase systems extraction with ILs could alleviate environmental pollution and improve the selectivity and the extraction yields of interesting compounds in the sample pretreatment processes in comparison to conventional organic solvents.[13-15]

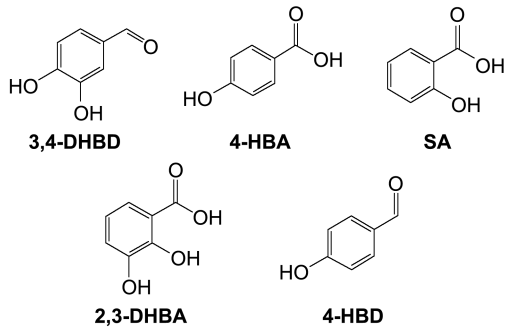

Figure 1. Molecular structures of five phenolic compounds.

There is seldom report about the extraction of bioactive compounds from marine plant. The tradition extraction methods such as heat-reflux extraction and Soxhlet extraction, which have some drawbacks including time-consuming, laborious, and using of a large amount of toxic and hazardous organic solvents. Onofrejová *et al.*[16] developed a pressurized-liquid with solid-phase extraction (PLE-SPE) method to extract of bioactive phenolic acids from algae. However, it requires a specific device loaded with a certain sorbent and a high-pressure delivery system that can be relatively expensive. It is also easily cross-contaminated when complicated sample matrices are used. Therefore, it is necessary to develop a simple, rapid, efficient extraction method.

The aim of the present paper is the development of a rapid and effective IL-based ultrasonic-assisted extraction (IL-UAE) approach for the extraction of phenolic compounds from *L. japonica*. The effects of some ultrasound-assisted extraction parameters including the choice of different ILs, pH, ultrasonic power and time were investigated to optimize the ultrasound-assisted extraction conditions.

Experimental

Reagents and Materials. *L. japonica* was purchased from local market. 3,4-DHBD, 4-HBA, SA, 2,3-DHBA and 4-HBD were obtained from Sigma-Aldrich (USA), and used

without further purification. 1-Ethyl-3-methylimidazolium tetrafluoroborate ([Emim][BF_4], 98%)1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim][BF_4], 98%), 1-hexyl-3-methylimidazolium tetrafluoroborate ([Hmim][BF_4], 98%), 1-butyl-3-methylimidazolium chloride ([Bmim][Cl], 98%) were purchased from C-TRI Company in Korea. Acetonitrile (ACN) and trifluoracetic acid (TFA) were obtained from Duksan Pure Chemical Co., LTD (Ansan, Korea). All the other reagents used in the experiment were HPLC or analytical grade. Double distilled water was filtered with a vacuum pump (Division of Millipore, Waters, U.S.A.) and filter (HA-0.45, Division of Millipore, Waters, U.S.A.) before use. All the samples were filtered by using a filter (MFS-25, 0.2 μm TF, WHATMAN, U.S.A.) before injection into the HPLC system.

Ultrasonic System. Ultrasonic system was used: ultrasonic (frequency 20 KHz, output power 200 W MAX , main source AC 220 V, Mirae Ultrsonic Tech. Co. Korea).

Chromatography. Chromatography was performed with a Waters 600 s multisolvent delivery system, Waters 616 liquid chromatography, and a Waters 2487 variable wavelength, dual-channel, UV detector (Waters Associates, Milford, MA, USA). A six-port Rheodyne injector (20-μL sample loop) was also used. Data processing was performed with Millennium 3.2 software resident in an HP Vectra 500PC. Compounds were separated on a 250 mm × 4.6 mm, 5 μm particle, OptimaPak C_{18} column (RS Tech, Daejeon, Korea). HPLC separation of organic acids was conducted using gradient elution ACN/H_2O/TFA (12.5/87.5/0.1, *v/v/v*) (A) changed linearly to ACN/H_2O/TFA (50:50:0.1, *v/v/v*) (B) within 50 min at a flow rate of 0.5 mL/min and the detection was carried out at a wavelength of 245 nm. Distilled water was filtered with a vacuum pump and filter (HA-0.45 μm; Millipore, Waters, USA) before use.

Extraction Process. The dried *L. japonica* was ground to powder by using a plant disintegrator. 0.5 g of sample powder was extracted with 10 mL different kinds of solvent, such as water, methanol and ILs ([Emim][BF_4], [Bmim][BF_4] and [Bmim][Cl]) in the above ultrasound cleaning bath, the temperature of which was controlled by the replacement between inlet and outlet water. By varying the solvent concentration, pH, ultrasonic power and time, solid/liquid ratio, the extraction conditions were optimized to obtain the best extraction efficiencies. After each extraction, the corresponding extract was filtrated through a 0.45 μm filter for the subsequent HPLC analysis.

Results and Discussion

Selection of ILs. The structure of ILs has significant influence on its physicochemical properties, which might greatly affect the extraction efficiency of target analyte,[17] owing greatly to their distinct multiple interactions with analytes and their dissolving ability for phenolic compounds. In order to evaluate the performance of ionic liquids incorporating the imidazolium cation in ultrasonic-assisted extraction process, the effects of anion and the cation on the extraction efficiency were investigated.

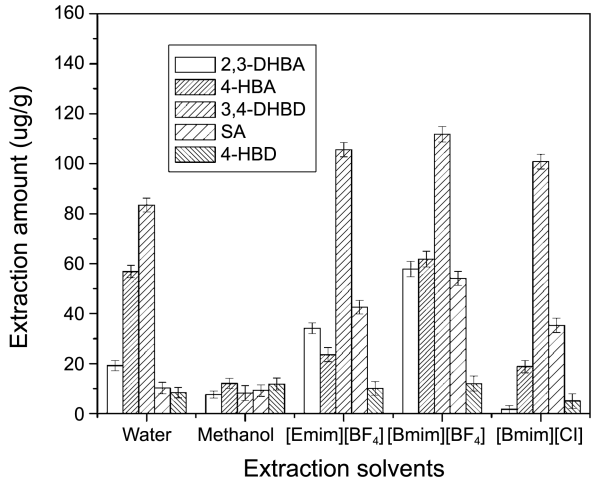

Figure 2. Effect of different extraction solvents on extraction efficiency.

It is well known that the choice of anion determines water miscibility of ILs.[18] From Figure 2, it can be seen that the addition of ILs to the extraction solvent obviously, especially 2,3-DHBA, 3,4-DHBD and SA, compared with the traditional solvent. On the other hand, the results of Figure 2 suggested that the cations and anions of ILs influenced the extraction yields of phenolic compounds. In the study, the 1-butyl-3-methylimidazolium ILs with two different anions (BF_4^-, Cl^-) were tested. As shown in Figure 2, the extraction efficiency of was decreased from BF_4^- to Cl^-. This was because [Bmim][BF_4] is more hydrophilic than [Bmim][Cl]. With the same anion of BF_4^-, [Emim][BF_4] and [Bmim][BF_4] were used to investigate the effects of the alkyl chain length on the UAE of phenolic compounds. The results indicated that the increasing alkyl chain length has increased the extraction efficiency, and the [Bmim][BF_4] was more efficient than [Emim][BF_4].

Effect of [Bmim][BF_4] Concentration on the Extraction Efficiency. Different concentrations of aqueous [Bmim][BF_4] solutions were prepared and their effects on

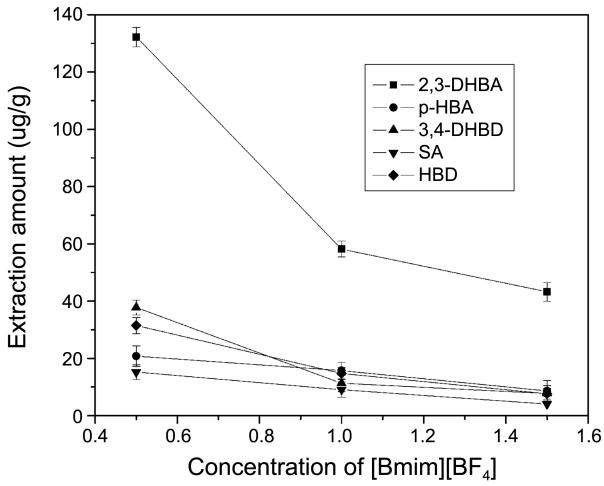

Figure 3. Effect of different concentration of [Bmim][BF_4] on extraction amount.

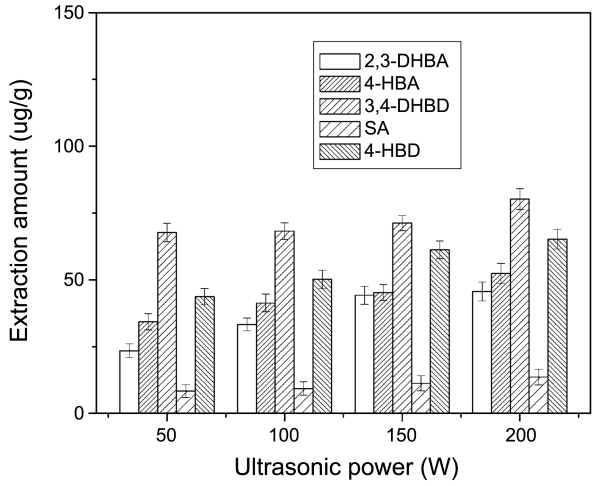

Figure 4. Effect of different ultrasonic power on extraction amount.

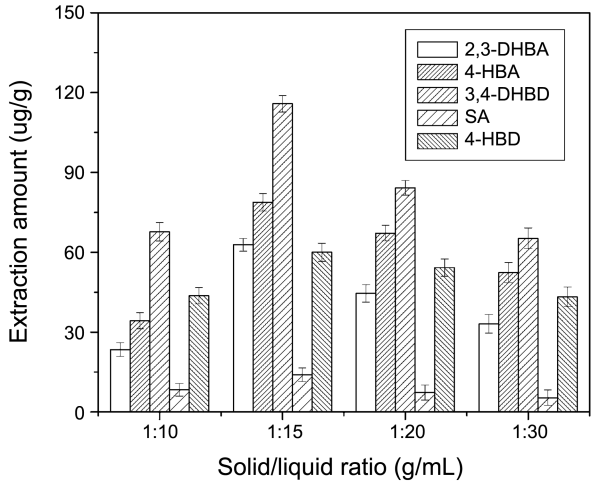

Figure 6. Effect of different solid/liquid ratio on extraction amount.

the extraction efficiencies of phenolic compounds from *L. japonica* were investigated. Figure 3 showed that the extraction efficiencies decreased with the increase in [Bmim]-[BF_4] concentration over the range of 0.5-1.5 mol/L, which can be explained by the fact that the excessive [Bmim][BF_4] increases the viscosity of solution and decreases the penetration of [Bmim][BF_4] into solid sample.[19] Based on this, 0.5 mol/L of [Bmim][BF_4] was adopted in the following study.

Effect of pH on the Extraction Efficiency. Generally speaking, the pH of the aqueous [Bmim][BF_4] solution indirectly influences the existences of phenolic compounds and the interaction between [Bmim][BF_4] and phenolic compounds. In this experiment, the effect of pH on the extraction efficiencies within the range of 1.25-7.00 was investigated and the results showed that extraction efficiencies of phenolic compounds decreased with the increase of pH. Higher pH easily causes the neutralization of phenols and the decrease of the H-bond between [Bmim][BF_4] and phenolic compounds. Therefore, the pH was set at 1.25 in the following study.

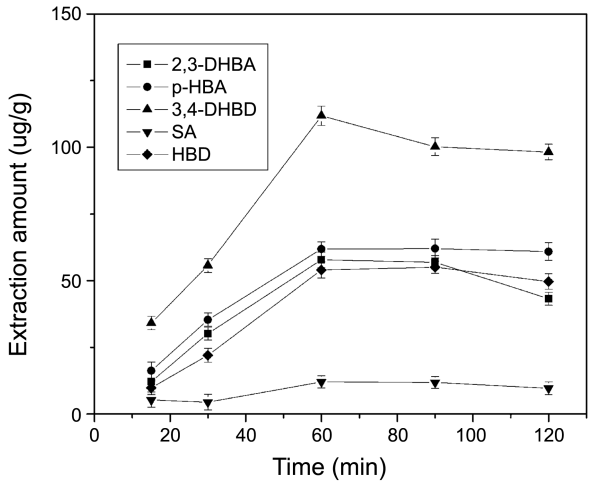

Figure 5. Effect of different extraction time of [Bmim][BF_4] on extraction amount.

Effect of Ultrasonic Power on the Extraction Efficiency. Ultrasonic power is believed to be the driving force for the complete dispersion of [Bmim][BF_4] into the solid sample. Figure 4 showed the effect of ultrasonic power on extraction efficiency. The results revealed that the increase of ultrasonic power led to the increase of extraction efficiency. The largest ultrasonic power of 200 W was chose to ensure the fully dispersion of [Bmim][BF_4] into *L. japonica*.

Table 1. Regression equations and detection limits of five phenols

Target compounds	Regression equation	Linear range (μg/mL)	r^2	Detection limit (μg/mL)
2,3-DHBA	Y=7.59+11523X	0.1 ~ 200.0	0.9995	0.07
4-HBA	Y=46.53+45373X	0.2 ~ 400.0	0.9996	0.11
3,4-DHBD	Y=8.53+10412X	0.3 ~ 600.0	0.9997	0.23
SA	Y=15.07+123027X	0.1 ~ 200.0	0.9998	0.03
4-HBD	Y=17.00+16667X	0.1 ~ 200.0	0.9997	0.21

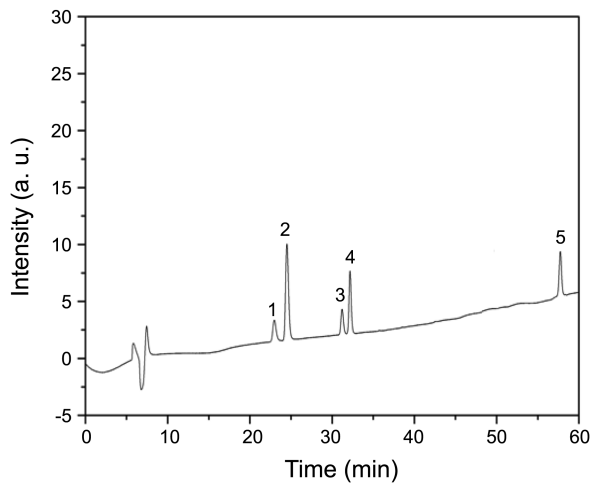

Figure 7. Chromatograms of five standard chemicals. (Concentration: 0.001 mg/mL, Injection volume: 10 μL. (1) 2,3-DHBA, (2) 4-HBA, (3) 3,4-DHBD, (4) 4-HBD, (5) SA).

Effect of Ultrasonic Time on the Extraction Efficiency. The ultrasonic time plays another predominant role in the ultrasound-assisted extraction. Figure 5 illustrated that the extraction efficiency increased with the increase in the ultrasonic time up to 60 min. When the ultrasonic time was longer than 60 min, the time effect was negligible. In view of this, 60 min was enough for the extraction procedure.

Effect of Solid/liquid Ratio on the Extraction Efficiency. A series of different solid/liquid ratio were investigated. A series of extractions were carried out with different solid-liquid ratios (1:10, 1:15, 1:20, and 1:30 g/mL) to evaluate the effect of the solid/liquid ratio. As shown in Figure 6, the extraction efficiency increased obviously with the increase of the solvent volume before the solid-liquid ratio reached 1:15, and then the efficiency was significantly decreased with the further increase of the solvent amount. Thus, a solid-liquid ratio of 1:15 for [Bmim][BF_4].

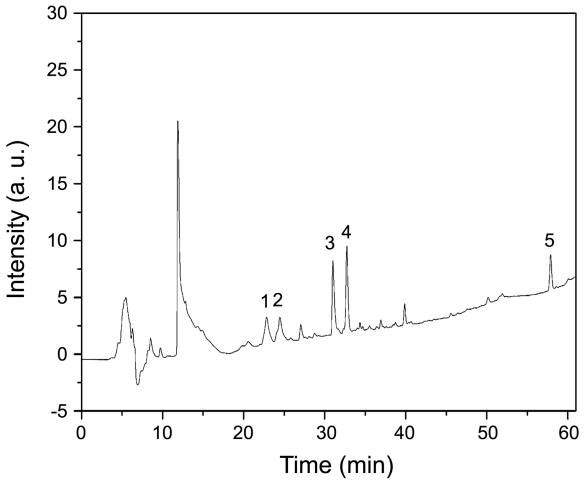

Figure 8. Chromatogram of *L. Japonica* extracted by 0.5 mol/L [Bmim][BF_4]. (Ultrasonic extraction: 1 h, ultrasonic power: 200 W, injection volume: 10 μL) (1) 2,3-DHBA, (2) 4-HBA, (3) 3,4-DHBD, (4) 4-HBD, (5) SA).

Table 2. Recovery of the five chemical compounds in three different levels

Phenols	Add amounts (μg/mg)	Found amounts (μg/mg)	Recovery (%)
2,3-DHBA	10	137.4	77.9
	50	169.3	83.4
	100	220.2	85.7
4-HBA	5	23.4	75.5
	10	27.9	80.1
	20	38.6	88.3
3,4-DHBD	5	40.5	77.1
	10	45.7	80.3
	20	53.6	86.8
SA	5	34.9	76.9
	10	28.4	80.0
	20	50.8	87.6
4-HBD	1	5.92	70.2
	2	7.06	75.3
	5	10.02	80.6

Validation of the Proposed Method. To evaluate the proposed ILUAE approach, some parameters such as linearity, reproducibility, and recovery were determined under the optimized conditions. Calibration curves were obtained by dissolving the standard phenolic compounds to mobile phase at seven concentrations. Linear regression equation, correlation coefficient and detection limit were shown in Table 1. To evaluate the accuracy of the present method, standard solution of phenolic compounds was added to *L. japonica* powder samples, at three levels. The chromatograms of standard chemicals and the *L. japonica* sample extracted [Bmim][BF_4] was shown in Figure 7 and Figure 8. The proposed IL-based UAE approach, satisfactory results were found, with recovery values between 75.5% and 88.3% (shown in Table 2).

Conclusion

In this study, ILs aqueous solution was proved to be a possible alternative solvent in the UAE of phenolic compounds from *L. japonica*. The optimization of extraction conditions proved that the [Bmim][BF_4] concentration, pH, ultrasonic power and ultrasonic time had great effect on the extraction efficiency. Compared with traditional solvent (water and methanol), ILs provides higher extraction efficiency. Moreover, considering the unique properties of ILs, the ILUAE method proposed will have a broad potentiality as an environmental friendly technique in sample preparation.

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