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Ultrasonic Assisted Extraction of Ferulic and *p*-Coumaric Acid using Hydrophobic Ionic Liquid 1-Butyl-3-methylimidazolium Hexafluorophosphate

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Ferulic and p-coumaric acids are essential phenolic acids found in plants and have traditionally been extracted with organic solvents. However, solvent-based extraction is hazardous and can be inefficient. Ionic liquids offer a promising alternative due to their customizable properties, improved efficiency, and selectivity in extraction. In this study, *p*-coumaric and ferulic acids were successfully extracted using the ultrasonic-assisted extraction (UAE) method in the presence of hydrophobic ionic liquid. The study aimed to assess the impact of different important parameters on the extraction efficiency of *p*-coumaric acid and ferulic acid. Under optimal conditions, the temperature, pH, volume ratio of ionic liquid to acid, and extraction time for *p*-coumaric and ferulic acid were 60°C, pH 2, 1: 1 v/v, and 3 mins, respectively, with an average extraction efficiency of more than 80% for both *p*-coumaric and ferulic acids. The optimal method achieved the maximum extraction efficiency in the shortest time compared to extraction using a conventional solvent. These findings indicate that an ionic liquid ultrasonic-assisted extraction (ILUAE) was an efficient method to reliably extract ferulic and *p*-coumaric acids in a reproducible and environmentally friendly manner.

Keywords: UAE; ionic liquid extraction; hydrophobic; ferulic; *p*-coumaric acid

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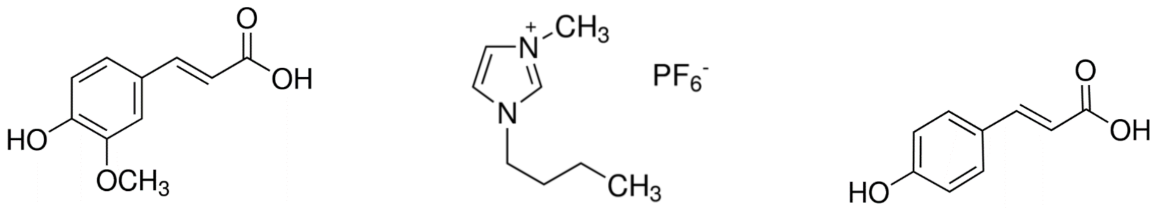
Phenolic acids (phenolcarboxylic acids) which are secondary plant metabolites belonging to polyphenol classes, can be described as a phenolic ring bearing at least one carboxylic acid functionality. These compounds have attracted significant attention because of their significant biological and pharmacological capabilities, including anti-inflammatory, antioxidant, antimutagenic, and anticarcinogenic actions [1]. Phenolic acids can be categorized into hydroxybenzoic acid and hydroxycinnamic acid, the latter being widely distributed in plants. The two most common hydroxycinnamic acids found in plant-based materials (e.g., fruits, vegetables, and cereals) are ferulic and *p*-coumaric acids [2, 3]. The chemical structures of ferulic and *p*-coumaric acids differ in terms of the presence of the methoxy group at C3 of the phenolic ring for ferulic acid as shown in Figure 1. These acids can be found predominantly bound to other structural components of the plant, such as cellulose and lignin, rather than in their free form [4].



Ferulic acid

1-Butyl-3-methylimidazolium hexafluorophosphate

p-coumaric acid

Figure 1. Chemical structure of ferulic acid, *p*-coumaric acid, and 1-Butyl-3-methylimidazolium hexafluorophosphate.

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In the actual preparation, direct extraction of ferulic and *p*-coumaric acids from plant sources such as lignocellulosic biomass from agriculture activities is favored over chemical synthesis. This may be due to the characteristics presented by both acids in natural sources, such as high concentration, widely distributed, renewable and cheap plant resources. However, in the process of obtaining ferulic and *p*-coumaric acids, conventional extraction techniques using organic solvents are usually associated with long extraction times and high organic solvent consumption [5]. Furthermore, organic solvents also possess several disadvantages not only to humans but to the environment as well, among which include volatility, flammability, and toxicity that limit their usage as extractants [6,7]. Based on these shortcomings, growing attention has been given to the development of an alternative extraction process with a "greener" and more sustainable approach, and for that, the use of ionic liquids (ILs) has been explored as a promising candidate in addressing the issue.

Ionic liquids (ILs) are composed only of organic cations (such as imidazolium and pyrrolidinium) and inorganic or organic anions (such as tetrafluoroborate, hexafluorophosphate, and bromide). These liquids are in a molten state at temperatures below 100°C [8-10]. ILs have gained significant recognition for their distinct characteristics, including thermal stability, minimal vapor pressure, nonflammability, low volatility, customizable viscosity, effective extractability, changeable miscibility, and polarity. Moreover, ILs have also been regarded as a designer and versatile solvent, as the combination of cation and anion can be tailored to the desired application [6, 11]. Utilization of ILs is not only limited to its application in chemical reactions but also as a novel extractant in various extraction and separation processes involving biomolecules, particularly phenolic chemicals [12–15].

Furthermore, the application of ILs coupled with some other assisted technologies, such as IL-ultrasonic-assisted extraction (IL-UAE), offers notable benefits in the domain of extraction and separation. This method stands out as a sustainable alternative that requires easy handling and is safe, economical, and reproducible as it can be operated at standard temperature and pressure [16 –18]. The UAE technique relies on the principle of acoustic cavitation, which involves the creation, enlargement, and sudden collapse of bubbles in a liquid. This mechanical activity disrupts the attractive forces between molecules in the liquid, resulting in the enhancement of the chemical activity such as the interaction between active compounds with the IL in the solution [19, 20].

This study aimed to develop a more sustainable and greener extraction approach for active compounds. To do this, ferulic and *p*-coumaric acids were chosen as the partitioning molecule using IL-ultrasonic assisted extraction. The choice of a hydrophobic ionic liquid is based on the moderate polarity of acids. This choice enables convenient isolation of the product from the reaction mixture after the reaction. For that purpose, hydrophobic IL, 1-butyl-3-imidazolium hexafluorophosphate was selected as a potential extractant, and combinatory effects of extraction parameters, namely time, temperature, pH and volume ratio of IL to acid, were evaluated and optimized.

EXPERIMENTAL

Chemicals and Materials

Ferulic acid and *p*-coumaric acid were acquired from Sigma Aldrich (USA). Meanwhile, the ionic liquid 1-butyl-3-imidazolium hexafluorophosphate ($[C_4mim]PF_6$) was obtained from Acros Organics (Germany). All other chemicals were utilized as supplied without additional purification.

Methods

Generally, 1.0 mL of 1-butyl-3-imidazolium hexafluorophosphate ionic liquid was mixed with 2.0 mL of the aqueous solution of ferulic acid and *p*-coumaric acid with a concentration of 1×10^{-4} M each under continuous stirring for 60 minutes. This method of extraction was obtained from Wang et al. 2018 with some modifications on the concentration. The reaction mixture was also subjected to ultrasound-assisted extraction using an ultrasonic probe dipped in a water bath to compare the efficiency of both extractions. The method that produced the highest yield was selected for the subsequent extraction process.

In the extraction process, four variables were evaluated based on a one-factor-at-a-time approach (OFAT). These variables included pH (1.0 – 8.5), solvent to acids ratio, V_{acid}: V_{IL} (1:0.2, 1: 0.4, 1: 0.6, 1: 0.8, and 1:1), temperature (25, 40, 50 and 60 ºC) and time (3, 5, 7 and 13 min). To better understand the influence of pH on the extraction, hydrochloric acid (HCl) and sodium hydroxide (NaOH) (1M) were used to adjust the pH of the reaction mixture. After the extraction was completed, the solution was allowed to equilibrate for at least 30 minutes for complete phase separation. After the separation of the phase, a certain amount of aqueous layer (upper phase) was collected and analyzed for residual phenolic acid concentration. The analyses were carried out using UV–Vis spectrophotometry (BIOWAVE II, Biochrom, England) at a wavelength of 310 nm and 286 nm for ferulic acid and *p*-coumaric acid, respectively, and calibration curves were properly established for this.

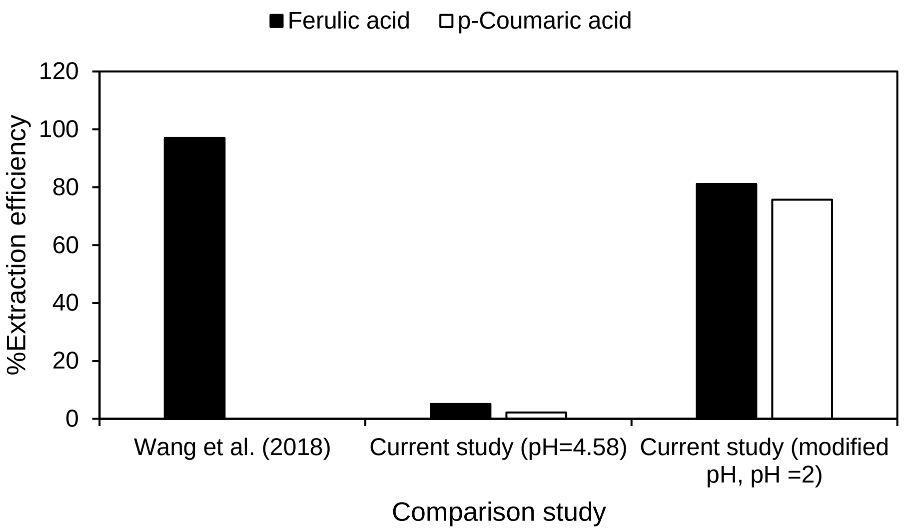

Figure 2. Comparative study of extraction efficiencies of ferulic acid and *p*-coumaric acid at different pH. Reaction conditions: V_{acid}: V_{IL} = 1:1, T = 30°C, and t = 60 min (Wang et al., 2018).

The content of phenolic acid in the ionic liquid phase (C_{IL}) and organic phase (C_w) was calculated by material balance (C_0-C_W), and the extraction efficiency of phenolic acid was calculated using the following equation.

$$\text{Extraction efficiency } (\%) = \frac{C_{IL}}{C_W^0} \times 100$$

where the C_0 and C_W are the initial and equilibrium concentrations of the phenolic acids in the aqueous phase, respectively.

RESULTS AND DISCUSSION

Figure 2 compares the extraction efficiencies of ferulic acid and *p*-coumaric acid at different pH conditions. The range of initial pH for ferulic acid and *p*-coumaric acid are 4.48 – 4.54 and 4.52 – 4.58, respectively.

The result of this study was compared with Wang et al. 2018 who performed a similar study for ferulic acid extraction. However, the efficiencies of extraction for both acids were very low, which led to further modification in the pH [21]. Ying et al. [21] reported that the efficiency of extraction for ferulic acid using the same ionic liquid was more than 85% when the pH was maintained at less than 3.67. Hydrochloric acid was added dropwise to reduce the pH to 2.00. The extraction efficiency increased significantly by more than 70% for both acids. The influence of pH was further studied in the pH range of 1.00 to 8.50. The results are shown in Table 1.

Table 1. Extraction efficiencies of ferulic acid and *p*-coumaric acid at different pH.

	% Extraction Efficiency	
pH	**Ferulic acid**	***p*-Coumaric acid**
1.00	91.14 ± 0.022	69.68 ± 0.011
2.00	91.14 ± 0.01	55.18 ± 0.015
3.00	79.74 ± 0.023	53.32 ± 0.019
4.11	7.59 ± 0.18	ND
5.16	1.27 ± 0.17	ND
6.12	ND	ND
7.10	6.32 ± 0.028	ND
8.50	1.86 ± 0.018	ND

ND: not detected

The extraction efficiencies for ferulic acid at pH levels higher than 4 were less than 10%, while p-coumaric acid was not detected, indicating that these acids are not stable in alkaline conditions. Organic acids, including *p*-coumaric and ferulic acids, can exist in various states based on the pH and their dissociation constants (pK_a). The pK_a values for p-coumaric acid are 4.32 (pK_{a1}) and 8.97 (pK_{a2}), whereas ferulic acid has 4.50 (pK_{a1}) and 8.92 (pK_{a2}). At pH levels below 4, these acids predominantly exist in their neutral forms, with undissociated carboxyl (-COOH) and hydroxyl (-OH) groups. This allows for strong hydrogen bonding with the ionic liquid, resulting in higher extraction efficiencies. Conversely, the strength of the hydrogen bonding between the ionic liquid and the acids decreased as the pH increased because the carboxyl and hydroxyl groups of the acids dissociated [22]. As a result, the extraction efficiency decreased. According to these findings, a pH of 2.0 was determined to be the most favorable pH value for the subsequent experiments.

Figure 3 compares the ultrasonic-assisted extraction efficiency of phenolic acids and conventional stirring extraction. The extraction using magnetic stirring was performed for 60 min to give a comparable percentage of extraction efficiency for both ferulic and *p*-coumaric acids.

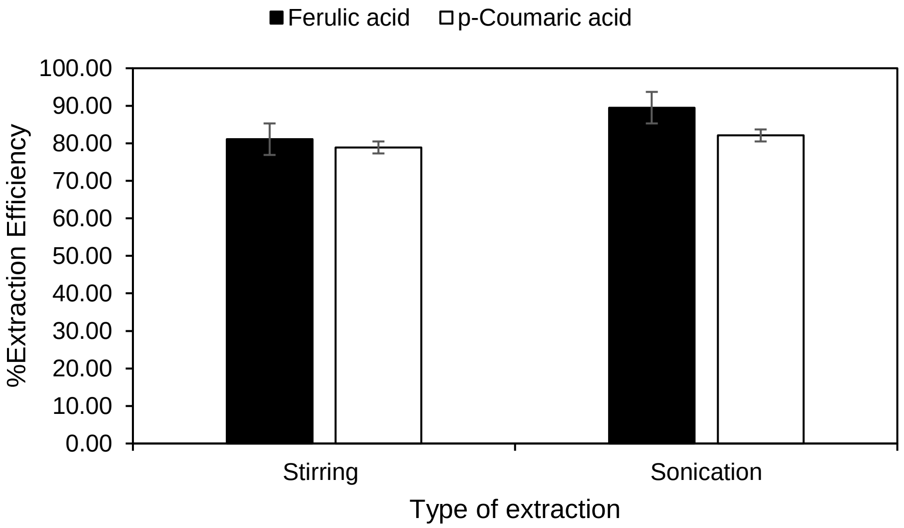

Figure 3. Comparative evaluation between the ultrasound-assisted and conventional stirring extractions of ferulic acid and *p*-coumaric acid using ionic liquid. Reaction conditions: V_{acid}: V_{IL} = 1:1, T = 30ºC, t = 60 min for stirring; 5 min for sonication and pH = 2.0.

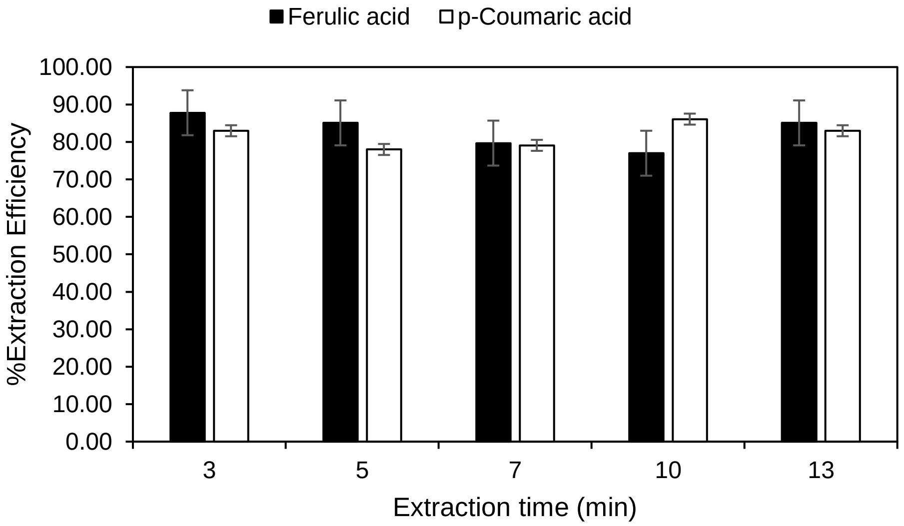

Figure 4. Extraction efficiencies of ferulic and *p*-coumaric acids at different extraction times. Reaction conditions: V_{acid}: V_{IL} = 1:1, T = 30ºC and pH = 2.0.

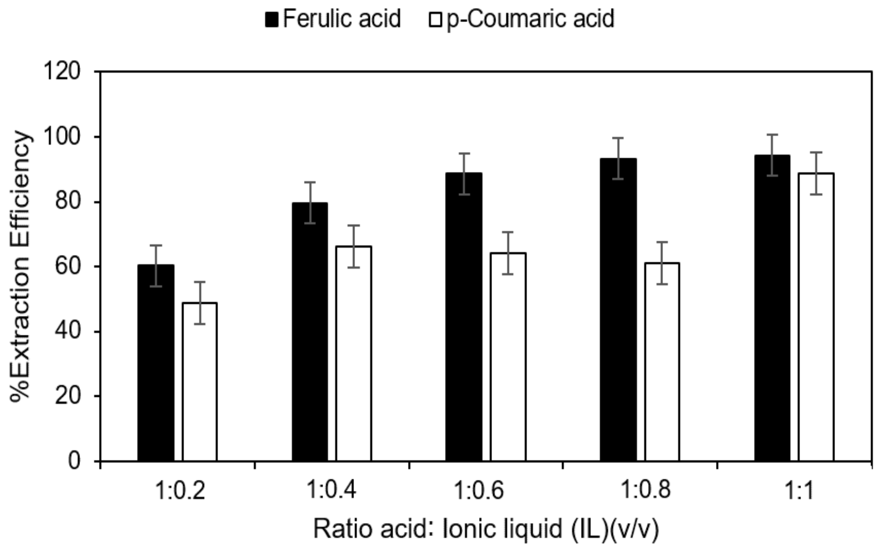

Figure 5. Extraction efficiencies of ferulic and *p*-coumaric acids at different solvent ratios. Reaction conditions: T = 30℃, t = 3 min and pH = 2.0.

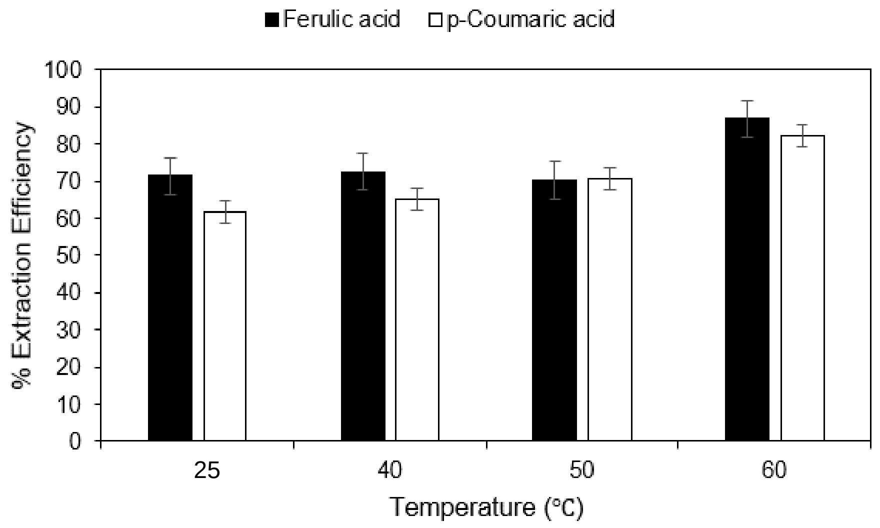

Figure 6. Extraction efficiencies of ferulic and *p*-coumaric acids at different reaction temperatures. Reaction conditions: V_{acid}: V_{IL} = 1:1, T = 30℃, t = 3 min and pH = 2.0

However, the results were still lower than the extraction efficiency obtained using ultrasonic-assisted extraction performed in just five minutes. The increase in extraction yield of desired chemicals during ultrasonic-assisted extraction can be attributed to the formation and compression of cavitation bubbles caused by sonication [23]. The degradation of these bubbles results in a rise in temperature and pressure, enhancing the diffusion process by enlarging the contact surface area between the analytes and solvent. This, in turn, accelerates the mass transfer of analytes to the solvent [24]. The utilization of ultrasonic-assisted extraction in ionic liquid has emerged as a highly effective method, particularly in terms of decreasing reaction time and streamlining procedures. The extraction was conducted across a range of extraction times time (3, 5, 7, 10, and 13 min), and the results are depicted in Figure 4.

The yield of both acids was observed to be the highest as fast as 3 min. Further prolonged extraction time showed no significant change in the concentration of acids extracted; therefore, the extraction time was set at 3 min for the subsequent experiment. The ratio of acids to ionic liquid (solvent) can be a crucial determinant for an efficient extraction process. Given the high cost of ionic liquid, it is crucial to minimize its usage while maximizing the production of desired chemicals. For each extraction, the volume of ionic liquid was altered while the volume of acids remained consistent. The experimental results in Figure 5 revealed that the amount of ferulic acid extracted increased gradually from 60.2% to 94.33% as the volume ratio of acid: ionic liquid increased from 1:0.2 to 1:1, respectively

However, the yield of *p*-coumaric acid was maintained below 65% for a volume ratio of 1:0.4 to 1:08 and increased drastically to 88.8% when an equal volume ratio of acid and ionic liquid was used. The increased extraction efficiency for both acids, as the volume of ionic liquid increased, might be due to the enhanced electrostatic interaction between the imidazolium cations of the ionic liquid and the hydroxyl groups of the phenolic acids [25]. A higher dosage of the extraction solvent provides a large number of active sites. It increases the surface area for the sorption of target compounds, which eventually promotes better extraction efficiency [15]. Figure 6 illustrates the impact of temperature on the extraction process of ferulic and p-coumaric acids. The temperature was found to be a key factor associated with extraction efficiency

An incremental improvement in efficiency was noted as the temperature rose from 26 to 60 ºC. Increasing the temperature during the extraction process helps reduce both the ionic liquid's surface tension and viscosity. Lower viscosity enhances the diffusion of active chemicals, including p-coumaric and ferulic acids, into the solvent. At the same time, reduced surface tension improves the wetting and penetration of the ionic liquid, especially when dealing with plant material. These changes facilitate better contact and interaction between the ionic liquid and the target compounds, leading to more efficient extraction [26]. Further increase in the temperature may degrade the acids. Therefore, the temperature was set maximum at 60 °C.

CONCLUSION

The present investigation revealed that the ferulic and *p*-coumaric acid has been successfully extracted using 1-butyl-3-imidazolium hexafluorophosphate, as a hydrophobic ionic liquid, with the aid of ultrasonic wave to assist the extraction. Moreover, the experimental parameters such as the initial pH of the solution, volume ratio (V_{acid}: V_{IL}), and temperature each play a vital role in both acids' extraction efficiencies; therefore, their optimization is necessary to achieve maximum extraction. The extraction efficiency was found to be the highest at lower acidic pH (1.0-2.0), minimum reaction time (3 min), maximum temperature (60° C), and equal volume ratio of acids to ionic liquid (1:1). These findings highlight the synergistic effect of ionic liquids and ultrasonic waves in the extraction process, showcasing their potential to improve extraction yields while adhering to green chemistry principles. The proposed IL-UAE approach not only enhances efficiency but also provides an alternative to conventional solvent extraction techniques. This represents a promising advancement in the field of extraction technologies, deserving further investigation and optimization. Future work should explore scaling up the extraction using this method and exploring its applicability to a broader range of compounds to assess its viability comprehensively.

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