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Comparative Study of Chlorogenic Acid Extraction from Different Plants

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Abstract
The objective of this study was to evaluate the efficiency of extraction of chlorogenic acid (CGA) from different plant matrices such as heather (Calluna vulgaris) flowers, green tea (Camelia sinensis) leaves and blueberry (Vaccinium myrtillus) fruits. *Ethanol-water mixture was compared with 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) and 1-butyl-3-methyl imidazolium tetrafluoroborate ([Bmim]BF_4). The highest CGA content was obtained in heather flowers using 60% EtOH aqueous solution, while [Bmim]Cl ionic liquid proved to be the good solvent for green tea and blueberries matrices. Statistically insignificant differences ($p < 0.05$) were observed with prolonged ultrasonic time and [Bmim]Cl for blueberry samples, but significant decrease using 60% EtOH and [Bmim]BF_4. HPLC analysis showed that CGA was degradated to caffeic acid under these conditions.*

Keywords: *Chlorogenic acid, Extraction, Plant materials, Ionic liquids.*

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I. INTRODUCTION

Over the last years, ionic liquid solvents (ILs), a class of chemicals composed entirely of an asymmetric large-size organic cation with an anion of weak coordination properties, have received explosive interest as alternatives to traditional organic solvents [1]. ILs are defined as substances with melting points below 100 ^oC and often show a broad temperature range over which they are liquid. Their unique physicochemical properties include very low to negligible vapour pressure, high thermal stability and conductivity as well as those which are tunable by proper cation-anion combination, such as density, viscosity, hydrophobicity, polarity and acid-base properties. ILs have been also recently proposed as superior extractants for separation of a variety of bioactive components [2, 3]. Among all ILs, alkylimidazolium cations combined with Cl^-, Br^-, BF_4^- or PF_6^- counter ions are the most often used, also for separation of phenolic acids from different plant samples [4-7]. ILs can interact with bioactive compounds via hydrogen bonding, π-π interactions, ion-dipole, ion-induced dipole and permanent dipoles interactions as well as dispersion forces.

Extracts from plant materials are a rich source of bioactive compounds for nutraceutical and pharmaceutical application, thus, further isolation and purification of these compounds are required. It should be remarked that although a large amount of literature sources focused on the optimization of the extraction procedures with ILs, very few considered the isolation of the solutes from the final IL solution. The most common isolation methods for polyphenolic compounds comprise back-extractions which consume more conventional organic solvents [5, 8-10] and the use of a microporous resin with ethanol-water mixture for elution [11].

In recent years there is growing interest in evaluating the availability of natural plant extracts as an alternative to the use of synthetic antioxidants by the food, pharmaceutical and cosmetic industries. Chlorogenic acid (CGA), a bioactive phenolic acid extensively distributed in plants, has attracted significant attention due to its wide spectrum of pharmacological properties including antioxidant, antibacterial, and antidiabetic activity, cardiovascular protection as well as reducing blood sugar [12, 13].

The objective of this study was to evaluate the efficiency of extraction of chlorogenic acid from different plant materials such as heather (*Calluna vulgaris*) flowers, green tea (*Camellia sinensis*) leaves and blueberry (*Vaccinium myrtillus*) fruits using ILs. Popular ethanol-water mixture was compared with two imidazolium-based ionic liquids (1-butyl-3-methylimidazolium chloride and 1-butyl-3-methylimidazolium tetrafluoroborate) as these Ils have been proposed as promising media for extraction of bioactive compounds.

II. EXPERIMENTAL

Chlorogenic acid (CGA) as well as the ionic liquids, 1-butyl-3-methylimidazolium chloride ([Bmim]Cl) and 1-butyl-3-methylimidazolium tetrafluoroborate ([Bmim]BF_4) were provided by Sigma-Aldrich. Ethanol was purchased from Merck (Darmstadt, Germany).

Fruits of wild growing blueberries and flowers of wild heather were collected from pine forest during September of 2016 in central Poland, Mazovia region. Drying of blueberry fruits was performed in laboratory dryer at 60 ^{o}C for 24 h. Heather flowers were air dried at ambient temperature (~20 °C). Green tea was purchased from the local marketplace.

The homogenized material (400 mg) was shaken with 20 mL an appropriate solvent for 1h at room temperature or placed in ultrasonic water bath (working frequency 37 kHz, power 80W) and sonicated for 5-20 min. Then, the extracts were filtered through Whatman no.1 filter paper. For a given sample three independent extractions using an appropriate solvent were carried out.

The amount of CGA was determined by Shimadzu LC system coupled to 8030 mass spectrometer. MS system was equipped with an ESI source operated in negative-ion mode. Nitrogen was used as the collision induced dissociation (CID) gas. The precursor ions for chlorogenic was monitored at *m/z* 353.0. That precursor ion was subject to CID and the daughter ions with *m/z* 191.0 was monitored. ZIC-HILIC column (100 x 2.1 mm, 3 µm) was used with water as eluent A and acetonitrile as eluent B. The mobile phase was delivered at 0.2 mL/min in gradient mode: 0-4 min. 98% B, 6 -7 min 90 % B, 8-8.- min 50% B, 13-20 min 98% B. Quantification was done from the calibration curve obtained in Selected Reaction Mode. Data are presented as a mean (in mg per 100 g of dry material weight) ± standard deviation.

III. RESULTS AND DISCUSSION

The review of literature revealed that pure ethanol extracted polyphenolic compounds with lower efficiency in comparison to hydroalcoholic solutions [14-18]. The presence of water is helpful to enhance swelling of plant material, which is favorable to increase the contact surface area between the plant matrix and the solvent, resulting in increase of the extraction yield. The extraction efficiency using ethanol-water mixture was compared with two ILs solutions. As the anion of ionic liquid may significantly influence the extraction yields [2], the 1-butyl-3-methylimidazolium cation with two types of anions (Cl^{-} and BF_4^{-}) were studied. These IL anions are strong and weak hydrogen-bond acceptors, respectively [17]. The IL water solutions at concentration of 0.5 mol/L were used as several studies have shown that the extraction efficiency increased with increasing of their concentration up to 0.4 - 0.6 mol/L reached latter plateau [2,5,7]. Plant dried matrices (heather flowers, tea leaves and blueberries fruits) were chosen as they contained different CGA concentration and different polyphenol patterns. The preliminary study was performed by simply infusion process (which is also named as maceration or soaking) conducted for 10-60 min at room temperature. The content of CGA in the extracts of three plant materials is presented in Table 1.

Table 1: Content of CGA in the studied plant materials using different extractants.

Solvent	Extraction time, min			
	10	20	30	60
Heather				
60% EtOH	105 ± 5.25	133 ± 5.31	261 ± 9.14	520 ± 2.80
[Bmim]Cl	4.88 ± 0.191	7.73 ± 0.309	9.71 ± 0.385	24.2 ± 0.726
[Bmim]BF_4				
Green tea				
60% EtOH	3.62 ± 0.134	4.70 ± 0.182	6.59 ± 0.260	9,10 ± 0.274
[Bmim]Cl	10.7 ± 0.428	11.6 ± 0.451	12.3 ± 0.492	12.8 ± 0.506
[Bmim]BF_4	2.64 ± 0.102	3.05 ± 0.0915	3.68 ± 0.137	5.75 ± 0.230
Blueberry				
60% EtOH	0.752 ± 0.0301	0.958 ± 0.0381	1.89 ± 0.0756	3.77 ± 0.189
[Bmim]Cl	9.10 ± 0.273	14.3 ± 0.481	23.5 ± 0.938	34,4 ± 1.36
[Bmim]BF_4	4.86 ± 0,202	6.60 ± 0.254	7.01 ± 0.276	14.2 ± 0.564

The yield of chlorogenic acid extraction from each plant material varies with the solvent used, obtaining the highest content in heather flowers for 60% EtOH aqueous solution. The higher transfer of CGA was observed for heather sample and [Bmim]BF_4 solution compared to [Bmim]Cl, that can be explained by better rupture of that plants` cell walls, allowing improved access to the active ingredients. From the other side, [Bmim]Cl ionic liquid proved to be good solvent for green tea and blueberries matrices as it extracted the highest amounts of chlorogenic acid. Extractability of the solvent mainly depends on the solubility of the compounds in the solvent system, the mass transfer kinetics of the product and the strength of the solute/matrix interactions. Higher efficiency of extraction was observed with the increase of time of this process.

The simple solid-liquid extraction process may require long extraction time and usually higher yields and faster extraction of bioactive compounds can be achieved through ultrasound-assisted extraction (UAE). Ultrasonic time is generally regarded as a predominant factor influencing the efficiency of extraction, however, the use of UAE may have a significant impact on the stability of the extract [14,18,19]. Figure 1 illustrates the effect of ultrasonic time on the obtained content of CGA.

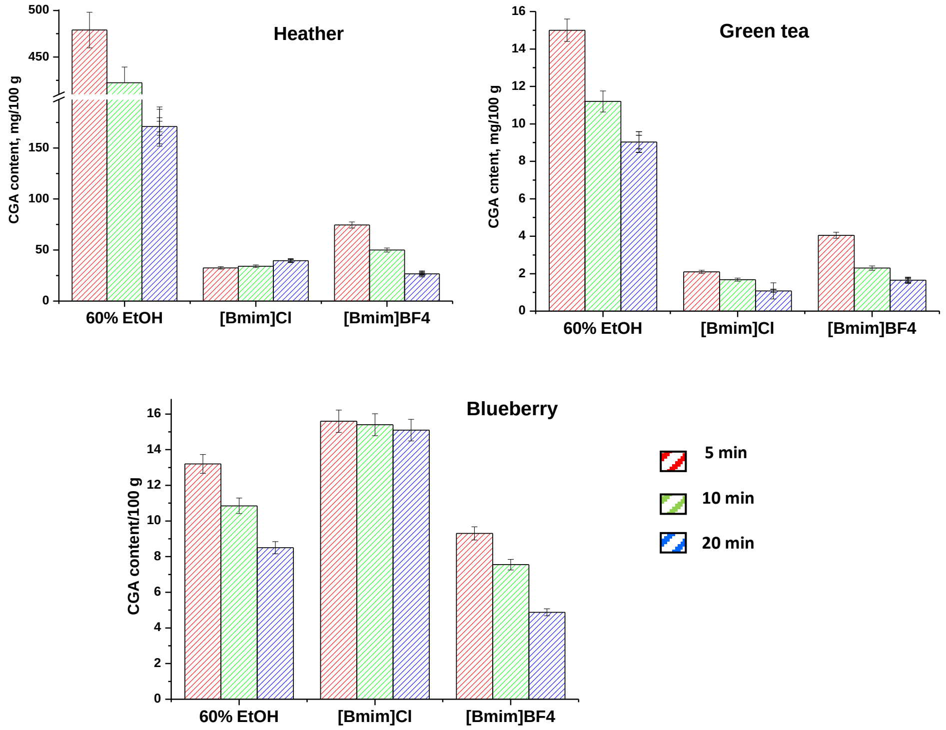

Figure 1: **Effect of sonication time on the content of chlorogenic acid using different extractants.**

For samples containing blueberry fruits statistically insignificant differences ($p < 0.05$) were observed with prolonged UAE and [Bmim]Cl solution, which exhibited the highest yield in simply maceration process, but significant decrease using 60% EtOH and [Bmim]BF_4. Similar behavior was observed for heather and green tea matrices using both ILs. Yang et al. [5] also reported reduced yield of CGA from ramie (*Boehmeria nivea* L.), a Chinese medicinal plant, with increasing ultrasonic treatment and acidic ionic liquids. According to Narita and Inouye [21], the stability of chlorogenic acid at elevated temperature is notably improved in the presence of epigallocatechin gallate, the main catechin present in tea leaves. The observed decrease of chlorogenic acid contents was due to its degradation under prolonged UAE conditions. Caffeic acid was formed as the product of CGA degradation process and its content was increasing with prolonged time of ultrasound treatment (Figure 2). It was found that several compounds (derivatives or the products of reaction with water) could be formed from CGA during the heating of its water solution [20,21]. Therefore, it is of critical importance to select efficient extraction procedure and maintain the stability of chlorogenic acid. Compared to the results obtained for simply maceration process (Table 1), the application of UAE significantly reduced extraction time with higher or similar chlorogenic acid yields.

Considering the above results, it can be concluded that the solvents used in these experiments extracted CGA with different efficiency from studied plant materials. The content of chlorogenic acid decreased in the order: 60% EtOH < [Bmim]BF_4 < [Bmim]Cl for heather flowers; [Bmim]Cl < 60% EtOH < [Bmim]BF_4 for green tea leaves and [Bmim]Cl < [Bmim]BF_4 < 60% EtOH for blueberry fruits. We are aware, that other extraction variables, such as a concentration of salt or solid/liquid ratio, should be additionally investigated and optimised. However, we would like to compare in the preliminary study the IL properties to already existing systems and verify if ILs are so useful for extraction replacing conventional solvents as this topic is extensively discussed in literature [1,2,23].

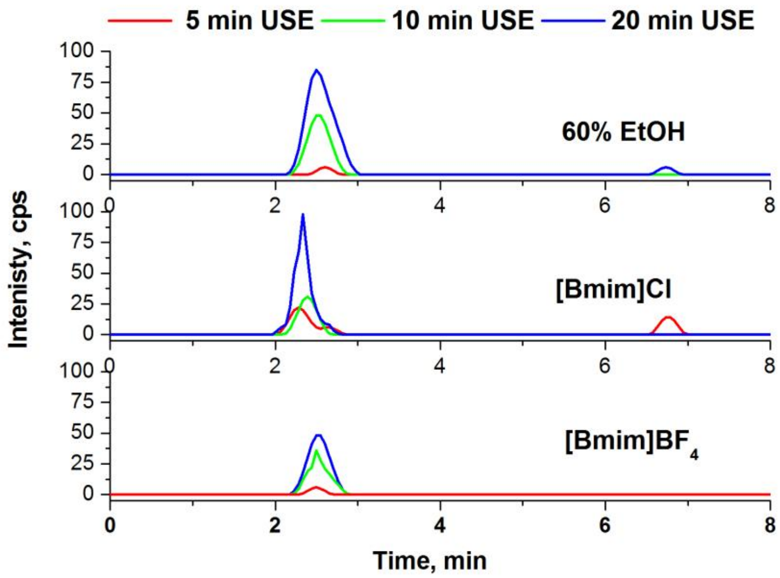

Figure 2: Peaks for caffeic acid recorded using CGA solutons in the presence of used extractants and sonicated for different times.

IV. CONCLUSION

These studies demonstrate the usefulness of imidazolium-based ionic liquids for the extraction of chlorogenic acid from green tea and blueberries matrices in comparison to widely used aqueous-ethanol solution. However, CGA extraction efficiency from heather flowers was higher for 60% EtOH than for the studied Ils. Thus, the overall opinion repeated in the published papers that ILs are promising and green solvent, and even be better than classical ones started to become doubtful. Their performance in sample preparation step depends on several factors such as the kind of plant material, solute to be extracted as well as the kind of ionic liquid used. Moreover, as the extracts from plant materials containing natural antioxidants can be used for nutraceutical and pharmaceutical application or even in food industry, quite easy step for further isolation of these compounds from IL solutions should be consider. Thus far little is known about toxicity of ionic liquids and their harm to human and environment.

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