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A novel combined process for extracting, separating and recovering flavonoids from flos sophorae immaturus

Runping Wang [a], Yuena Chang [a], Zhijian Tan [b], Fenfang Li [a,*]

[a] College of Chemistry and Chemical Engineering, Central South University, Changsha 410083, Hunan, PR China
[b] Institute of Bast Fiber Crops, Chinese Academy of Agricultural Sciences, Changsha 410205, Hunan, PR China

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ABSTRACT

The hydrotropic characteristic of ionic liquids can be used to recover bio-molecules from aqueous media and ionic liquid based-aqueous biphasic systems have been extensively applied to the separation and purification of value-added compounds. According to the hydrotropy effect and the phase separation ability, n-1,3-dibutylimidazolium propionate was chosen from several imidazolium carboxylate ionic liquids to carry out the novel combined process integrating extracting, separating and recovering flavonoids from flos sophorae immaturus with the aid of aqueous biphasic systems. The optimum separation conditions derived from the response surface analysis were potassium citrate concentration 26.12%, pH 9.83 and temperature 41.89 °C, with the predicted extraction efficiency 100.27% and measured one 99.68%. The flavonoids in the ionic liquid-rich phase were precipitated out and recovered by the dilution of using pure water as an anti-solvent and the cooling of refrigerator, producing the maximum recovery rate 82% or so and establishing the appropriate dilution ratio 5 in the involved recovery systems. The extracts presented good scavenging activity, namely, 62% on the 2, 2-diphenyl-1-picrylhydrazyl radical and 85% on the hydroxyl radical from the well-known Fenton reaction when the sample concentration was approximately 0.125 mg/mL, elucidating the combined process efficient and feasible.

1. Introduction

Flos Sophorae Immaturus (FSI) is the dried flower buds of *Sophora japonica* L., mainly growing in the Loess Plateau and the North China Plain. As a traditional Chinese medicine, FSI contains rich flavonoids which are polyphenols ubiquitously existing in nature with many potential biological activities of anti-inflammatory [1,2], anti-cancer [3], anti-allergy [4], anti-platelet aggregation [5], cardiovascular protection [6] and anti-oxidation [7]. Simultaneously, rutin is a main ingredient in FSI flavonoids and its content is quite high. Flavonoids are extracted usually by conventional solvents such as hot water, alkaline liquor and organic solvent. The involved procedures in conventional solvent extractions of flavonoids are not only multistage, laborious, time and energy consuming, but also may cause some adverse effects on bioactive molecules and environment because of high temperature, alkalinity, toxicity or volatility. Therefore, the application of safer and greener extractants possessing improved performance can be taken into account in the academic and industrial fields.

A new era on the investigation of ionic liquids (ILs) as potential candidates for the extraction of value-added compounds from natural sources has come [8]. ILs are a molten salt composed entirely of organic cations and inorganic or organic anions, usually presenting liquid at room temperature or near room temperature. Owing to their excellent physiochemical properties such as negligible vapor pressure, high thermal and chemical stability, low toxicity and a wide range of solubility, ILs taken as "greener" solvent have been extensively used for various extractions of value-added compounds from biomass [9–15] in addition to separation techniques of electrophoretic methods, gas chromatography and liquid chromatography [16]. Furthermore, ILs have proved to be an efficient extractant in the recovery of a wide variety of natural compounds, providing enhanced extraction yields and significant reduction of the extraction times and solvent consumption [8,17,18]. Bogdanov et al. applied IL-supported solid–liquid extraction of glaucine from Papaveraceae and proposed a plausible extraction mechanism [11], and the results gave a considerable advantage to the IL-supported extraction procedure due to the faster mass transfer. Chowdhury et al. have used a protic and distillable IL ($[N_{1100}][N(C_1)_2CO_2]$) at room temperature to improve the extraction of tannins from Acacia catechu and Terminalia chebula [12]. Many authors applied the IL-based microwave-assisted extraction for the extraction of a

* Corresponding author.
E-mail address: lfflqq@csu.edu.cn (F. Li).

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series of polyphenolic and flavonoid compounds from various natural sources and obtained better extraction efficiency [13–15]. Especially, an anti-solvent induced precipitation proved successful for the separation and recovery of neutral compounds [19]. Simultaneously, Cláudio' group reported the magic of aqueous solutions of ILs in promoting the dissolution of hydrophobic bio-molecules with ILs as a powerful class of catanionic hydrotropes, and thus proposed that the hydrotropy induced by ILs can be used to recover solutes from aqueous solutions by precipitation only using water as anti-solvent [20].

Ionic liquid based aqueous biphasic systems (ILABS) were first discovered and used to the separation process by Gutowski et al. [21]. Compared with typical polymer aqueous biphasic systems (ABS), ILABS attracted more and more attention from researchers in virtue of some unique properties such as shorter phase separation time, lower viscosity, tunable polarity, harder emulsification and mild operating conditions [22]. Santos et al. compared the extraction ability of the ILABS and conventional polymer/salt-based ABS for two antioxidants, eugenol and propyl gallate, and found that the maximum extractive performance of the former could reach 100% for both antioxidants [23]. ILABS are generally composed of ILs and inorganic salts such as phosphate, carbonate and sulphate. Huang et al. and Lin et al. investigated the extraction of bovine serum albumin with choline-like and imidazole ILs-phosphate-based ABS [24,25]. Louros et al. assessed the extraction ability of the ILABS composed of phosphonium-based ILs and K_3PO_4 solutions for amino acids, food colourants and alkaloids [26]. Lai and Tan et al. conducted the IL-based ultrasound-assisted extraction and the corresponding ABS technology for analysis of caffeoylquinic acids from Flos Lonicerae Japonicae [27]. We previously reported the simultaneous extraction and recovery of pectin and flavonoids from Ponkan peels based on choline amino acid ILs and corresponding ILABS and got better extraction yields, and furthermore the flavonoids recovery from the IL-rich phase was achieved by using ethanol as anti-solvent [28].Tan et al. applied the $[C_4mim]BF_4/Na_2SO_4$ ILABS to isolate and purify anthraquinones from aloe leaves with the maximal extraction efficiency 92.1% [29]. In addition, they also investigated the IL-based ultrasonic-assisted extraction of secoisolariciresinol diglucoside from flaxseed with further purification by an aqueous biphasic system, and the maximum extraction efficiency was 93.35% [30]. In recent years, the ILABS involving organic salts have been reported and applied to extract and concentrate bioactive molecules and pharmaceuticals [23,31,32]. Shao et al. applied the IL-trisodium citrate dehydrate-based ABS to extract sulfonamides in milk [31]. Lu et al. and Santos et al. used the IL-potassium citrate ABS to extract cytochrome *c* and antioxidants [23,32]. These organic salts, such as acetate, propionate and citrate, do little harm to human and environment because of nontoxicity and ease degradation. Nevertheless, to the best of our knowledge, there are few reports on a combined process integrating aqueous extraction of value-added compounds from natural sources with ILs as a hydrotropic agent, elimination of corresponding impurities with organic salt-based ILABS, and recovery of objective compounds and ILs with only water as an anti-solvent.

In this work, we designed a combined process for extracting and recovering flavonoids from FSI based on the imidazolium carboxylate ILs and corresponding organic salt-based ILABS. The carboxylate anion of the above mentioned ILs contributed to better dissolution of cell-wall cellulose [33] and release of the target molecules; the imidazolium cation possessed better phase separation ability compared with the benign bio-derived cholinium cation in that the former had lower density charge than the latter as reported in the literature [34]. Thus, a series of imidazolium carboxylate ILs were used to investigate the hydrotropy effect on rutin that is a main component of FSI flavonoids and simultaneously construct the ILABS with potassium citrate. According to the results of hydrotropy effects and phase diagrams, n-1,3-dibutylimidazolium propionate ($[C_4bim][Pro]$) was chosen to extract flavonoids from FSI, which were subsequently separated and purified by the ILABS formed with potassium citrate to remove polysaccharide and other impurities. Potassium citrate concentration, temperature and pH significantly affected the extraction efficiency of flavonoids with the ILABS and the optimal separation conditions were assessed by central composite design (CCD) of response surface methodology (RSM). The FSI flavonoids in the IL-rich phase precipitated out by cooling and dilution of using only water as an anti-solvent, accompanied with the recovery of the IL, and then the factors affecting the recovery rate of flavonoids were analyzed. Aiming to further elucidate the combined process applicable and biocompatible for the extraction and recovery of value-added compounds from natural sources, the scavenging ability of various antioxidants including the FSI extracts and standard antioxidants (ascorbic acid and butylated hydroxytoluene) was measured on the DPPH radical and the hydroxyl radical from the well-known Fenton reaction.

2. Materials and methods

2.1. Chemicals and raw material

1-Methylimidazolium, 1-ethylimidazolium, 1-isopropylimidazolium, n-1-butylimidazolium and n-butyl bromide are of analytic grade and supplied by a company of Jiangxi province. Butylated hydroxytoluene (BHT), ascorbic acid (Vc) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Aldrich. Rutin standard sample, hydrogen peroxide and anion exchange resins were obtained from Aladdin, Shanghai, China. Potassium citrate, acetic ether, diethyl ether, hydrochloric acid, sodium hydroxide, salicylic acid and ferrous sulfate were of analytic grade and from Sinopharm Chemical Reagent Co., Ltd., Shanghai, China. FSI was purchased from a drugstore located in Changsha and ground into powders in accordance with the experimental demand.

2.2. Preparation of ionic liquids

A series of imidazolium carboxylate ILs were synthesized and purified according to the reference method with some modifications on the solvents used for purification, i.e., acetonitrile and methanol was replaced by diethyl ether [35]. The detailed information about the synthesis was attached to the Supporting Information. All purified ILs were characterized by ^1H NMR and elemental analysis after vacuum drying and the purity reached 96 wt%. The results of characterization are presented in Figs. S2–S6 and Table S1 of Supporting Information. The related information of the ILs involved in this work is listed in Table 1, and their chemical structures are depicted in Fig. 1.

Table 1
Names, abbreviations and state at room temperature for the ionic liquids involved.

Ionic liquids	Abbreviation	State at room temperature
n-1-Buty-3-methylimidazolium propionate	$[C_4mim][Pro]$	Colorless transparent liquid
n-1-Buty-3-ethylimidazolium propionate	$[C_4eim][Pro]$	Colorless transparent liquid
n-1-Buty-3-isopropylimidazolium propionate	$[C_4pim][Pro]$	Colorless transparent liquid
n-1,3-Dibutylimidazolium propionate	$[C_4bim][Pro]$	Colorless transparent liquid
n-1,3-Dibutylimidazolium lactate	$[C_4bim][Lac]$	Light yellow transparent liquid