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Tailor-made hydrophobic deep eutectic solvents for cleaner extraction of polyprenyl acetates from *Ginkgo biloba* leaves

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ABSTRACT

Deep eutectic solvents (DESs), especially hydrophilic DESs, have been considered green substitutes to conventional organic solvents for bioactive compounds extraction. This study provided a practical example demonstrating the efficiency of hydrophobic DESs as designer solvent to extract polyprenyl acetates from *Ginkgo biloba* leaves. A hydrophobic ternary DES named as MCO gave the highest extraction yield for polyprenyl acetates during the initial screening. It was tailor-made from methyl trioctyl ammonium chloride, capryl alcohol and octylic acid at a molar ratio of 1:2:3 by systematically optimizing the DES component type, molar ratio and number. With MCO-based extraction, the operational factors influencing the extraction yield were further optimized by statistical method. The model for the extraction of polyprenyl acetates was well established and verified. Extraction yield of 84.11 ± 0.7366 was obtained at the optimal conditions, which was much higher than those of conventional hydrophobic organic solvents (petroleum ether and *n*-hexane). Moreover, excellent recovery of polyprenyl acetates from MCO extraction solution was obtained by macroporous resin AB-8 or DM130 adsorption. This study demonstrated that not only hydrophilic DESs but also hydrophobic DESs can be adopted as solvents for bioactive compounds extraction from biomass.

1. Introduction

Since the "twelve principles" were applied to the green chemistry, the "green" was endowed new characteristics in chemistry (Francisco et al., 2013). Generally speaking, green chemistry means to design chemical processes or products that eliminate or reduce the generation or utilization of hazardous substances (Chemat et al., 2012). In this context, seeking green solvents as substitutes for non-green solvents becomes a prior target (Abou-Shehada et al., 2016).

Ionic liquids (ILs) as green solvents have become a research hotspot due to their unique properties (Huang et al., 2015). Although green and security, the application of ILs is obstructed because of complicated preparation procedure and high cost. Deep eutectic solvents (DESs) have appeared as new green solvents with the potential to overcome the drawbacks of ILs (Xu et al., 2015). Most DESs are commonly made up of two or more non-toxic, biodegradable, inexpensive, and non-flammable components that are able to connect with each other through hydrogen-bond (Paiva et al., 2014). As with ILs, DESs are recognized as designer solvents with a wide choice range of components (Durand et al., 2016). Due to distinct, adjustable and green characteristics, DESs can often be used to replace conventional volatile organic solvents in many physical and chemical processes such as extraction. There are lots of reports about hydrophilic DESs as extraction medias to extract flavonoids, phenolic acids, polyphenols and other bioactive compounds from biomass (Dai et al., 2013; Gu et al., 2014; Tang et al., 2014; Park et al., 2014; Bi et al., 2013; Qi et al., 2015; Li et al., 2015; Tang et al., 2015; Nam et al., 2015; Mouratoglou et al., 2016; Bakirtzi et al., 2016). However, only two papers were published recently with the aim of preparing hydrophobic DESs (Ribeiro et al., 2015; Van Osch et al., 2015). And, these two studies focused on the extraction of model biomolecules from aqueous solutions. As far as we know, extraction of any bioactive compounds from real plant material employing hydrophobic DESs have not been reported.

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In this study, an actual example was provided to demonstrate that the hydrophobic DESs could be used as tunable solvents to extract bioactive substances from plant biomass efficiently. For this purpose, *Ginkgo biloba* leaves were selected because of the high content of bioactive compounds, such as polyprenyl acetates, flavonoids and terpene trilactones, which have strong pharmacological effects on regenerative anemia, liver diseases, diabetes and central nervous system diseases (Kasradze et al., 2003; Zhang et al., 2015; Tao et al., 2014). We aimed to evaluate the possibility of hydrophobic DESs as tunable solvents to extract polyprenyl acetates from *Ginkgo biloba* leaves. In such a framework, hydrophobic DESs were designed to verify the designability after initial screening. Following that, the extraction methods and conditions relevant to the extraction efficiency were examined by response surface methodology (RSM). Furthermore, the recovery of target polyprenyl acetates from hydrophobic DES solution was undertaken by macroporous resins.

2. Materials and methods

2.1. Materials

The *Ginkgo biloba* leaves used in this study were purchased from Chinese Herb Transaction Center (Bozhou, China). The leaves were dried at 65 °C for 6 h, and then pulverized. The water content of the *Ginkgo biloba* leaves powder was 3.17% (w/w, determined according to the GB 5009.3–2010 of Chinese National Standards). The pulverized leaves were sieved between 30- and 40-mesh, and then stored in a desiccator.

Methyl trioctyl ammonium chloride (≥99.0%) was obtained from Adams Reagent Co., Ltd (Shanghai, China). Decyl alcohol (≥98.0%), hexyl alcohol (≥98.0%), cyclohexanol (≥97.0%), cis-9-octadecenoic acid (≥99.0%), myristic acid (≥99.0%), palmitic acid (≥97.0%), and ricinoleic acid (≥98.0%) were purchased from Shanghai Aladdin Bio-chem Technology Co., Ltd (China). 1-hexadecanol (≥99.0%), capryl alcohol (≥99.0%), 1-tetradecanol (≥99.0%) and dodecyl alcohol (≥98.0%) were obtained from Sinopharm Chemical Reagent Co., Ltd (China). Octanoic acid (≥99.0%), capric acid (≥99.0%), and hexanoic acid (≥99.0%) were supplied by Shanghai Macklin Biochemical Co., Ltd (China). The standards of polyprenyl acetates (≥99.0%) were prepared by the preparative HPLC method (presented in the Electronic Supporting Information).

Methanol, isopropanol and *n*-hexane of chromatographic grade were provided by Tedia Co Inc (Shanghai, China). Deionized water was prepared by a Milli-Q® Ultrapure Water System (Millipore, Billerica, MA). Other chemicals and reagents used in the experiment were analytical reagent grade.

The macroporous resins (HPD-17, D101, DM130, HPD-450, ADS-17 and AB-8) used to recover the target Ginkgo polyprenyl acetates from the DES extraction solution were donated by the Cangzhou Bon Adsorber Technology Co., Ltd (Cangzhou, China), and pretreated before use according to the instructions provided by the manufacturer.

2.2. HPLC analysis of polyprenyl acetates

HPLC analysis was performed on an Elite P1201 HPLC system, which was equipped with a SinoChrom ODS-BP C18 column (4.6 × 200 mm, 5 μm) and a 1201 UV detector (Elite, China). Mixture of isopropanol/methanol/*n*-hexane/water (45/22/25/3, v/v) was used as mobile phase. The column temperature and flow rate were controlled at 27 °C and 1.0 mL/min, respectively. 10 μL of sample was injected. The absorbance at 210 nm was detected (Tang et al., 2015; Van Beek and Montoro, 2009; Ezanka and Votruba, 2001; Carlson et al., 2000). The content of polyprenyl acetates was calculated by means of a calibration curve established with a regression equation $y = 1179.4x+12.746$ ($R^2 = 0.9991$) (Fig. S1 in the Electronic Supporting Information).

2.3. Hydrophobic DESs preparation

In this work, methyl trioctyl ammonium chloride (N81-Cl) was chosen as the hydrogen bond acceptor (HBA) to prepare hydrophobic DESs. 18 different alcohols or aliphatic acids were selected as hydrogen bond donors (HBDs). The mixture of HBA and HBD was heated and stirred at 80 °C to form homogeneous liquid DES. Table 1 gives the list of the prepared DESs.

2.4. Extraction of polyprenyl acetates from Ginkgo biloba leaves using hydrophobic DESs

During DES screening and tailoring, 80 mg of *Ginkgo biloba* leaves powder was mixed with 0.80 mL of extraction solvent in 2 mL microfuge tube. The mixture was treated briefly on a vortex mixer, and then extracted in an air-bath shaker at 250 rpm and 25 °C for a time period of 5 min. The extraction solution was centrifuged for 10 min at 10,000 rpm. Certain amount of supernatant was withdrawn and diluted for four-fold with the HPLC mobile phase. The parallel experiments were carried out for three times, and the polyprenyl acetates were quantified and the deviations were evaluated.

2.5. Hydrophobic DESs extraction of Ginkgo polyprenyl acetates employing different methods

After initial DES screening and tailoring, the most efficient DES was adopted as the extraction solvent to compare the extraction efficiency of different extraction methods. Heating, stirring, water-bath shaking, air-bath shaking and ultrasonic methods were compared for the extraction. 80 mg of *Ginkgo biloba* leaves powder was extracted with 0.80 mL of the hydrophobic DES by heating at 60 °C and 0 rpm, stirring at 150 rpm and 25 °C or 60 °C, water-bath shaking at 150 rpm and 25 °C or 60 °C, air-bath shaking at 250 rpm and 25 °C or 60 °C, ultrasonic treating at 200 W and 25 °C or 60 °C.

2.6. Experimental design and statistical analysis

Single factor experiments were firstly performed to obtain appropriate variable range for experimental design based on the response surface methodology, presented in the Electronic Supporting Information. After that, main factors were optimized to attain the highest extraction yield of the target polyprenyl acetates. Central composite design (CCD) combined with RSM was employed to study the effects of temperature, extraction time and solvent to solid ratio at five levels (−2, −1, 0, 1, 2) on the extraction yield of polyprenyl acetates, which was represented as the response. 20 experiments including six replicates at center points were performed in random order. Table 2 showed the coded levels and actual values of the variables. A second order polynomial model was fitted based on the experimental data.

The experimental design and regression analysis was carried out with Design-Expert 8.0.5 software (State-Ease Inc., MN, USA). F-test was checked to investigate the statistical significance of the regression coefficient. The fitness of the proposed model was estimated by assessing coefficient of determination (R^2), lack of fit, and F-value based on analysis of variance (ANOVA).

2.7. Recovery of polyprenyl acetates

The recovery of Ginkgo polyprenyl acetates from DES was