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Tailoring and recycling of deep eutectic solvents as sustainable and efficient extraction media

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ABSTRACT

The present study demonstrates that deep eutectic solvents (DESs) with the highest extractability can be designed by combining effective DES components from screening diverse DESs. The extraction of polar ginseng saponins from white ginseng was used as a way to demonstrate the tuneability as well as recyclability of DESs. A newly designed ternary DES (GPS-5) composed of glycerol, L-proline, and sucrose at 9:4:1 was used as a sustainable and efficient extraction medium. Based on the anti-tumor activity on HCT-116 cancer cells, it was confirmed that GPS-5 was merely an extraction solvent with no influence of the bioactivity of the ginsenosides extracted. Excellent recovery of the extracted saponins was easily achieved through solid-phase extraction (SPE). Recycling of the DES was accomplished by simple freeze-drying of the washed solutions from the SPE. The extraction efficiencies of the DESs recycled once, twice, and thrice were 92%, 85%, and 83% of that of the freshly synthesized solvent.

1. Introduction

Over the past two decades, much attention has been paid to ionic liquids (ILs) as sustainable alternatives to hazardous organic solvents [1]. ILs are a class of fluid that is formed from the combination of anions and cations with melting points below 100 °C [2]. ILs can be synthesized either from eutectic mixtures of metal halides and organic salts, or from those of discrete ions [2]. More recently, deep eutectic solvents (DESs), have been recognized as a novel class of sustainable solvents to replace common organic solvents or even ILs [1–3]. DESs are fluid systems formed from a eutectic mixture of two or more components that are naturally occurring, safe, and inexpensive components. While melting points of DESs are much lower than the individual components, most DESs are liquid between ambient temperature and 70 °C [4]. Although DES components can contain a variety of anionic and/or cationic species, they can be associated with each other through intermolecular hydrogen bonding [4]. DESs are considered superior to ILs due to their biodegradability, non-toxicity, and low costs for synthesis, in addition to the tuneability, negligible volatility, and wide polarity range that are shared by ILs [3,5,6]. DESs have been used in various research fields such as catalysis, organic synthesis, electrochemistry, material chemistry, and extraction processes [1–4].

Extraction of natural products from herbal medicines using green, safe, and efficient solvents is important in pharmaceutical and biochemical research fields [7–10]. Because bioactive natural products vary greatly in polarity, maximized extractability can be achieved by tuning the polarity of the extraction solvent. Although it is known that the properties of DESs can be tailored by changing the components and their molar ratios [3], studies showing the true tuneability of DESs as extraction solvents are very limited. In our previous study, we demonstrated the tuneability of DESs as designer solvents for selective and efficient extraction of bioactive natural products. Using *Flos sophorae* as a model system, we demonstrated that flavonoids could be efficiently extracted using a tailored DES that was newly synthesized by combining two effective DES components, glycerol and L-proline.

In this study, we tested a hypothesis that our previous strategy was generally applicable to a wide variety of classes of bioactive natural products. The previous study was focused on the extraction of one common class of natural products, flavonoids, which are relatively nonpolar. Applicability to a completely different class of natural products would support the expandability of our previous strategy. For this purpose, we employed ginseng as a model system, which is one of the most popular and valuable traditional

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herbal medicines. We designed a DES with the highest extraction efficiencies for the major bioactive compounds in ginseng, called ginsenosides, which are saponin-based and exist in a wide range of polarities.

Ginseng is known to possess many active components with useful effects including anti-aging, anti-diabetic, anti-tumor, and tranquilizing activities, most of which are attributable to ginsenosides [11–14]. The composition of ginsenosides varies depending on the species, processing method, growth environment, etc. [15] In this study, white Korean ginseng (*Panax ginseng* C.A. Meyer) was selected for experiments due to its easy procurement and because it is reported to contain mostly ginsenosides that are relatively polar such Rg_1, Re, Rf, Rb_1, Rc, and Rb_2 [16–23]. A large number of methods have been reported for the extraction of ginsenosides from white ginseng [24–32]. In general, organic solvents such as methanol, ethanol, and their aqueous solutions have been commonly used in combination with various extraction methods including heat reflux extraction (HRE), pressurized liquid extraction (PLE), ultrasound-assisted extraction (UAE), and supercritical fluid extraction (SFE). Although some of these reported methods have displayed high extraction efficiencies for ginsenosides, these methods generally require large quantities of organic solvents, long extraction times, and large amounts of energy.

The aim of this study was to tailor DESs and optimize extraction conditions to maximize ginsenoside extraction efficiencies.

2. Materials and methods

2.1. Chemicals, reagents, and equipment

Compounds used for DES preparation included choline chloride (≥98.0%), glycerol (≥99.5%), L-proline (≥99.0%), xylitol (≥99.0%), citric acid (≥99.5%), adonitol (≥99.0%), betaine (≥99.0%), D-(+)-galactose (≥99.0%), D-(−)-fructose (≥99.0%), D-(+)-glucose (≥99.5%), DL-malic acid (≥99.0%), and sucrose (≥99.5%), all of which were obtained from Sigma–Aldrich (St. Louis, MO, USA). Analytical standards of ginsenosides including Rg_1, Re, Rf, Rb_1, Rd, and Rc (for chemical structures, see Supplementary Fig. S1), all of which had a purity of 95% or higher based on HPLC, and finely pulverized white ginseng powder (diameter < 355 μm) produced from authenticated 6-year old *Panax ginseng* C.A. Meyer, were kindly provided by Prof. Jeong Hill Park (College of Pharmacy, Seoul National University, Seoul, Korea). HPLC-grade acetonitrile, water and methanol were purchased from J.T. Baker (Phillipsburg, NJ, USA). 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and formic acid (≥98.0%) were obtained from Sigma–Aldrich. Doubly deionized water was prepared using a Milli-Q water purification system (Millipore, Bedford, MA, USA). Analytical-grade ethanol and pH 0–14 universal pH-indicator strips were purchased from Merck KGaA (Darmstadt, Germany). SPE cartridges (OASIS HLB 6 cc, 200 mg) were purchased from Waters (Milford, MA, USA).

Centrifuges, models 1580MGR and Gyrospin, were obtained from Gyrozen (Incheon, Korea), while the Eppendorf 1524 was from Eppendorf (Hamburg, Germany). An ultrasonic bath (Powersonic 410) and a freeze dryer (model FD8508) were obtained from Hwashin Technology (Seoul, Korea) and Ilshin Biobase (Yangju, Korea), respectively.

2.2. Preparation of analytical standard solutions

Each ginsenoside stock solution of Rg_1, Re, Rf, Rb_1, and Rc was prepared in methanol at 1 mg mL^{-1} and stored at −20 °C. Standard working solutions were prepared by diluting the stock solutions with water and were used for analytical method validation including linearity and assay precision and accuracy.

2.3. Procedures for DES preparation

DESs were synthesized using a freeze-drying method as previously described [7]. Water added in the mixture was then removed by lyophilization for 24 h or longer until the mixture reached a constant weight.

2.4. LC–UV analysis for the quantification of extracted ginsenosides

Liquid chromatography coupled to ultraviolet detection (LC-UV) was performed using a PerkinElmer LC system (Norwalk, CT, USA) equipped with a PerkinElmer interface 600 series link, a quaternary pump (series 200), an auto-sampler (series 200), and a UV-visible detector (series 200). TotalChrom Workstation software was used for system operation and data management. The detection wavelength was 203 nm. Standard compounds and extracts were chromatographed on a Waters Xbridge phenyl column (5 μm, 4.6 mm × 150 mm) from Waters (Milford, MA, USA) at a flow rate of 1.0 mL min^{-1} at 25 °C. The mobile phase consisted of water (A) and acetonitrile (B), and the binary linear gradient elution was as follows: 0–20 min, 20–22% B; 20–23 min, 22–28% B; 23–45 min, 28–35% B; 45–55 min, maintained at 35% B for 10 min. The system was returned to the initial conditions within 1 min and equilibrated for 20 min before subsequent injections. The standards and extracts diluted in water were filtered through a 0.45 μm membrane filter (Whatman, Piscataway, NJ, USA) prior to injection. The established LC-UV method was validated in terms of linearity, precision, and accuracy, and the validation results are summarized in Supplementary Table S1. The calibration curve for each ginsenoside was plotted as peak area versus concentration of each ginsenoside standard. Assay precisions and accuracies were determined using quality control (QC) samples prepared at three different concentrations (low, middle, and high) for intra-day ($n = 3$) and inter-day ($n = 3 \times 3$) assays within one day and on three separate days, respectively.

2.5. UHPLC–Q-TOF–MS analysis for the qualitative analysis of extracted ginsenosides

An Acquity UPLC system (Waters Co., Milford, MA, USA) was composed of a binary solvent delivery system and a cooling autosampler maintained at 4 °C. Ginsenoside standards and ginseng extracts were chromatographed on an Acquity UPLC BEH C18 column (50 mm × 2.1 mm, 1.7 μm) from Waters at a flow-rate of 0.35 mL min^{-1} at 40 °C. A linear gradient system was employed for elution using a mobile phase consisting of (A) 0.1% formic acid in water and (B) 0.1% formic acid in acetonitrile: 0–20 min, 10–90% B; 20–21 min, 90–100% B, followed by washing with 100% B for 1 min. The extract was diluted with water to produce a four-fold dilution of the original extract and was filtered through a 0.2 μm membrane filter (Whatman) prior to injection.

Mass spectrometric analysis was conducted using a Waters Acquity Xevo G2 Q-TOF tandem mass spectrometer (Waters Co., Manchester, UK) equipped with an electrospray ionization interface in positive and negative ion mode, which was controlled by Masslynx software (version 4.1, Waters Co., Milford, MA, USA). The experimental conditions were described elsewhere [7]. Peak identification of the ginseng extract was performed based on accurate mass measurements in comparison to standard compounds and values reported in the literature [23,33–35].

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