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Betain and Alcohol-based Deep Eutectic Solvents for Vitexin Extraction from Binahong (*Anredera cordifolia*) Leaves

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Deep eutectic solvents (DESs) consisting of betaine as the hydrogen donor acceptor and a propanediol or a butanediol as the hydrogen bonding donor were used to extract vitexin from the leaves of *Anredera cordifolia* (Ten.) Steenis, known in Indonesia as binahong used traditionally to treat wounds. The vitexin extraction yields depend strongly on the molecular structure of the hydrogen bonding donor used and the betaine to alcohol molar ratio. The highest extraction yield of 0.024%, w/w vitexin in binahong leaves, was obtained at room temperature using the mixture of betaine and 1,3-propanediol in 1:4 mole ratio. For each set of DESs having a common HBD, the highest extraction yield was consistently obtained using the DES having betaine to alcohol molar ratio of 1:4. A higher extraction yield of 0.042% was obtained as the extraction temperature was increased to 40 °C. This study shows that DESs made of betaine and polyalcohol are prospective green solvents for extraction of bioactives such as vitexin from plants.

Keywords: deep eutectic solvent, extraction, betain, vitexin, binahong leaves, *Anredera cordifolia*

INTRODUCTION

The most commonly used solvents in the extraction processes are conventional organic solvents, unfortunately, most of these solvents have high volatilities and possess hazardous properties on human health. Organic solvents could affect nerve system, reproductive system, liver and kidney, as well as, induce cancers (Baker 1994). Furthermore, many organic solvents are not environmentally friendly as they are highly volatile. Much research effort has been done to develop a new class of green solvent for extraction, such as ionic liquids (ILs). ILs have unique characteristics, such as negligible volatility at room temperature, adjustable physicochemical properties, phase behavior that enable them to dissolve various substances, as well as selectivities that can be adapted for extraction and separation (Earle & Seddon, 2000). However, the use of ILs as solvents in the extraction of bioactives up to the present is still quite limited due to the toxicities and high synthesis cost of ILs.

More recently, another development effort that has gained significant attention is the development of deep eutectic solvents (DESs) having similar physical characteristics to ILs. DESs consist of a solid organic salt such as choline chloride and one or more organic compounds such as polyalcohols, acting as the hydrogen bonding acceptor (HBA) and the hydrogen bonding donor (HBD), respectively. The intermolecular hydrogen bond formed between the HBA and the HBD present in an appropriate composition, stabilizes the resulting liquid eutectic mixture, having a melting point lower than those of its constituents. In this regard, DESs have been regarded as designer solvent because their physicochemical properties can be adjusted for optimum extraction by selecting the right HBA-HBD combination and composition. Like ILs, DESs have negligible vapor pressure, so that they can be reused after extraction to reduce pollution and costs. DESs consist of primary metabolites of living cells or natural products (Dai et al. 2013a) are preferable in the extraction of bioactive compounds because they belong to the generally recognized as safe (GRAS) category.

A herbal plant that is currently popular in terms of utilization is binahong (*Anredera cordifolia*). The flavonoids contained in the leaves of *A. cordifolia* are known to possess antioxidant and antibacterial activities to cure or prevent various diseases, including cancer, shortness of breath, stroke, and gout (He et al. 2016). One of the flavonoids contained in the leaves of binahong is vitexin (8-glucopyranosyl-4'5'7-trihydroxy-flavone) that can be used as an antidepressant drug (Can et al. 2013).

The focus of the present study is the effect of the polyalcohol used as the HBD and extraction temperature on the extraction yield of vitexin. To see the effect of molecular structure of the HBD used for extraction yields, four polyalcohols were investigated: 1,2-propanediol, 1,3-propanediol, 1,2-butanediol, and 1,3-butanediol.

MATERIAL AND METHODS

Chemicals and Plant Material

Betaine (98%), 1,2-propanediol (99%), 1,3-propanediol (99%), 1,2-butanediol (99%), 1,3-butanediol (99%), and Folin-Ciocalteau reagent were purchased from Sigma Aldrich (Singapore). HPLC grade acetonitrile and methanol were obtained from Smart Lab. The vitexin standard (>99.8%) was purchased from Aktin Chemical Inc., China.

Preparation of Binahong Leaves and DESs

Binahong leaves were sorted, cleaned, and dried in ambient air without exposure to sunlight for 7 days. The dried binahong leaves were ground and the resulting powder was segregated with a mesh filter and stored in an airtight container bottle protected from sunlight to increase its shelf-life. DESs was prepared by mixing accurately weighed betaine and polyalcohols in 1:2 to 1:5 mole ratios. The solid and liquid mixture was heated and stirred using a hotplate stirrer set at temperatures of 50 or 80 ˚C for 30-90 min until a stable clear DES liquid was obtained.

Extraction of Vitexin

Accurately weighed simplicia (0.2 g) was mixed with DES (2 g) in a test tube.

Extraction was carried out using in a thermo shaker set to stirring speed of 500 rpm at three temperatures 27, 40, and 55 ˚C for 240, 90, and 90 min, respectively. The resulting suspension was filtered using a filter paper to obtain a clear DES extract prior to quantitative analysis carried out in triplicates.

Quantification of Vitexin

The quantitative analysis of vitexin extracted into the DES phase was carried out using an HPLC (High Performance Liquid Chromatography) apparatus. The eluent used was a mixture of methanol, acetonitrile, and orthophosporic acid solution (0,1%-v/v at pH of 2.5) in a volume ratio of 20:20:60 vol-%. A calibration curve was prepared using a standard solution of vitexin. Prior to injection, each DES sample was diluted with ethanol and filtered using a 0.45 µm microfilter. The elution time for each sample was 11 min.

FTIR Analysis

The hydrogen bond formed between betaine and the polyalcohols was analyzed using a Fourier Transform Infra Red (FTIR) apparatus. Liquid samples (0.5 mL) were scanned in the wavenumbers range of 4000-400 cm^{-1} for 1-5 min. Prior to analysis, solid betaine samples (2 mg) and KBr (200 mg) were grind to obtain uniform particle size less than 2 µm in diameter. The mixed particles were then fed into a pelletizer to produce a transparent pellet suitable for FTIR analysis.

Viscosity and Nile Red Polar Parameter of DESs

The viscosity of the DESs was measured using a Cannon-Fenske Viscometer 200 and 350 suitable for 20-100 cSt and 100-500 cSt range, respectively. The polarity of the DESs was determined as the Nile red polar parameter calculated from the solvatochromic shift of the Nile red dye obtained using UV-visible spectrophotometry in the range of 400-700 nm (Ogihara et al. 2004).

RESULTS AND DISCUSSION

Table 1 gives the composition of DES used in this study. It can be seen that DES rich in HBD, having betaine to polyalcohol molar ratio of 1:4 or 1:5, required the least amount of time to become a stable homogenous liquid. In contrast, DES with the low polyalcohol to betaine molar ratio need much more time to become homogeneous, as long as 22 days for DES 4-3. This observation indicates the effect of the high melting point of betaine (301 ˚C). The eutectic point in DES can be achieved because of the interaction between the solid ammonium salt and the liquid polyalcohols forming a hydrogen bond.

Table 1. DESs used in this study.

Polyalcohol (HBD)	Betaine to HBD molar ratio	Code	Stirring (day)
1,2-propanediol	1:2	DES 1-2	10
	1:3	DES 1-3	3
	1:4	DES 1-4	1
1,3-propanediol	1:3	DES 2-3	7
	1:4	DES 2-4	1
	1:5	DES 2-5	1
1,2-butanediol	1:3	DES 3-3	17
	1:4	DES 3-4	2
	1:5	DES 3-5	1
1,3-butanediol	1:3	DES 4-3	22
	1:4	DES 4-4	4
	1:5	DES 4-5	1

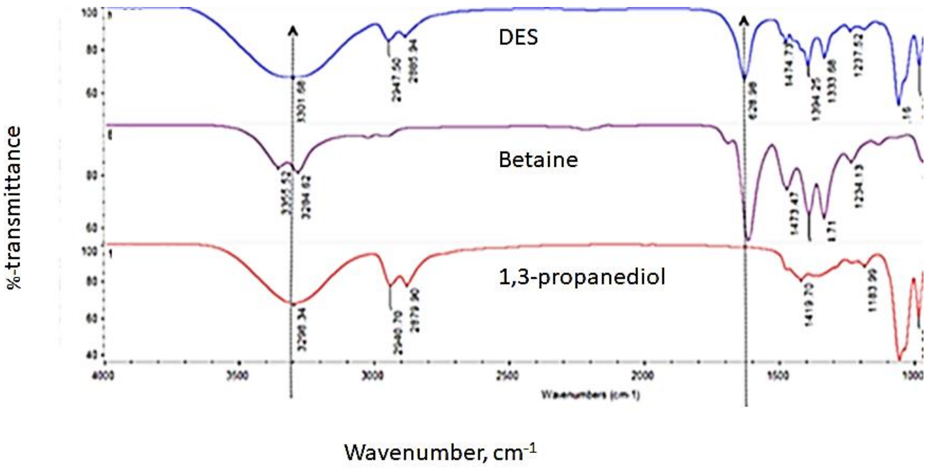

Fig. 1: FT-IR spectra of betaine, 1,3-propanediol, and their DES mixture

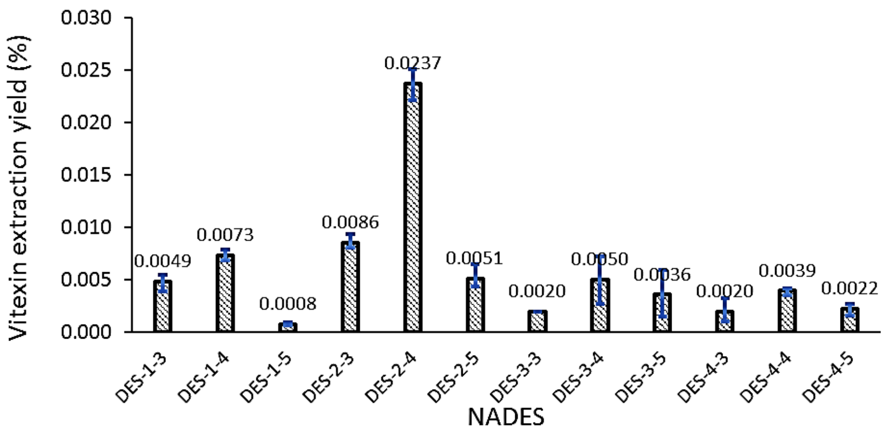

Fig. 2: Vitexin extraction yield in % (w/w) for each DES

To detect the presence of hydrogen bonds, FTIR spectra of betaine, 1,3-propanediol, and their mixture were obtained and shown in Figure 1. The peak at 3298 cm^{-1} attributed to the stretching vibration of the OH functional group of the 1,3-propanediol was shifted to 3302 cm^{-1} after formation of DES 2-5. The expected redshift to a lower wavenumber due to hydrogen bond formation in DES (Zhu et al. 2017) was not observed. Instead, the peak shifted slightly to a higher wavenumber of 3302 cm^{-1} that might be due the presence of the betaine's absorption peaks at 3355 and 3285 cm^{-1}. Meanwhile, the stretching vibration of the C=O functional group was also slightly shifted to a higher wavenumber, an indication that the carboxylate anion in betaine is interacting with the OH functional group of the polyalcohol.

The vitexin extraction yields were calculated as the percent ratio of the vitexin mass extracted into DES to the mass of binahong leaf powder, as in Eq. (1).

The data obtained after extraction at 27 ˚C for 4 h are shown in Figure 2. The highest yield of 0.024% was obtained using DES 2-

$$vitexin\ extraction\ yield\ (\%) = \frac{mass\ of\ extracted\ vitexin}{mass\ of\ binahong\ leaves\ powder}\ x\ 100 \qquad (1)$$

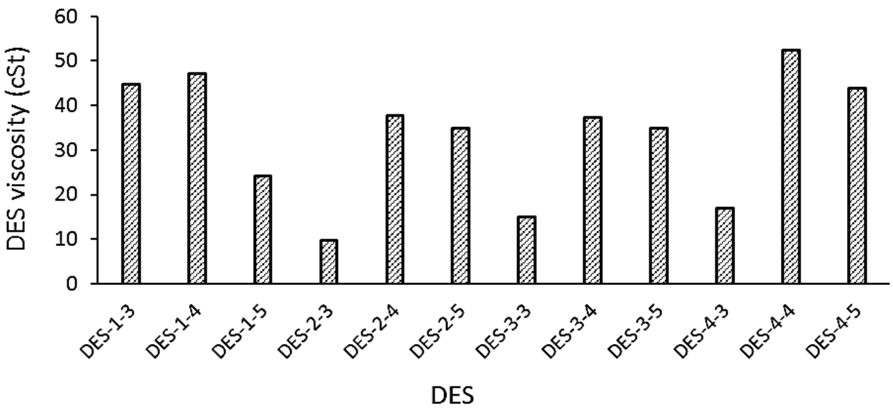

Fig. 3: Viscosity of DESs used for extraction

4 consisting of betaine and 1,3-propanediol in 1:4 molar ratio. It can be seen that for the propanediol-DESs, the position of the OH functional group in the alkane chain affects the extraction yield.

Higher yields were obtained if the OH functional groups of propanediol are in the position 1,3 rather than in position 1,2: DES 2-3 > DES 1-3; DES 2-4 > DES 1-4. A good HBD has a sufficient distance between the OH functional group and less branches (1,3-propanediol), as more branches (1,2-propanediol) will cause a steric hindrance that will inhibit the interaction of the bioactive compound with the carboxylate functional group COO^- of the betaine salt. It was also noted that regardless of the DES used, the highest extraction yields were always obtained using DES having betaine to polyalcohol molar ratio of 1:4, the molar ratio with the highest viscosity (Figure 3).

Figure 4 shows that yields increased significantly from 0.024 to 0.042% (w/w) as the extraction temperature was increased from 27 to 40 °C. At higher temperature, the viscosity of DES would decrease (Dai et al. 2013b), leading to higher diffusion and vitexin solubility in the solvent. However, further increase of extraction temperature further to 55 °C did not improve the extraction yield as vitexin would be decompose when exposed to high temperatures for an extended period of time.

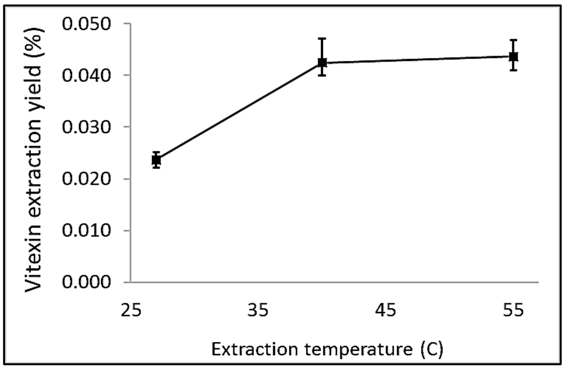

Fig. 4: Vitexin extraction yield as a function of extraction temperature (DES 2-4)

CONCLUSION

Vitexin from binahong leaves was successfully extracted at 27 °C using betaine-based DES with 1,3-propanediol as the hydrogen bonding donor in 1:4 molar ratio, afforded the highest vitexin extraction yield of 0.024%. The extraction yields depend strongly on the molecular structure of the hydrogen bonding donor and the betaine to alcohol molar ratio. For each set of DESs having a common HBD, the highest extraction yield was consistently obtained using the DES having betaine to alcohol molar ratio of 1:4. A significantly higher extraction yield of 0.042% was obtained by increasing the extraction temperature to 40 °C. This study shows that DESs made of betaine and polyalcohol are prospective green solvents for extraction of bioactives such as vitexin from plants.

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