1,2-Propanediol - Betaine as Green Solvent for Extracting α-Mangostin from the Rind of Mangosteen Fruit: Solvent Recovery and Physical Characteristics

K Mulia, Y Yoksandi, N Kurniawan, I F Pane and E A Krisanti*

Chemical Engineering Department, Faculty of Engineering, Universitas Indonesia, Depok, 16424, Indonesia

*E-mail: elsakm@che.ui.ac.id

Abstract. Deep Eutectic Solvents (DESs) are emerging as alternative green solvents. Unlike ionic liquids, DESs are more environmentally friendly and non-toxic, so they are more suitable to be used in pharmaceutical and food production processes. Few studies reported that DESs, which are liquid at room temperature, can be applied for extracting bioactive compounds from plants. In this study, DESs were formed by mixing betaine as the hydrogen bond acceptor with 1,2-propanediol as as the hydrogen bond donors. The aims of the study were to evaluate the extraction condition of mangostins from the mangosteen (Garcinia mangostana L.) rind using 1,2-propanediol-betaine DES and the recovery of solvent by separating the mangostin from DES after extraction. The extractions were conducted in a temperature range of 27-75 °C and the extracts were analyzed using high performance liquid chromatography. The highest α-mangostin extraction yield of 4.14 % (g/g) was obtained using 1,2-propanediol betaine DES at 55 °C. Back extraction of mangostins from DES using ethyl acetate can regain mangostins as much as 88.4% (g/g). The results showed the potential of 1,2-propanediol betaine DES as solvent for extraction of mangostin at mild condition with easy solvent recovery.

Keywords: Betaine, deep eutectic solvents, green solvent, mangostin, propanediol

1. Introduction

Organic solvents such as methanol, ethanol, chloroform and ethyl acetate are known as solvents for extracting bioactive compounds from plant. However, due to their toxicity and volatility these organic solvents are considered contributing the environmental pollution [1-3]. Green solvents that give minimum negative impact to the environment and human health are emerging become the focus of many researchers now days for replacing the conventional organic solvents. Previously, Ionic liquids (IL) received much attention as alternative green solvents, due to their low melting point, nearly complete non-volatility at ambient condition, chemical and thermal stability, non-flammability, high conductivity, and good solubilizing capacity of organic compounds [4,5]. However, the high toxicity of ionic liquid reduces its potential for pharmaceutical product application [6,7].

Deep eutectic solvents (DES), that have similar physical characteristic phase behavior as ionic liquids, have recently been investigated extensively. Deep eutectic solvents (DES) which are more environmentally friendly than ionic liquids, have negligible vapor pressure, with tailored polarity and selectivity have received more attention recently [8,9]. DES is a solvent composed of a quaternary ammonium salt as hydrogen bond acceptor (HBA) and urea, carboxylic acids, or polyols as hydrogen bond donor (HBD) compound. Intermolecular hydrogen bonds between HBA and HBD in certain composition resulting in a eutectic mixture, that has a lower melting point than that of each individual component and remains as liquid at very low temperature [10-12]. Diagrammatic representation of eutectic mixture of HBA and HBD is shown in figure 1.
Figure 1. Diagrammatic representation of eutectic mixture HBA-HBD.

Certain mixtures of primary metabolites found in many kinds of organisms formed kind of deep eutectic solvents, which was called Natural Deep Eutectic Solvent (NADES). These solvents are present in nature and play a role in cellular processes of living organism [5,6]. Being non-toxic and environmentally friendly solvent make NADES suitable as potential solvents for various applications in food, cosmetic and pharmaceutical industries. In general, DES and NADES have similar physicochemical properties, such as strong ability to dissolve protic molecules, low vapor pressure, and miscibility with water [13].

In the present research, DES were prepared by mixing betaine (trimethyglycine) as HBA with 1,2-propanediol as HBD, to extract α-mangostin from the rind of mangosteen fruit (Garcinia mangostana L.). Mangosteen is a tropical fruit tree grows in Southeast Asia and its fruit rind has been used widely as traditional medicine. Some studies reported that the fruit rind contains secondary metabolites, i.e. oxygenated and prenylated xanthones [14]. Xanthones is reported to have antioxidant, antitumoral, anti-inflammatory, antiallery, antibacterial, antifungal and antiviral activities [15,16,17]. The most abundant xanthone in the mangosteen rind known as α-mangostin. The molecular structure of α-mangostin is shown in figure 2. Other xanthones isolated in significant amount from the mangosteen rind are β-mangostin, γ-mangostin, gartanin and 8-deoxygartanin [18].

Figure 2. Some of xanthones from mangosteen rind

The objectives of this study were to evaluate the optimum condition to extract α-mangostin from the dried mangosteen rind powder using 1,2-propanediol - betaine DES. This type of polyol was used as an HBD since their DES showed better extraction yields compared to DES of acids or sugars [19]. The physical characteristics of DES were also determined as to study its effect on the extraction yield. The recovery of DES after the extraction in order to separate the valuable α-mangostin from DES was also studied. The results will be important information for assessing whether 1,2-propanediol-betaine DES could be considered as a green alternative solvent that are sustainable, and rapidly extracting xanthones from the plant.
2. Experimental

2.1. Chemical and Materials
Betaine anhydrous (>98%), and 1,2-propanediol (99%), and HPLC grade acetonitrile and ethanol were purchased from Sigma Aldrich. Pure standard α-mangostin (>99.8%) was purchased from Aktin Chemical Inc. China. Mangosteen fruit was bought in Solo, Central Java, and had been identified as *Garcinia mangostana* L. by the Bogoriense Herbarium, Research Center for Biology, Indonesian Institute of Sciences (LIPI).

2.2. Preparation of Mangosteen Rind Powder
Dried mangosteen rind were chunked, crushed into powder using an electric grinder. The powder was dried using an oven (65 °C, 40 minutes) to reduce the water content. The dried powder was screened through a 20-mesh sieve to obtain a uniform size powder and kept in a sealed container to avoid air and direct sunlight.

2.3. Preparation of 1,2-Propanediol Betaine DES
DES was made up by mixing 1,2-propanediol in 1:3 molar ratio to betaine in a capped glass bottle, to prevent the entry of air that can degrade the compounds, heated to 50 °C, and agitated at 500 rpm for 60 minutes. The agitation at this condition was repeated several times a day. The viscosity of DES was measured using a Cannon-Fenske Viscometer at 40 °C. The thermal behavior of 1,2-propanediol betaine DES was also studied using Differential Scanning Calorimetry (DSC) and Thermogravimetry Analysis (TGA). The polarity of DES was determined using solvatochromic Nile Red as a probe to measure the absorbance of DES at 400-700 nm UV-VIS spectrometer. The wavelength of maximum absorbance is used to calculate the polarity that is represented by $E_{\text{NR}}$ [20,21].

2.4. Extraction of α-Mangostin from Mangosteen Rind Powder
The extraction of α-mangostin was conducted by mixing powder of mangosteen rind with DES in a weight ratio of 1:10 in a capped tube. The extraction was conducted in thermosthaker in one to four hours with an agitation speed of 500 rpm [19]. The two phases were then centrifuged for 15 minutes at 2000 rpm, and the residue was separated using a 0.45μm filter to obtain the supernatant. The experiments were conducted triple and similar experiment using ethanol as solvent at the same extraction condition was also tested for comparison.

2.4.1. Quantitative analysis of α-mangostin in DES. The mangostin extracts were analyzed using High Performance Liquid Chromatography (HPLC) operated under similar condition as reported by [22]. The instrument used was Shimadzu LC-20AD Prominance UFLC. The column used was C18 with 250 mm in length and 4.6 mm diameter. The elution was carried out by isocratic elution system at a total flow rate of 1 mL min$^{-1}$ at room temperature (25-28 °C). The mobile phase consisted of equivalent 0.1% (v/v) ortho-phosphoric acid and acetonitrile. The mobile phase was filtered through a 0.45 μm filter with a vacuum filter. Sample injection volume was 0.1 mL with UV-Vis detector wavelength set at 244 and 320 nm. Before injected, the sample was first diluted with methanol with uniform dilution factor for all samples, and then filtered through a 0.45 μm membrane.

2.4.2. Effect of extraction temperature. Similar procedure for extraction of α-mangostin was conducted for studying the effect of temperature. Series of experiment using temperatures of 27, 37, 55, 65, and 75 °C were conducted in four-hour extraction.

2.4.3. Recovery of DES and α-mangostin. The supernatant contained DES and α-Mangostin was separated via back extraction using ethyl acetate with volume ratio of 1:1, triple. Mixture of DES with ethyl acetate was stirred in one hour and then separated. The upper part, the ethyl acetate fraction, was collected and analyzed using HPLC to determine the α-mangostin content and yield of recovery of α-mangostin from DES.
3. Results and Discussion
Preparation of DES by mixing betaine powder and 1,2-propanediol liquid took about two days, by stirring the mixture for one hour three times a day. The stable mixture is a colorless viscous solution. The schematic diagrammatic representation of the DES formation of 1,2-propanediol and betaine was shown in figure 3.

![Diagrammatic representation of 1,2-propanediol - betaine DES](image)

**Figure 3.** Diagrammatic representation of 1,2-propanediol - betaine DES

The physical properties determined are shown in table 1. The viscosity of DES was much higher than ethanol. The high viscosity of DES might be caused by the extensive hydrogen bonds network formed intermolecularly between HBA and HBD molecules, and in lesser extent from van der Waals and electrostatic interactions, that inhibiting the free movement of the components within DES [23,13].

| Solvent                  | Polarity ENR (kcal/mol) | T boiling/ decomposition (°C) | Viscosity (cP) |
|-------------------------|-------------------------|------------------------------|----------------|
| DES (Betaine-1,2-propanediol) | 50.16                   | 189.8                        | 37.09          |
| Ethanol                 | 52.17                   | 78.2a                        | 1.07b          |

The polarity of DES as well as ethanol was measured and the values are not quite different. It seems the extraction yield is significantly affected by viscosity when the polarity of DES is slightly polar. The DSC/TGA assays showed the data of vaporization temperature at 189.8 and 298.4 °C which are belong to 1,2-propanediol and betaine, respectively. The data is closed to the boiling point of pure 1,2-propanediol at 187.8 °C and decomposition point of betaine at 293 °C reported by Lide [25]. Below those temperatures, no peaks present in the spectra, which means no vaporization or decomposition occur. The results indicated that DES of 1,2-propanediol and betaine is a stable eutectic mixture that can be used as solvent in the extraction at temperature higher than room temperature, as long as it is still lower than its vaporization/decomposition temperature. Compare to ethanol, the extraction cannot be performed at higher temperature since ethanol is a volatile solvent as shown in table1.

3.1. Extraction of α-Mangostin from the Rind Powder
The extraction of α-mangostin was conducted in different time periods and temperatures. Ethanol was also used in the extraction for comparison.

3.1.1. Time of extraction. The extractions were conducted at 1, 2, 3, and 4 hours-periods at 27 °C. The results showed in Figure 4 indicated the α-mangostin extracted from the simplicia was higher in the ethanol than in 1,2-propanediol betaine DES. The extraction yield using ethanol after four hours was 3.3%, while it was 2% using 1,2-propanediol betaine DES. This has been predicted due to the more viscous of DES than ethanol. When the viscosity is high, the movement of the components in the solvent is reduced as well as the penetration ability to penetrate the matrix of mangosteen rind powder.
Figure 4. The $\alpha$-mangostin extracted from the rind of mangosteen at various times of extraction

The $\alpha$-mangostin extracted increased with longer time of extraction, however around three to four hours the % extracted leveled off. This result indicated that in one-step extraction around four hours, the extraction reached an equilibrium state. Hence, other extractions at different temperatures were conducted in four hours.

3.1.2. Temperature of extraction. The extractions were performed in four hours at 27 up to 75 °C as shown in Figure 5. The viscosity of DES decreased with increasing extraction temperature. The movement of molecules within DES is higher when the viscosity is low. However, when the temperature was increased more than 55 °C, yield of extraction lowered. It seems that when the temperature is higher than 55 °C, the movement of DES molecules to penetrate the matrix become easier, but also might break the hydrogen bonds formed between the $\alpha$-mangostin with DES. The results in Figure 5 shows the optimum temperature for extracting $\alpha$-mangostin from the powder of mangosteen rind.

The extraction of $\alpha$-mangostin using 1,2-propanediol betaine DES increased from 2% to 4.1% when the temperature increased from 27 to 55 °C. Thus, by increasing the extraction temperature to 55 °C, the extraction yield using DES can surpass the extraction yield using ethanol in one-step extraction. This result indicated that when DES is used as an extraction solvent, the temperature can be set at a higher temperature and the yield will increase significantly. This can be done since the mixture of 1,2-propanediol and betaine has higher vaporization/decomposition temperature than conventional organic solvent, ethanol (table 1).

Figure 5. The $\alpha$-mangostin extracted as a function of extraction temperature in four hours.
3.2. Recovery of DES and α-mangostin

The recovery of DES from the extract phase was studied in order to have a sustainable green solvent. Using ethyl acetate in three-step back extraction of used DES, the α-mangostin recovered from the DES reached 88.4 % (w/w). This data indicates that DES can be recovered and to be used repeatedly to extract α-mangostin from the rind powder of mangosteen. Other methods of recovery of DES were in progress in order to use more environmentally friendly techniques.

4. Conclusions

DES that prepared from 1,2-propanediol and betaine in a molar ratio of 3:1 could extract α-mangostin from the rind of mangosteen fruit as much as 4% (w/w) at 55 °C in four-hour one-step extraction. Viscosity and temperature seemed to influence the formation of hydrogen bonds between the α-mangostin and the DES, as well as between the components within DES itself, which are marked by the value of extraction yield in various temperature and time of extraction. The back extraction of used DES using ethyl acetate can recover the α-mangostin up to 88.4% (w/w). The results indicate the potential of 1,2-propanediol betaine DES as a sustainable green solvent that can extract active compounds from plant.

Acknowledgments
The authors gratefully thank the DRPM of the Universitas Indonesia through PITTA project No. 771/UN2.R3.1/HKP.05.00/2017.

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