International Journal of Technology (2015) 7: 1211-1220
ISSN 2086-9614

SELECTED NATURAL DEEP EUTECTIC SOLVENTS FOR THE EXTRACTION OF α-MANGOSTIN FROM MANGOSTEEN (*Garcinia mangostana* L.) PERICARP

Kamarza Mulia[1*], Elsa Krisanti[1], Felita Terahadi[1], Sylvania Putri[1]

[1] *Department of Chemical Engineering, Faculty of Engineering, Universitas Indonesia, Kampus UI Depok, Depok 16424, Indonesia*

(Received: June 2015 / Revised: September 2015 / Accepted: September 2015)

ABSTRACT

This research considers the application of Natural Deep Eutectic Solvents (NADES) as green solvents for the extraction of bioactive compounds, mainly α-mangostin, from the pericarp of mangosteen (*Garcinia mangostana* L.). Extractions were carried out using NADES consisting of choline chloride, a quarternary ammonium salt, and four hydrogen bond donors: 1,2-propanediol, citric acid, glycerol, and glucose. The highest α-mangostin extraction yield of 2.6 % (w/w) in dried pericarp was obtained using a mixture of choline chloride and 1,2-propanediol in 1:3 mole ratio. The presence of hydrogen bonding was indicated by the broadening of the OH peak in the infra-red spectra of the NADES used. The polarity and viscosity data of NADES were determined to describe the solubility of α-mangostin. The decomposition and glass transition temperatures were determined in order to study their thermal behavior and stability. The results of this study suggest that NADES made of choline chloride and diol-based hydrogen bond donors are effective for the extraction of bioactive compounds from the mangosteen pericarp.

Keywords: α-mangostin; Deep eutectic solvent; Extraction; Garcinia mangostana L.; NADES

1. INTRODUCTION

The large-scale usage of conventional organic solvents has been associated with a negative impact on environment with related health and safety issues. Although organic solvents are able to extract bioactive substances with high yields, these solvents have high volatility factor or a tendency to vaporize and some of them have toxicity properties that leave excess residue in the extracts (Dai et al., 2013a; Azmir et al., 2013). Green solvents are required urgently to replace hazardous organic solvents (Bi et al., 2013), resulting in what is known as green extraction processes (Chemat et al., 2012). Initially, ionic liquids (ILs) appeared to be green solvents that are non-hazardous and reduce waste because they have negligible vapor pressures (Earle & Seddon, 2000; Earle et al., 2006). Even though ILs are commonly used in various fields, synthetic ionic liquids are not yet used as solvents in the pharmaceutical industry. This is due to issues related to high toxicities and irritating properties of some ILs, and also to high synthesis costs of ILs in general (Dai et al., 2013b; Choi et al., 2011; Angell et al., 2012).

Deep eutectic solvents (DES) are a new type of solvent that have similar physical characteristics as ionic liquids. DES is a mixture of two or more compounds that consists of a hydrogen bond acceptor (HBA) and hydrogen bond donors (HBD) that form intermolecular hydrogen bonds with each other, having a lower melting point than those of the individual components. Most of

* Corresponding author's email: kmulia@che.ui.ac.id, Tel.+62-21-7863516, Fax. +62-21-7863515
Permalink/DOI: http://dx.doi.org/10.14716/ijtech.v6i7.1984

these solvents will be in liquid form at room temperature (Pena-Pereira & Namieśnik, 2014). Abbott et al. (2004) first reported that DES can be formed between quaternary ammonium salts, such as choline chloride with a range of amides, carboxylic acids and alcohols. Bi et al. (2013) reported that DES could be used to extract flavonoid compounds, such as myricetin and amentoflavone, from *Chamaecyparis obtuse*. As a solvent for extraction, DES can be reused after extraction due to its negligible volatility, so it is more environmentally friendly and this can reduce operating costs (Maugeri et al., 2012; Hayyan et al., 2013). The existence of Natural Deep Eutectic Solvent (NADES) was reported as being a mixture of various cellular constituents (primary metabolites) from all kinds of organisms. Therefore, NADES is DES that contain natural components that are abundant, having diverse chemical properties, biodegradability and low toxicity (Dai et al., 2013a; Dai et al., 2013b).

Mangosteen (*Garcinia mangostana* L.) is a tropical tree originating from Indonesia that is widely used in traditional medicine. Phytochemical studies have shown that mangosteen pericarp contain secondary metabolites, such as oxygenated and prenylated xanthones. These xanthones are reported to have antioxidant, antitumoral, anti-inflammatory, antiallergy, antibacterial, antifungal and antiviral activities (Akao et al., 2008; Yu et al., 2007; Kondo et al., 2009). Many xanthones have been isolated from mangosteen pericarp, α-mangostin shown in Figure 1 being the most abundant. Other xanthones isolated from mangosteen pericarp in significant amounts are β-mangostin, γ-mangostin, gartanin and 8-deoxygartanin (Pedraza-Chaverri et al., 2008). It is reported that a potent anti-proliferated activity of xanthones (α-mangostin, β-mangostin, γ-mangostin, and methoxy β-mangostin) from the pericarp of mangosteen is effective against human leukemia HL 60 cells, and colon cancer DLD-1 cells (Akao et al., 2008; Aisha et al., 2012a; Aisha et al., 2012b). The efficacy of mangostin could be improved when the active compound is administered in the form of a controlled release formulation, in which the bioactive compounds are released only in the targeted areas within the human body (Ahmad et al., 2012; Ahmad et al., 2013).

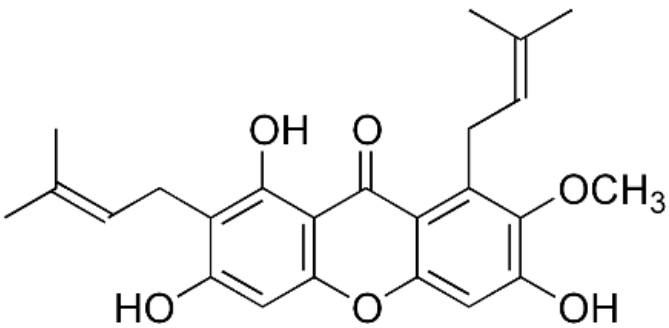

Figure 1 Molecular structure of α-mangostin

In this study, NADES is used for the extraction of the mangostins from the pericarp of mangosteen. The NADES used were mixtures of choline chloride (ChCl) and an organic acid (citric acid), a sugar (glucose), and two polyalcohols (glycerol and 1,2-propanediol). The extracts were obtained using a shaking method and analyzed using High Performance Liquid Chromatography (HPLC) following the procedure reported by Pothitirat (2009). In addition, ultrasonic-assisted extraction was also carried out for comparison. The physicochemical properties of NADES obtained in this study were polarity using Nile Red as the solvatochromic probe, viscosity using a Brookfield viscometer, infrared spectra using Fourier Transform Infrared (FT-IR) spectroscopy, and thermal behavior using Differential Scanning Calorimetry (DSC)

2. EXPERIMENTAL

2.1. Chemicals

Analytical grade choline chloride (> 98 %), 1,2-propanediol (> 99 %), glycerol (> 99 %), citric acid, d-(+)-glucose (> 99.5 %), and Nile Red were purchased from Sigma Aldrich. HPLC grade acetonitrile and ethanol were purchased from SmartLab and standard α-mangostin was purchased from Aktin Chemical Inc. (China).

2.2. Preparation of Raw Material

Mangosteen pericarp was separated and cleaned from its edible parts; the tough outer skin was peeled; and its inner part was cut and dried at room temperature. After drying, the dried material was ground using an electric grinder and the powder obtained was dried using an oven (65^{o}C, 40 min) to reduce the water content. The dried powder that passed through a 20 mesh sieve was stored in a sealed container to avoid contact with air and exposure to direct sunlight.

2.3. Preparation of NADES

NADES used in this study are mixtures of choline chloride and a HBD having a certain mole ratio as listed in Table . These NADES were prepared by heating mixtures that consists of solid-liquid and solid-solid compounds at 50 and 80^{o}C under constant stirring, respectively. Stirring was continued for a period from 30–90 min until a clear solution was formed (Dai et al., 2013b).

Table 1 NADES used in this study

Code	HBA	HBD	HBA:HBD Mole Ratio
DES-1	choline chloride (ChCl)	1,2-propanediol	1:1
DES-2		1,2-propanediol	1:2
DES-3		1,2-propanediol	1:3
DES-4		citric acid	1:1
DES-5		citric acid	2:1
DES-6		glycerol	1:1
DES-7		glycerol	3:2
DES-8		glucose	1:1
DES-9		glucose	5:2

2.4. FT-IR Analysis

FT-IR spectroscopy analysis was performed to analyze the presence of molecular interactions between choline chloride and HBD. Perkin Elmer Spectrum 1000 FT-IR Spectrophotometer was used in this study. Aliquots of 0.5 mL liquid samples were scanned in the wavelength range of 4000-400 cm^{-1}.

2.5. NADES Physicochemical Properties Tests

Physicochemical properties of NADES were studied by performing polarity, viscosity and calorimetry tests. The polarity of NADES were determined following the procedure reported by Reichardt (1994) and Ogihara et al. (2004). Nile Red dye was used as the solvatochromic probe and the change of color was detected by a UV-Vis Spectrometer in the wavelength range of 400–700 nm to determine λ_{max} in nm. The solvent polarity E_{NR} was calculated using the formula shown in Equation 1:

$$E_{NR}\left(\frac{kcal}{mole}\right) = \frac{h_c.N_A}{\lambda_{max}} = \frac{28,591}{\lambda_{max}} \quad (1)$$

The viscosity test was performed using a Brookfield viscometer at room temperature with LV spindle no.1 at 6, 12, and 30 rpm. DSC analysis was performed at the National Nuclear Energy Agency of Indonesia and recorded at temperature range of 50 to 400°C at a rate of 20°C/min.

2.6 Extraction of α-mangostin with NADES

The extraction of α-mangostin using NADES was carried out by mixing aliquots of 0.2 g mangosteen pericarp powder and 2 g NADES in a sealed extraction tube. The extraction was carried out at room temperature by shaking the tubes at 65 shake/min. The suspension was then centrifuged for 15 min at 3000 rpm and filtrated through a filter paper to obtain the supernatant. The percentage of α-mangostin extracted was determined using HPLC several times in 24 h to find the optimum extraction time. This extraction procedure was repeated for the NADES tested in triplicate.

2.7 HPLC Analysis

HPLC analysis was performed using the HPLC Shimadzu Prominence UFLC system equipped with LC-20AD pump, SPD-20A 230 V UV-Vis detector, Rheodyne injector with a 20 µL loop, and a C-18 column (250 mm long and 4.6 mm diameter). The sample injection volume was 8 µL with and the isocratic elution was used at a flow rate of 1 mL/min at room temperature. The mobile phase consisted of 0.1 % (v/v) orthophosphoric acid and acetonitrile. The mobile phase was filtered through a 0.45 µm filter with a vacuum filter and sonicated before use. UV-vis detector wavelength was set at 244 and 320 nm. Prior to injection, the sample was first diluted with methanol with uniform dilution factor for all samples, and then filtered through a 0.45 µm membrane (Sartorius filter syringe, NY).

3. RESULTS AND DISCUSSION

3.1. NADES Structure Analysis with FT-IR

In order to detect conformational changes that occur due to the presence of hydrogen bonds formed between the anion of choline chloride with the hydroxyl groups of the HBD depicted in Figure 2, the FT-IR spectra of pure compounds as well as NADES were obtained and analyzed. Figure 3 shows the IR spectrum of ChCl showing a strong and wide hydroxyl (OH) peak in 3650–3200 cm^{-1} range. In addition, there are also peaks indicating the presence of a specific group of quaternary ammonium compounds, in the range of 900–980 cm^{-1}. The absorption peak which shown at the wavelength of 953.7 cm^{-1} is suspected to be the peak of the C-N group of choline chloride. Generally, changes in the structure can be seen from the widening or narrowing of the peaks involving OH groups, the shift of C-H and C=O peak of acids, or loss of the representative group peak of pure substances.

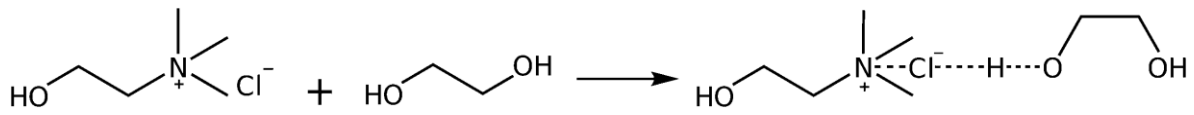

Figure 2 Hydrogen bonding between the anion of choline chloride and the hydroxyl group of an HBD

Figure 3 also shows that the absorption peak of the hydroxyl groups of the two NADES consists of choline chloride and 1,2-propanediol in a molar ratio of 1:2 and 1:3. Further examination of the three spectra revealed that the peak of the OH group in 1,2-propanediol at 3357 cm^{-1} is shifting and widening. This is due to the formation of hydrogen bonds in NADES. At a molar

ratio of 1:3, the alcohol group peaks became stronger and wider due to the increasing number of hydrogen bonds formed. Similar phenomena were found in ChCl: citric acid (1:1) NADES and ChCl: glycerol (1:1) NADES. The OH group peak of choline chloride was present, but shifted in NADES. In the 1,2-propanediol IR spectrum, the absorption of the carbon-hydrogen (CH) sp3 group can also be seen in the 2960 to 2850 cm^{-1} range. In the ChCl:1,2-propanediol NADES, this CH peak is more clearly visible for the 1:3 molar ratio mixture. This change is also due to the effect of the formation of hydrogen bonding that occurs in NADES.

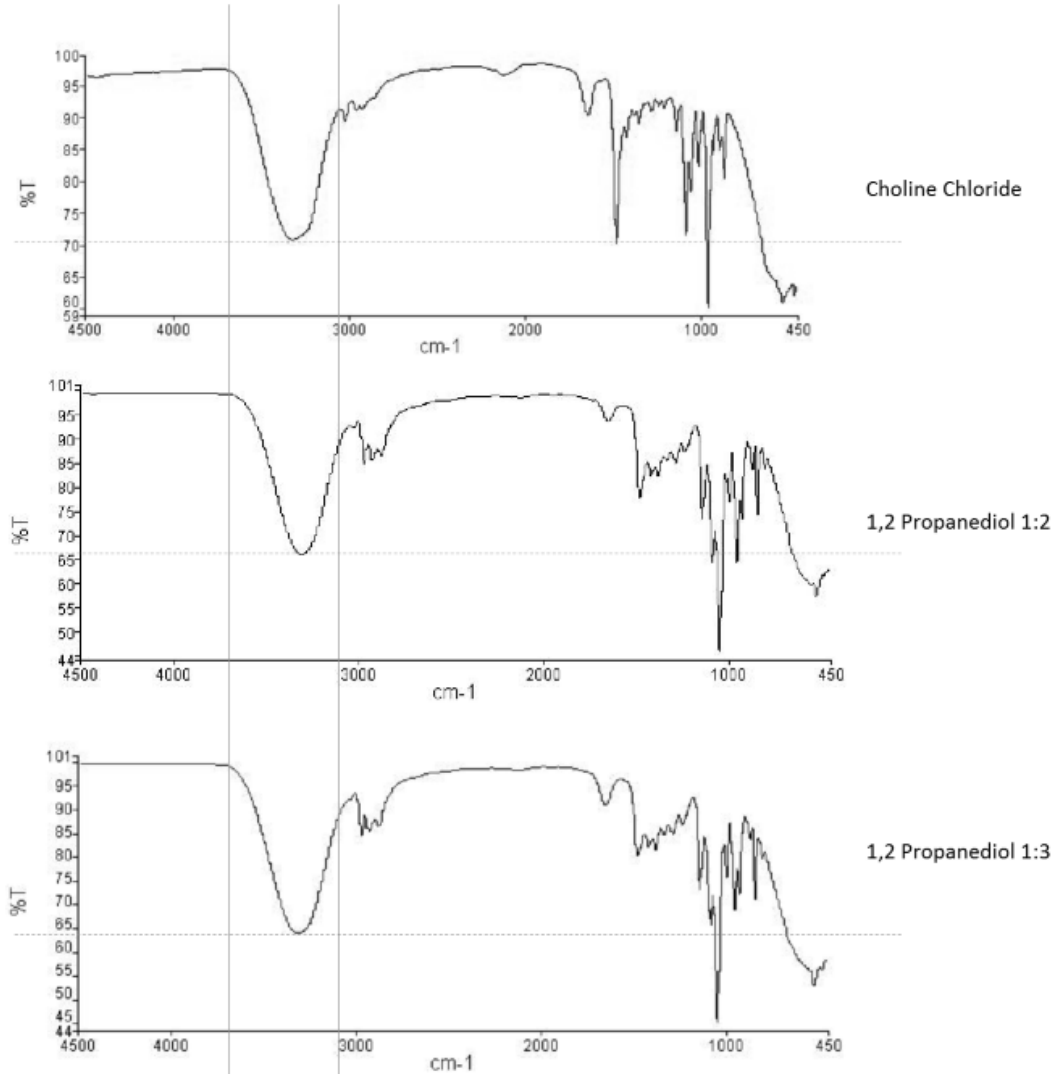

Figure 3 FT-IR spectra of choline chloride, ChCl:1,2-propanediol (1:2), and ChCl:1,2-propanediol (1:3)

3.2. Physical Properties of NADES

Physical properties of NADES were determined to see their effect on the NADES preparation and on the extraction yields. Polarity and viscosity are common parameters used to describe the effect of solvents on the solubility of the solute, while the glass transition temperature (T_g) and decomposition temperature (T_d) were determined to study thermal behavior and the stability of NADES within a certain temperature range. The test results are summarized in Table 2.

The formation of the colored Nile Red - NADES complex took some time, because of the viscous nature of NADES. Higher solvent polarity parameter (E_{NR}) values indicate a less polar compound. It is noted that the NADES used in this study have slightly different polarities, in the range of the polarity of water and methanol having E_{NR} values of 48.21 and 51.89 kcal/mole, respectively (Dai et al., 2013b). In E_{NR} scale, the order of polarity is water ~ DES-

4/5 ~ DES-8/9 > ethanol > DES-6/7 > DES-1/2/3. NADES with 1,2- propanediol and glycerol as HBD are found to be less polar than ethanol. NADES having citric acid and glucose as HBD are more polar than ethanol, having similar E_{NR} to water.

Table 2 Physical properties of NADES and ethanol

Code	Viscosity (cP)	T_g (^oC)	T_d (^oC)	E_{NR}* (kcal/mole)
DES-1	37.10	152	322.85	56.50
DES-2	35.20			56.73
DES-3	31.60			56.62
DES-4	82.60	110	287.36	48.05
DES-5	77.50			47.65
DES-6	46.80	290	-	55.62
DES-7	38.80			55.52
DES-8	42.17	175	285.85	47.49
DES-9	27.83			48.71
Ethanol	1.07			51.61

* E_{NR} was calculated using Equation 1.

Table 2 also shows that the viscosities of NADES with polyalcohols (1,2-propanediol and glycerol) as HBD are lower than the viscosities of other HBD. Moreover, NADES that contains HBD with more OH- groups are more viscous, which might be due to the formation of hydrogen bonds with choline chloride as indicated by their E_{NR} values. The DSC analysis shows that NADES with citric acid and glucose as HBD have lower decomposition temperatures than those of NADES with 1,2-propanediol. The higher decomposition temperature of NADES with propanediol as HBD indicates that this NADES has higher thermal stability. The decomposition temperature of NADES in the range of 285-322°C, is affected by the stability of its HBD (Abbas, 2010). The DSC analysis also shows that choline chloride NADES with different HBD compounds have different glass temperatures.

3.3. Extraction of α-mangostin with Shaking Method

Extraction was carried out by shaking NADES at frequency of 65 shakes/min and at room temperature (~27^{o}C). Figure 4 shows the extraction yields obtained in 24 hours using NADES ChCl-1,2-propanediol (1:2) and ethanol as the reference solvent. After two hours, extraction with NADES showed a significant increase. After four hours, the α-mangostin concentration obtained from extraction began to stabilize. Based on this result, subsequent shaking time was set to 6 h.

The long extraction time is due to the viscous nature of NADES that make it difficult to break its intermolecular bonding to form new bonds with mangostin compounds. As a comparison, ethanol can extract mangostin faster than NADES ChCl-1,2- propanediol (1:2). This is because ethanol has a lower viscosity than those of NADES, therefore, with the kinetic energy resulted from the shaking, intermolecular bonding within ethanol active groups were more easily broken to form new bonds with mangostin active groups. Low viscosity solvents have higher diffusion coefficients so that the rate of extraction increases. The influence of viscosity (η) on the diffusion coefficient (D) is expressed by the Stokes-Einstein equation as shown in Equation 2:

$$D = \frac{k.T}{6.\Pi.\eta.r} \quad (2)$$

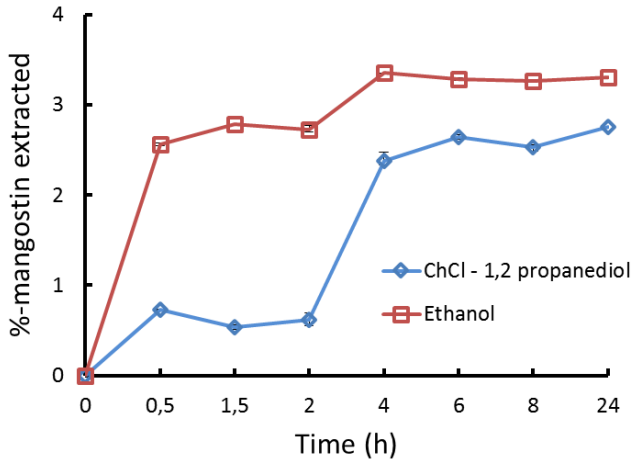

Figure 4 Effect of time on mangostin yield using ChCl-1,2-propanediol (1:2 mole ratio) as NADES and ethanol as reference solvent

where η is inversely proportional to D, k is the Boltzmann constant, and r is the radius of the solvent molecule. It can be inferred that NADES with a higher viscosity and a larger molecule than ethanol will have lower diffusivity values, and therefore, need more time to penetrate the plant matrix. The mangostin might be absorbed on the NADES by physical adsorption and chemical interactions, such as van der Waals' forces, dipole moment, and electrostatic interaction. Polarity might also play a role in extraction efficiency of mangostin.

3.4. Effects of Types and Compositions of NADES on Extraction Yields

Extraction using NADES listed in Table 2 was carried out by shaking at room temperature for 6 h and the results are shown in Figure 5. The data obtained clearly shows that mixtures of choline chloride in combination with 1,2-propanediol are able to extract α-mangostin in much higher quantities (up to 2.6 %) compared to the other DES containing citric acid, glycerol, and glucose (< 0.5 %). The highest α-mangostin extraction yield was obtained using a mixture of ChCl salt and 1,2-propanediol with a molar ratio of 1:3 (DES-3). One of the factors that influence the extraction yield is the polarity of the solvent used. In the polarity scale, DES-1 to DES-3 have the highest E_{NR} value or the least polar among the HBD tested, and therefore, most suitable for the extraction of the non-polar compounds such as mangostins. This is consistent with the theory of "like dissolves like", where a compound will be more easily dissolved by solvents with similar polarity as it has a similar intermolecular force.

Figure 5 also shows the decreasing amount of α-mangostin extracted as the 1,2-propanediol to ChCl ratios decreases from 3 to 1 in DES-3 to DES-1, respectively. This is because the addition of charge carried by ChCl in NADES mixture makes the cohesive energy between molecules in NADES become stronger due to the increasing number of Cl^- ions from ChCl. The intermolecular interactions in NADES molecules that have more ChCl than the amount of HBD tend to be stronger than in NADES with a balanced ratio between ChCl and HBD, so the solute experiences a higher degree of difficulty to break in the intermolecular bond between NADES molecules.

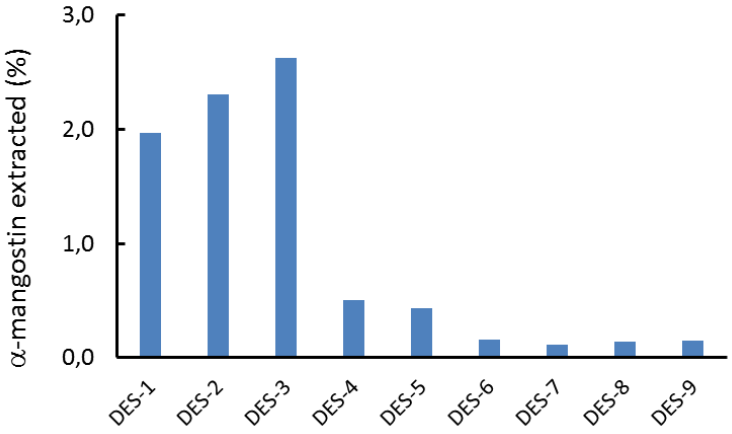

Figure 5 Effects of types and compositions of NADES on extraction result

Although polarity is usually the main factor affecting the extraction ability of a solvent, in this case, it is difficult to observe the extraction trend only in terms of polarity. The polarity results using the Nile Red test showed that almost all NADES tested have a molar transition energy value (E_{NR}) similar to each other. There is no direct relationship between polarity and extraction yield of mangostin. Thus, it can be assumed that in this case, polarity is not the only factor that influences the interaction between the NADES with α-mangostin from the mangosteen pericarp. The effect of viscosity might play an important role in influencing the extraction results. This is because the energy received by the matrix plant and NADES in the shaking method is too small, so it is not enough to help the active compounds in the samples to diffuse into the solvent. NADES with low viscosity tends to give better results either because the flow of active compounds through solvent system is not blocked or better solvent penetration occurs into the matrix plant. This could explain the high extraction results using NADES with 1,2-propanediol HBD.

Other factors to be considered are branches present in the alcohol hydrogen bond donors used. The alcohol HBD with more branches exerts more steric hindrance, affecting the interaction of mangostin with chloride ions of ChCl. Steric hindrance caused by the formation of a rigid conformation of NADES means it has a high surface tension so that a higher energy input is required for the active compound to interact with the chloride ions in NADES (Bi et al. 2013). This explains why NADES with glycerol (DES-6 and 7) whose viscosity and polarity are similar to NADES with 1,2-propanediol (DES-1 to DES-3), extract lower amounts of α-mangostin. Glycerol itself as a pure compound has a simple structure, but its OH groups are adjacent to each other which results in a compact structure. Thus, there is a barrier for chloride ions to be able to bind freely with all OH groups contained in glycerol, so NADES formed using glycerol also has a dense and rigid conformation which makes difficult to interact with α-mangostin. For NADES with 1,2-propanediol as HBD, the OH group in 1,2-propanediol might be locked in a certain position, but the alkane chain might rotate freely. This explains why extraction with NADES of ChCl-1,2-propanediol gave the highest yield of α-mangostin.

4. CONCLUSION

NADES can be used to extract mangostin from the pericarp of the mangosteen fruit with yields similar to that obtained using ethanol as the reference organic solvent. NADES consisting of choline chloride and 1,2-propanediol give α-mangostin extraction yields up to 2.6 % (w/w), much higher than those of NADES containing other hydrogen bonding donors (citric acid, glycerol, and glucose). This is due to the favorable physicochemical properties of this NADES:

slightly polar, less viscous, and low steric hindrance of 1,2-propanediol having only a short branch. NADES application for mangostin extraction showed the potential of this green solvent for the extraction and isolation of bioactive compounds from natural materials.

5. ACKNOWLEDGEMENT

The authors are grateful for the financial support from Indonesia Biological Sciences Research Grant Program contract number CRDF Global USA, IDB 1-80760-DE-14.

6. REFERENCES

Abbott, A.P., Boothby, D., Capper, G., Davies, D.L., Rasheed, R.K., 2004. Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. *Journal of the American Chemical Society*, Volume 126(29), pp. 9142–9147

Ahmad, M., Yamin, B.M., Lazim, A.M., 2012. Preliminary Study on Dispersion of α-Mangostin in PNIPAM Micro gel System. *Malaysian Journal of Analytical Sciences*, Volume 16(3), pp. 256–261

Ahmad, M., Yamin, B.M., Lazim, A.M., 2013. A Study on Dispersion and Characterisation of Alpha-mangostin Loaded pH Sensitive Microgel Systems. *Chemistry Central Journal*, Volume 7(85), pp. 1–6

Aisha, A.F.A, Abu-Salah, K.M., Ismail, Z., Majid, A.M.S.A, 2012a. In Vitro and in Vivo Anti-colon Cancer Effects of Garcinia Mangostana Xanthones Extract. *BMC Complementary and Alternative Medocone*, Volume 12(104), pp. 1–10

Aisha, A.F.A, Abu-Salah, K.M., Siddiqui, M.J., Ismail, Z., Majid, A.M.S.A, 2012b. Quantification of α-, β-and γ-mangostin in Garcinia Mangostana Fruit Rind Extracts by a Reverse Phase High Performance Liquid Chromatography. *Journal of Medicinal Plants Research*, Volume 6(29), pp. 4526–4534

Akao, Y., Nakagawa, Y., Nozawa, Y., 2008. Anti-cancer Effects of Xanthones from Pericarps of Mangosteen. *International Journal of Molecular Sciences*, Volume 9(3), pp. 355–370

Angell, C.A, Ansari, Y., Zhao, Z., 2012. Ionic Liquids: Past, Present and Future. *Faraday Discussions*, Volume 154, pp. 9–27

Azmir, J., Zaidul, I.S.M., Rahman, M.M., Sharif, K.M., Mohamed, A., Sahenab, F., Jahurul, M.H.A., Ghafoor, K., Norulaini, N.A.N., Omar, A.K.M., 2013. Techniques for Extraction of Bioactive Compounds from Plant Materials: A Review. *Journal of Food Engineering*, Volume 117(4), pp. 426–436

Bi, W., Tian, M., Row, K.H., 2013. Evaluation of Alcohol-based Deep Eutectic Solvent in Extraction and Determination of Flavonoids with Response Surface Methodology Optimization. *Journal of Chromatography A*, Volume 1285, pp. 22–30

Chemat, F., Vian, M.A., Cravotto, G., 2012. Green Extraction of Natural Products: Concept and Principles. *International Journal of Molecular Sciences*, Volume 13(7), pp. 8615–8627

Choi, Y.H., Spronsen, J.V., Dai, Y., Verberne, M., Hollmann, F., Arends I.W.C.E, Witkamp, G.J., Verpoorte, R., 2011. Are Natural Deep Eutectic Solvents the Missing Link in Understanding Cellular Metabolism and Physiology? *Plant Physiology*, Volume 156(4), pp. 1701–1705

Dai, Y., Witkamp, G.J., Verpoorte, R., Choi, Y.H., 2013a. Natural Deep Eutectic Solvents as a New Extraction Media for Phenolic Metabolites in *Carthamus tinctorius* L. *Analytical Chemistry*, Volume 85(13), pp. 6272–6278

Dai, Y., Spronsen, J.V., Witkamp, G.J., Verpoorte, R., Choi, Y.H., 2013b. Natural Deep Eutectic Solvents as New Potential Media for Green Technology. *Analytica Chimica Acta*, Volume 766, pp. 61–68

Earle, M.J., Seddon, K.R., 2000. Ionic Liquids. Green Solvents for the Future. *Pure and Applied Chemistry*, Volume 72(7), pp. 1391–1398

Earle, M.J., Esperança, J.M.S.S., Gilea, M.A., Lopes, J.N.C., Rebelo, L.P.N., Magee, J.W., Seddon, K.R., Widegren, J.A., 2006. The Distillation and Volatility of Ionic Liquids. *Nature*, Volume 439, pp. 831–834

Hayyan, M., Hashim, M.A., Hayyan, A., Al-Saadi, M.A., AlNashef, I.M., Mirghani, M.E.S., Saheed, O.K., 2013. Are Deep Eutectic Solvents Benign or Toxic? *Chemosphere*, Volume 90(7), pp. 2193–2195

Kondo, M., Zhang, L., Ji, H., Kou, Y., Ou, B., 2009. Bioavailability and Antioxidant Effects of a Xanthone-Rich Mangosteen (Garcinia mangostana) Product in Humans. *Journal of Agricultural and Food Chemistry*, Volume 57(19), pp. 8788–8792

Maugeri, Z., Leitner, W., de María, P.D., 2012. Practical Separation of Slcohol-ester Mixtures using Deep-Eutectic-Solvents. *Tetrahedron Letters*, Volume 53 (51), pp. 6968–6971

Ogihara, W., Aoyama, T., Ohno, H., 2004. Polarity Measurement for Ionic Liquids Containing Dissociable Protons. *Chemistry Letters*, Volume 33 (11), pp. 1414–1415

Pedraza-Chaverri, J., Rodríguez, N.C., Ibarra, M.A., Rojas, J.M.P., 2008. Medicinal Properties of Mangosteen (Garcinia mangostana). *Food Chem Toxicol*, Volume 46 (10), pp. 3227–3239

Pena-Pereira, F.P., Namieśnik, J., 2014. Ionic Liquids and Deep Eutectic Mixtures: Sustainable Solvents for Extraction Processes. *ChemSusChem*, Volume 7 (7), pp. 1784–1800

Pothitirat, W., Gritsanapan, W., 2009. HPLC Quantitative Analysis Method for the Determination of a-Mangostin in Mangosteen Fruit Rind Extract. *Thai Journal of Agricultural Science*, Volume 42 (1), pp. 7–12

Reichardt, C., 1994. Solvatochromic Dyes as Solvent Polarity Indicators. *Chemical Reviews*, Volume 94 (8), pp. 2319–2358

Yu, L., Zhao, M., Yang, B., Zhao, Q., Jiang, Y., 2007. Phenolics from Hull of Garcinia mangostana Fruit and Their Antioxidant Activities. *Food Chemistry*, Volume 104 (1), pp. 176–181