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Optimization Parameters of Natural Deep Eutectic Solvent Based on Choline Chloride and Fructose for Extraction of Polyphenol from *Spilanthes acmella*

Fajar Amelia Rachmawati Putri[1*], Askal Maimulyanti[1], Isna Nurhidayati[1], Bella Mellisani[1], R. Wiwi Widarsih[1], Fitria Puspita[1], Anton Restu Prihadi[2]

[1.] Department of Analytical Chemistry, AKA Bogor Polytechnic, Pangeran Sogiri Street 283, Tanah Baru, Bogor Utara, Bogor, West Java 16154
[2.] Department of Quality Assurance of Food Industry, AKA Bogor Polytechic, Pangeran Sogiri Street 283, Tanah Baru, Bogor Utara, Bogor, West Java 16154

**Corresponding author: fajarameliarputri@gmail.com*

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Abstract: Natural deep eutectic solvents (NADES) have grown in popularity as an environmentally benign alternative to conventional solvents for extracting natural compounds. In this current research, choline chloride was employed as a hydrogen bond acceptor (HBA) and was mixed with fructose which roled as a hydrogen bond donor (HBD) to construct NADES. NADES was deployed to separate the phenolic compounds from *Spilanthes acmella* (*S. acmella*) by utilizing an eco-friendly means under prime conditions. Corresponding to the outcomes of the research, the most gainful states of extraction were achieved at a sample weight-to-NADES volume ratio of 1:50, a period of extracting process of 60 min, water content of 20%, and a stirring speed of 80 rpm.

Keywords: green extraction, NADES, *Spilanthes acmella*, polyphenol

1. INTRODUCTION

One strategy for altering the consumption of organic solvent to extract a compound is by practicing green extraction. Numerous researches have been performed to encourage sustainable extraction by producing green solvents. This aims to reduce the widespread employment of toxic organic solvents in the process of separating a chemical from its mixture (Abbott et al., 2004). One example of using an organic solvent is the maceration process which is commonly applied to extract substances from nature. Organic solvents used in this process are varied, including acetone, methanol, dichloromethane, hexane, ethyl acetate, and acetone. The procedure of maceration has limitations of requiring a long period of operation as well as utilizing a significant amount of solvent, both of which are damaging to human health and the environment. Furthermore, to obtain a solvent-free extract, this extraction method demands the removal of solvent and purification phase. Therefore, innovation to develop solvents to facilitate the isolation of biologically active elements is needed to develop more environmentally friendly techniques. This breakthrough is aimed at obtaining natural extracts that include minimal residual solvents that contaminate the environment.

A potential method for implementing green chemistry is making use of a eutectic solvent, widely referred to as deep eutectic solvent (DES). This so-called green solvent is the diversified class of solvents that share many similarities with ionic liquids (Ge et al., 2017). DES is a eutectic mixture of acids and bases that belong to the Lewis or Brønsted group and, therefore, has the capability to bind cations or anions. The formation of DESs is typically through the reaction of a hydrogen bond donor (HBD) or metal salt with quaternary ammonium salt. A special characteristic of DESs is short in

temperature of melting due to asymmetrical ions and enormous size with weak lattice energy (Smith et al., 2014). Since the incorporation of natural ingredients and performing on green solvent principles, the term DES transformed into the natural deep eutectic solvent (NADES). Different kinds of compounds compose NADES such as carboxylic acids, choline chloride, and diverse HBD compounds including glycerol, succinic acid, sugars, citric acid, and amino acids. NADES has many resemblances to ionic liquid however NADES is less challenging to synthesize, harmless, substantial in viscosity, naturally recyclable, and undergoing spontaneous purification (Owczarek et al., 2016; Paiva et al., 2014).

These aforementioned positive characteristics help to ensure the ability of NADES to serve as a green extraction medium of botanical secondary metabolites with prospects of utilization in the industries of food and pharmaceutical.

Nevertheless, there is a limitation to applying the separation of target compounds using NADES because every HBA and HBD compound must be first set up appropriately before the process of extraction. Many types of research that have been conducted revealed that NADES was beneficial to separate a broad selection of metabolite compounds in the plant such as flavonoids (Vo et al., 2023), terpenoids (Rodríguez-Llorente et al., 2020), phenolics (Palos-Hernández et al., 2022), and alkaloids (Torres-Vega et al., 2020). Despite that, the application aspect of NADES to withdraw the target compound is currently limited due to its effectiveness. The good anticipation of NADES comes from the feature that is an eco-friendly solvent hence promoting the chance of it as a benign extraction medium for obtaining the target compound that is significant to building products of cosmetics and pharmaceuticals.

Spilanthes acmella (*S. acmella*) or in Indonesia called jotang originally came from Brazil and had some merit as a medicinal and ornamental plant for some decades. The therapeutic interest of this plant gained its fame due to each fraction of the *Spilanthes genus* has been utilized such as roots, leaves, stems, and flowers (Deka and Kalita, 2005; Rios-Chavez et al., 2003; Tan et al., 2004). *S. acmella* spreads in an area that is high in temperature and lies in the equator line (Sana et al., 2014). As a herbal remedy, the extract of *S. acmella* flower contains antipyretic chemicals (Chakraborty et al., 2010). This antipyretic activity has been discovered in pyrexia-causing microorganisms. The specific substance that plays an important role in this antipyretic function is flavonoid (Narayana et al., 2001). Another foremost compound that frequently embodied in flower extract is spilanthol. It is renowned for its multifaceted medicinal properties. Spilanthol demonstrated functions as an anesthetic (Barbas et al., 2016), acts as a potent antioxidant (Nabi and Shrivastava, 2016; Wu et al., 2020), exhibits anti-inflammatory effects (Gupta et al., 2012), and significant antibacterial and antimicrobial activities (Arora et al., 2011).

A variety of methods have been utilized to extract components from *S. acmella*. These methods include maceration techniques using ethanol and water (Charu et al., 2022; Wu et al., 2020), supercritical CO_2 extraction (Barbas et al., 2016), as well as methanol (Afzal et al., 2022). Additionally, the active components of *S. acmella* were separated using a Soxhlet extraction procedure with an organic solvent (Nabi & Shrivastava, 2016). For the purpose of getting the medicinal extract from renewable sources, the entire extraction steps are expected to adhere to green chemistry principles. In this current investigation, the introduction of NADES was conducted to capture the target compound contained in *S. acmella* by the green chemistry approach. Since

NADES is a harmless solvent generated from natural materials, the selection of NADES was promoted to be applied in pharmaceutical uses. Moreover, an initial investigation of each component that impacted the process of extraction was organized to discover the most favorable circumstances for obtaining the target substances.

2. MATERIAL AND METHODS

2.1 NADES Preparation

The synthesis of NADES was performed through the mixture in a specific mole ratio of HBD and HBA compounds. The substances that were assigned as HBA and HBD were choline chloride and fructose, respectively. HBA and HBD had a mole ratio of 1:2. The formation of NADES was executed via heating at 120-150ºC in solid-solid and solid-liquid phases. This stage was done along with the constant process of stirring at 1400 rpm for as long as 30-120 min to achieve a clear solution (Dai and Row, 2019).

2.2 NADES Utilization for Extraction of Polyphenol Contained in *S. acmella*

The active compounds in *S. acmella* were found using 10 mL of NADES for 1 g of dehydrated sample. This mixture was then stirred at room temperature for 30 min at a steady speed. The supernatant of this mixture was obtained by filtering using filter paper and continued by chemical analysis.

2.3 Polyphenol Content Measurement

Measurement of polyphenol concentration was referred to the technique established by Chen et. al. (2014). This technique used Folin-Ciocalteu to determine the level of polyphenol contained in *S. acmella*. The extract was diluted at a ratio of 1:10 where 500 μL of extract was mixed with 2 mL of Na_2CO_3 7.5% (w/v) and 2.5 mL of Folin-Ciocalteu reagent (1:10). incubation in a space with no light for 2 hours at ambient temperature. The solutions were measured for their absorbances at 760 nm employing a UV-Vis spectrophotometer. The standard solutions used in this measurement were mixed with gallic acid at varied concentrations between 10-100 mg/L. The absorbance was calculated to be reported as gallic acid equivalent (GAE) per gram dry weight.

2.4. Optimizing Parameters of Extraction

The optimization of extraction parameters was done to identify the most suitable environment for NADES to isolate polyphenols in *S. acmella*. This exploratory stage was built up from prior research (Chen et al., 2014). The following factors were considered to be analyzed for their impact on the extraction process: (a) water content, (b) ratio of sample-to-NADES volume, (c) contact length, and (d) power of stirring.

a. Water content effect

The analysis of the addition of water was performed by varying the amount of water from 20% to 60% with a 10% interval.

b. The implication of the sample-to-NADES-volume ratio

The ratios used in this research were 1:10, 1:20, 1:30, 1:40, and 1:50 to assess their contribution to the extraction process.

c. The influence of extraction duration

The optimal extraction duration was determined by increasing the length from 30 to 150 min with gradual increments of 30 min.

d. The impact of stirring power

By modifying the power by raising it from 50 to 175 rpm, the implication of stirring speed was evaluated.

3. RESULTS AND DISCUSSION

3.1 Relationship between Sample Weight-to-NADES Volume Ratio and Polyphenol Extraction

Based on the variation of the ratio of sample weight to NADES volume, Figure 1 indicates that the most substantial amount of polyphenol was attained at 1:50. This significant value was attributed to the ease of sample dissolution in NADES at ratio 1:50 that promoted NADES to optimally interact with polyphenols (Hu et al., 2023). At lower ratios, NADES was not able to effectively contact polyphenols since the sample was not fully diluted in NADES. This condition provoked poor content of polyphenols and weak escalation at ratios under 1:50. The finding also suggested that NADES was still far from its polyphenol binding saturation.

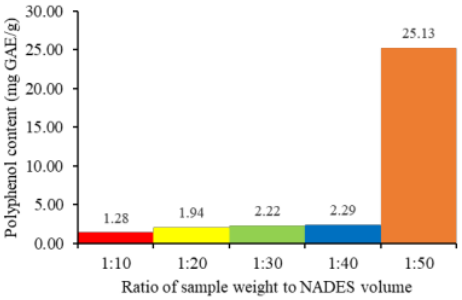

Figure 1. The relationship graph of sample weight to NADES volume ratio on results of extraction.

3.2 Correlation of Extracting Duration on Polyphenol Content

Along with the increment of time, the level of extracted polyphenol intensified until it arrived at the highest point of 60 min of extracting time (Figure 2). Polyphenol concentration slowly declined as the extraction period was longer than 60 min, reaching the noticed minimum level at 150 min with the polyphenol content of 14.13 mg GAE/g. The yields illustrated that the efficiency of polyphenol extraction decreased with the extension time of extraction. This outcome denoted that a 60-min extraction duration was continued to be used in the next stages since it provided some preferable conditions such as relatively short usage of energy, polyphenol stability remained desirable, and resulting prominent level of polyphenol. On the contrary, the decrement of extracted polyphenol was observed at extended times of extraction since labile polyphenol were generated. The instability of polyphenol induced by the process of decomposition created an alteration in the chemical structure of the active substance (Ali et al., 2019; Rebocho et al., 2022). Degradation of polyphenol occurred through the hydrolysis between NADES and polyphenol. An additional variable that contributed to the decrement in the effectiveness of extraction was the re-adsorption of polyphenol in the sample because there was an equilibrium in the process of extraction and adsorption.

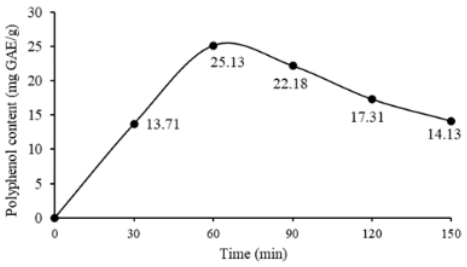

Figure 2. The correlation of extraction duration on extracted polyphenol.

3.3 Influence of Speed of Stirring to Polyphenol Content

Figure 3 exhibited that the elevation power of stirring to 80 rpm enhanced the isolation of polyphenol at its peak, yielding a concentration of polyphenol of 25.75 mg GAE/g. The result also confirmed that the equilibrium was achieved. It meant that boosting the speed of stirring displayed no advantageous effect on the efficiency of polyphenol extraction. In other words, faster stirring speed resulted slight content of polyphenol. According to Figure 3, after the

equilibrium point was passed, the extraction of polyphenol reduced, and then tended to show no substantial change in the content of extracted polyphenol. The tendency conveyed that NADES was saturated hence slowing the extraction of polyphenol.

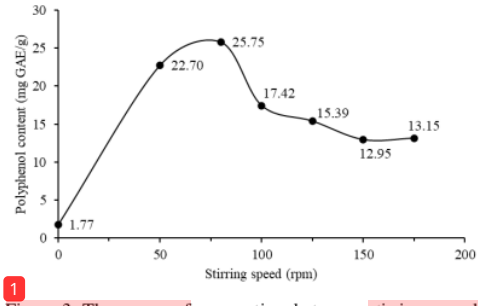

Figure 3. The curve of connection between stirring speed and polyphenol content.

3.4 The Influence of Water Content on Concentration of Extracted Polyphenol

The incorporation of water into the polyphenol extraction process aimed not only to reduce the viscosity but also to alter the density and polarity of NADES. This adjustment enhanced the extraction efficiency by optimizing the solvent properties, leading to improved solubility and separation of polyphenolic compounds. The adjustment was plausible since NADES predominately comprised hydrogen bond acceptors and donors, which can be modified by the addition of water. This alteration allows for a fine-tuning of the solvent's physical and chemical properties as well as enhancing the extraction efficiency of polyphenolic compounds (Hikmawanti et al., 2021; Ling and Hadinoto, 2022; Oomen et al., 2020). The water incorporation into NADES brought in the effectiveness degradation of polyphenol extraction. This dilution effect may hinder the solvent's ability to solubilize and extract polyphenolic compounds efficiently.

Based on the results depicted in Figure 4, the lowest water content of 20% resulted in the highest polyphenol concentration, measured at 25.75 mg GAE/g. This finding indicated that an increase in water content reduced the efficiency of polyphenol extraction. In other words, the volume of water added directly influenced the efficacy of polyphenol extraction. The water content of 20% weakened the hydrogen bond network and reduced NADES viscosity, which assisted the distribution of polyphenols in the solvent, thereby enhancing extraction efficiency. When the water content was between 30-40%, there was no significant change in polyphenol content, as the hydrogen bond network in NADES remained largely intact despite the addition of water. It meant that the active compound maintained hydrogen bonds with NADES while also binding to water molecules due to its high water solubility (Hu et al., 2023). As the water content increased to 40%, the efficiency of polyphenol extraction decreased since molecules of water destroyed the hydrogen bond network in NADES. At higher amounts of water, polyphenol were prevented from forming hydrogen bonds with NADES (Ali et al., 2019).

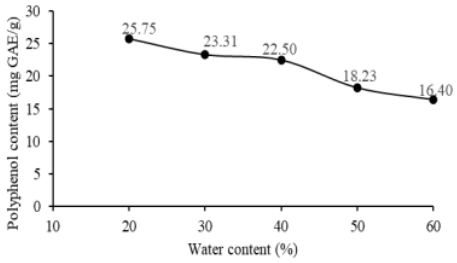

Figure 4. The influence of water content on polyphenol content.

4. CONCLUSION

In the present investigation, the most beneficial environment for NADES consisted of choline chloride and fructose as an agent to isolate polyphenol compounds was determined at a level of

water of 20%, 1:50 of the sample weight-to-NADES ratio, agitation rate of 80 rpm, and 60 min of extracting period.

5. ACKNOWLEDGMENT

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