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

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Abstract: Natural deep eutectic solvents (NADES) have become popular 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 had the role of a hydrogen bond donor (HBD) to construct NADES. This extracting agent was deployed to separate the polyphenolic compounds from the stem of Jotang (*Spilanthes acmella*) under prime conditions. The extracted polyphenol was subsequently calculated based on the Folin-Ciocalteu method using gallic acid as the standard solution by UV-Vis spectrophotometer at 760 nm. Corresponding to the research outcomes, 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. The optimized parameters were conceived as the most gainful because they exhibited the most prominent level of polyphenol were described as follows: ratio of sample weight-to-NADES volume and extraction time showed polyphenol content of 25.13 mg GAE/g each, while the others, stirring speed and water content revealed the polyphenol concentration of 25.75 mg GAE/g respectively.

Kata Kunci: green extraction, NADES, period of extracting, polyphenol, sample weight-to-NADES, *Spilanthes acmella*, stirring speed, volume ratio, water content

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1. INTRODUCTION

One strategy for altering organic solvent consumption is to extract a compound by practicing green extraction. Numerous research studies 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 (Winterton, 2021). 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, can 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 the short 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 or in Indonesia called Jotang originally came from Brazil and had some merit as a medicinal and ornamental plant for some decades. The pharmacological activities of this plant gained its fame due to each fraction of the *Spilanthes genus* has been utilized such as leaves, stems, and flowers (Yadav et al., 2023). *S. acmella* spreads in an area that is high in temperature and lies in the equator line (Sana et al., 2014). As an herbal remedy, the extract of *S. acmella* revealed a wide range of biological functions such as antioxidant, anti-inflammatory, antimicrobial, and diuretic (Sharma and Arumugam, 2021; Shivanda et al., 2023). This antipyretic activity has been discovered in pyrexia-causing microorganisms. The specific substance that plays an important role in this antipyretic function is flavonoid (Vishwanathan et al., 2001). Another foremost compound that is 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 (Balieiro et al., 2020), and significant antibacterial and antimicrobial activities (Peretti et al., 2021).

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 Preparation of Sample

The stems of *S. acmella* were harvested from Cimahpar Village, Bogor City in February 2023. The plants were identified by morphology and planted by one member of the research team. Stem samples of *S. acmella* were dehydrated for 1 week at room temperature without exposure to sunlight. The air-dried samples were later finely chopped and kept in a plastic container to prevent contact with air and sunlight.

2.2 NADES Preparation

NADES was synthesized 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 120-150^{o}C 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).

2.3 NADES Utilization for Extraction of *S. acmella*

1 g of air-dried stem of *S. acmella* was mixed with 10 mL of NADES. This mixture was then subjected to specific parameters that were portrayed in procedure 2.4. The supernatant was obtained by filtering through filter paper.

2.4 Optimizing Parameters of Extraction

The extraction parameters were optimized 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 addition of water was analyzed 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.

2.5 Polyphenol Content Measurement

The measurement of polyphenol concentration was referred as 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). ~~i~~ncubation 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 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. The polyphenol content was examined by the following equation:

$$C = C_1 \text{ x } \frac{V}{m}$$

Where C = content of polyphenol stated in mg GAE/g, C_1 = gallic acid concentration defined from the curve of calibration in mg/L, V = volume of extract expressed in L, and m = the weight of stem extract of *S. acmella* in g.

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.

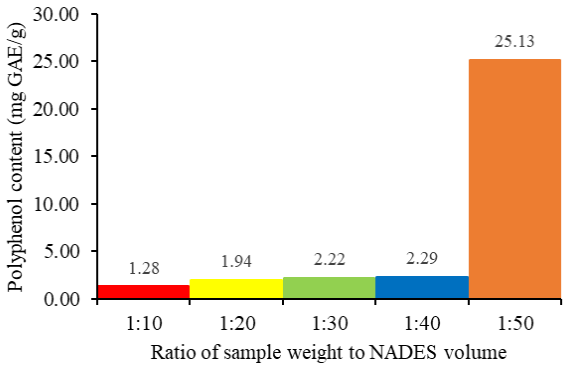

Figure 1. The relationship graph of sample weight to NADES volume ratio on results of extraction.

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.

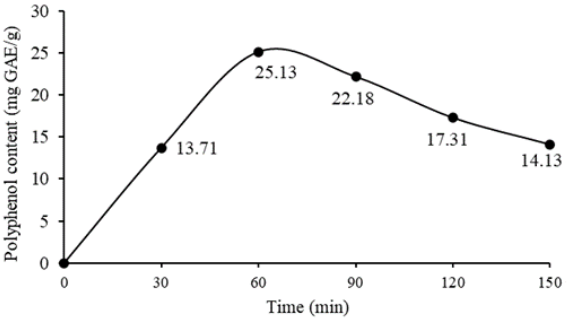

Figure 2. The correlation of extraction duration on extracted polyphenol.

3.3 Influence of Speed of Stirring to Polyphenol Content

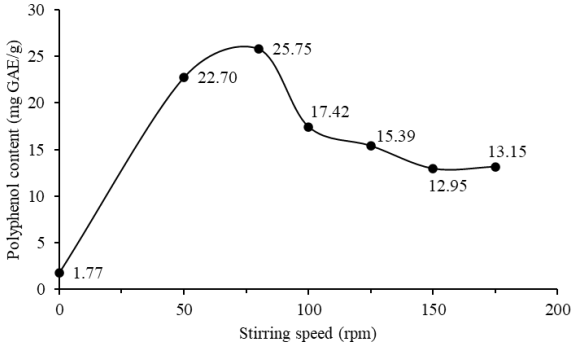

Figure 3. The curve of connection between stirring speed and polyphenol content.

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.

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 degradation of the polyphenol extraction effectiveness. 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. The active compound maintained hydrogen bonds with NADES while also bonded 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 was prevented from forming hydrogen bonds with NADES (Ali et al., 2019).

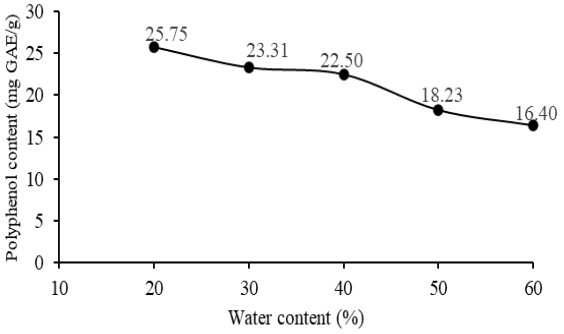

Figure 4. The influence of water content on polyphenol content.

The method proposed in this research was considered beneficial since the polyphenol contained in *S. acmella* stem was able to be isolated in 60 min of extraction with a stirring speed of 80 rpm. This condition was highly mild compared to the previous study carried out by Lalthanpuii et al. (2020). In their investigation, chloroform and Soxhlet apparatus with a capacity of 5 L was employed and finished in 72 h to extract target compounds of *S. acmella*. The most plenteous compound found in this sample was alkylamide.

Another organic solvent commonly applied in the extraction of *S. acmella* is ethanol. Charu et al. (2022) compared ethanol and water extracts of *S. acmella* that were obtained from a 24-h extraction process at room temperature. The ethanol extract displayed more remarkable biological activities, with the most dominant compound is n-Hexadecanoic acid. The extended time of extraction showed that the aforementioned techniques were incapable of applying the green chemistry approach.

Methanol was also customary to be taken as an extracting agent, as Swargiary et al. (2019) had done. The powdered-form sample of *S. acmella* was soaked in 80% methanol. After 24 h, the solution was filtered and the fresh solvent was added. The replication of this process was done four times. The phenolic content of *S. acmella* exhibited a level of circa 40 mg/g. Although the content was higher than in this present study (23-25 mg/g in 10 mL NADES), the use of organic solvent was extremely plentiful, therefore it failed to employ the green extraction principle.

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.

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