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Total Phenolic Content of Natural Deep Eutectic Solvent (NADES) Extraction in Comfrey (Symphytum officinale)

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A B S T R A C T

Objective: The most common type of phytochemical is phenolic compounds. These compounds are mostly found in the plant kingdom, and extraction is one of the first and most important stages before any use. The green chemistry trend has encouraged the researcher to discover and generate innovative solvents in extraction of phenolic compound (PC) as strategy to toxic and potentially hazardous organic solvents. Natural Deep Eutectic Solvents (NADES) have recently been used as phenolic compound extraction solvents. The objective of this study is to determine the total phenolic contents of the NADES extract from comfrey (Symphytum officinale). Methods: This study used choline chloride-urea, choline chloride-glycerol and choline chloride-sucrose combination of NADES for extraction phenolic compound in comfrey. The extraction was helped using Ultrasound Assisted Extraction (UAE). The extracted material was examined using a UV-Vis spectrophotometer. Results: The results showed that the total phenol of choline chloride-urea, chloride-glycerol and choline chloride-sucrose NADES comfrey extraction was 2.069, 1.566, and 1.906 mg GAE /gram of dry powder, respectively. Conclusion: Based on the result, it can be concluded type of NADES combination showed different level of phenolic extraction. The best phenolic extraction was showed in combination of choline chloride-urea

Kata kunci:

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A B S T R A K

Latar belakang: Fenol adalah senyawa kimia yang paling umum ditemukan dalam tanaman. Proses ekstraksi berperan penting dalam pemisahan senyawa fenol dari tanaman sebelum dimanfaatkan. Saat ini penelitian banyak difokuskan pada proses ekstraksi yang ramah lingkungan. Salah satunya adalah menggunakan Natural Deep Eutectic Solvent (NADES) dalam proses ekstraksi senyawa fenol. Tujuan penelitian ini adalah menghitung kadar fenol total dalam ekstrak NADES tanaman komfrey. Metode: Penelitian ini menggunakan NADES yang berasal dari kolin klorida-gliserol, kolin klorida-urea dan kolin klorida-sukrosa. Proses ekstraksi menggunakan Ultrasound Assisted Extraction (UAE) untuk mempercepat proses ekstraksi. Penentuan kadar fenol total diukur menggunakan spectrophotometer UV-Vis. Hasil: Kadar fenol total dalam ekstrak NADES masing-masing adalah 1.566, 2.069 and 1.906 mg GAE /gram sampel. Kesimpulan: Berdasarkan hasil yang diperoleh dapat disimpulkan bahwa kombinasi masing-masing NADES memberikan kemampuan ekstraksi senyawa fenol yang berbeda-beda. Kadar fenol total terbaik dihasilkan oleh kombinasi NADES kolin klorida-urea.

INTRODUCTION

The Boraginaceae family's comfrey (*Symphytum officinale* L.) is a traditional herb plant. (Sahidin & Sudharmono, 2019). This plant, commonly known as knit bone, has proven effective for reducing wounds, sprains, and bone fractures in traditional medicine (Smith & Jacobson, 2011). Previous research has shown that the presence of various secondary metabolite in comfrey contributes to its medicinal characteristics (Savic et al., 2015). Phenolic acid was one of the most concerning metabolites discovered in comfrey. Phenolic acid compound of comfrey have been reported for antioxidant activity, anti-inflamation, antihypertension and antiproliferative (Oliviu et al., 2017; Seigner et al., 2019; Trifan et al., 2018; Vladic et al., 2019). High performance liquid chromatography (HPLC) analysis of *Symphytum officinale* herb and roots indicated the presence of twenty phenolic acids, including salvianolic acid, rosmarinic acid, *p*-coumaric, caffeic acid and *m*-hydroxybenzoic acids (Nastić et al., 2018; Salehi et al., 2019; Trifan et al., 2018).

In an early research, comfrey was extracted using organic solvents such as methanol and ethanol. The organic solvent was capable of extracting phenolic acid from comfrey (Trifan et al., 2018; Vladic et al., 2019). These methods need extensive treatment hours, volatile, flammable and high temperatures, and the solvents used are toxic to human health and the environment; moreover, the extracts must endure solvent removal and purification treatments before for using. The use of conventional organic solvents such as ethyl acetate, chloroform, hexane, acetone or methanol has restricted the use of green chemical strategies (Barbieri et al., 2020). The demand for alternative and environmentally friendly extraction procedures is currently in popular, as this will be extremely valuable in order to reduce both extraction time and solvent consumption through the implementation of profitable strategies that conform to Green Chemistry criteria (Bonacci et al., 2020).

Green solvents have been proposed as organic solvent substitutes to prevent the use of toxic materials solvents. Green solvents was biodegradable, non-toxic and biocompatible solvents (Barbieri et al., 2020). Nowadays, Ionic Liquid (IL) and Natural Deep Eutectic Solvent (NADES) has been developed as green solvents (Razboršek et al., 2020). Ionic Liquid (IL) showed problem such as expensive, complex manufacturing, high water solubility and low biodegradability (Kalhor & Ghandi, 2019). Meanwhile, Natural Deep Eutectic Solvent (NADES) was made from cheap materials and easy to prepared (Dai et al., 2013; El Achkar et al., 2019). NADES are obtained by combining a hydrogen bond acceptor (HBA) and a hydrogen bond donor (HBD), which mixed in precise molar ratios become a liquid with a melting point that is significantly lower than a single component. The liquid form is clear and stable in the room temperature (Alcalde et al., 2019).

Many investigation about the use of NADES in the extraction of phenolic compounds have been reported. The use of NADES to extract phenolic compounds from olive oil is extremely promising (Bonacci et al., 2020). Furthermore, the extraction of phenolic components from rosemary yielded promising results when compared to standard solvents (Barbieri et al., 2020). The phenolic compounds isolation from dried chokeberries using combination of choline chloride-fructose presented 36.15 ± 3.39 mg gallic acid / g dry weight (Razboršek et al., 2020). Based on the explanation, the purpose of this research was to evaluate the total phenolic compound of NADES extract from comfrey (*Symphytum officinale L*). Three NADES were produced and employed in the extraction, using choline chloride as HBA component and glycerol, urea and sucrose as HBD component in addition to water.

METHOD

Materials and Instrument

Comfrey (*Symphytum officinale* L.) used in this study harvesting from Karanganyar, Central Java, Indonesia. It was determined at the Biology Laboratory, Gajah Mada University. The solvents were methanol pro-analysis (Merck, Germany), Aqua pro Injection (P.T. Ikapharmindo), Choline Chloride (SigmaAldrich, USA), Glycerol (PT Molex Ayus, Indonesia), Urea (SigmaAldrich, USA), Sucrose (Brataco), gallic acid as standard (Merck, Germany), NaOH (Merck, Germany) and Folin-Ciocalteu reagent (Merck, Germany). Total phenolic compounds were evaluated using Spectrophotometry UV-Vis set (UV-1800, Shimadzu).

Preparation of NADES

In this work, NADES were paired using choline chloride as a hydrogen acceptor (HBA) component. Glycerol, urea, and sucrose are used as hydrogen donor components. The molarity ratio used is 1:2, with 30 % of water content. The combination is blended in a glass instrument and heated at 80°C until it becomes a transparent liquid.

Comfrey extraction

The extraction was assisted by Ultrasound Assited Extraction (UAE) at 50°C for 45 minutes. The amount of powder to solvent is 1:20. The acquired extract was centrifuged and filtered to separate the NADES extract.

Total phenol content assay

Indonesian Herbal Pharmacopoeia explained the total phenol content assay. It used to determine the phenolic content in NADES extract. In a 25 ml volumetric flask, 2 ml of NADES extract was dissolved in methanol p.a. Then, 1 ml of each mixture dissolved in methanol extract was collected and mixed with 5 ml of Folin-Ciocalteu, 4 ml of 1% of NaOH, and incubated at room temperature for 1 hour. UV-Vis spectrophotometry was used to determine the absorbance of each test solution.

RESULTS AND DISCUSSION

This research used 3 types of NADES combination for extraction. The combination of NADES is choline chloride-urea, choline chloride-glycerol and choline chloride-sucrose. The characteristic of NADES solvent for extraction is present in Table 1.

NADES was formed by combining two or three materials in a eutectic combination, which, when blended at the proper molar ratio, has a lower melting point and becomes a liquid solvent. Hydrogen bond acceptors (HBAs) and hydrogen bond donors (HBDs) mixture are present in these transparent liquid mixtures (Razboršek et al., 2020). NADES was produced in this investigation by heating and stirring

(Espino et al., 2019). NADES component creates by hydrogen bond interactions and Van derWalls interactions (Kalhor & Ghandi, 2019; E. L. Smith et al., 2014). In addition, choline chloride-based NADES for comfrey extraction were tested in this study.

Table 1
Characteristic of NADES

NADES composition	Molar ratio	Water addition (%)	Characteristic
Choline chloride-glycerol	1:2	30%	Transparant liquid
Choline chloride-urea	1:2	30%	Transparant liquid
Choline chloride-Sucrose	1:2	30%	Thick light yellow liquid

NADES was prepared in the same day for testing. All sample was extracted using Ultrasound Assisted Extraction (UAE) under the same condition. The ratio of sample-solvent was 1:20 for extraction. The temperature was set at 50^{0}C for 50 minutes.

Total phenolic content of comfrey NADES extract was analyzed using Folin Ciocalteu assay. This approach is the most often used to determine total phenolic content in plants extraction since the preparation is easier and the Folin Ciocalteau reagent is required because phenolic compounds may react with Folin Ciocalteau reagent to create a detectable solution absorbance. The absorbance was measured by Spectrophotometer UV-Vis. The Folin-Ciocalteu assay is an electron transfer-based test that provides reducing capability represented as phenolic content. The solvent chosen for extraction influences the total phenolic content and yield of phytochemicals.

The external calibration was carried out using various amounts of gallic acid. The total phenolic content was determined as gallic acid in milligrams per gram sample using the gallic acid calibration standard curve. The results are given in milligrams per gram of dry powder.

The absorbance wavelenght of total phenolic content was set at 730 nm. Series concentration of gallic acid was treated with Folin Ciocalteau reagent and NaOH. After incubation, the mixture was measured at 730 nm. The relationship between absorbance and the concentration of standard was plotted in calibration curve. The calibration curve obtained from the standard (gallic acid) analysis was linear with y = 0.0176x-0.0206 and R =0.98.

The equation of calibration curve was used to measure the total phenolic content in comfrey NADES extracts. NADES choline chloride-urea gived the highest level of phenolic content 2.068 mg gallic acid per dry weight powder. Moreover, chloride-glycerol and choline chloride-sucrose was 1.566 and 1.906 mg gallic acid per dry weight powder, repectively.

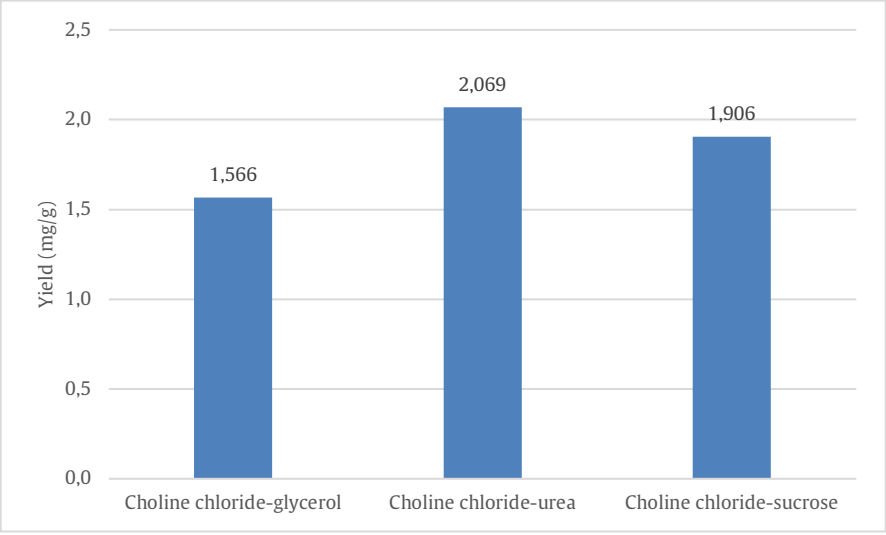

Figure 1. Total phenol content of comfrey in various NADES

In generally, the extraction parameters implemented (solvent/liquid ratio, extraction technique, temperature) and also the physico-chemical properties of the solvent used may influence the extraction capacity for multiple types of phenolic compounds in plant extracts. Therefore, the chemical composition of a specific NADES is critical for its capacity to extract phenolic compounds since it influences properties such as physicochemical interactions, polarity, viscosity and solubility (Razboršek et al., 2020). Comfrey (*Symphytum officinale*) present some phenolic such as salvianolic acid, rosmarinic acid and caffeic acid (Trifan et al., 2018; Vladic et al., 2019).

Phenolic compound

LIMITATION OF THE STUDY

This study needs improvement such as activity assay and stability test of NADES extraction.

CONCLUSIONS AND SUGGESTIONS

The combination of choline chloride-urea is the best combination for phenolic extraction of comfrey (*Symphytum officinale*).

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ETHICAL CONSIDERATIONS

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Conflict of Interest Statement

There is no conflict of interest in this study

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