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Extraction of Polyphenols from Turmeric (*Curcuma longa*), Red Ginger (*Zingiber officinale*), and Aromatic Ginger (*Kaempferia galanga* l) Using Natural Deep Eutectic Solvent Based on Choline Chloride and Sugar

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Abstract: Indonesia is a tropical country rich in biodiversity, including a wide variety of herbal plants. Herbs and spices are abundant in Indonesia and have been used in local customs since ancient times. Turmeric, ginger, and curcuma are rhizomes used as spices in Indonesian cooking and natural medicine. This study investigates the efficiency of natural deep eutectic solvents (NADES) synthesized from choline chloride and various sugars—fructose, glucose, maltose, and xylose in extracting polyphenolic compounds from Indonesian rhizomes: turmeric (*Curcuma longa*), red ginger (*Zingiber officinale*), and aromatic ginger (*Kaempferia galanga* L). The NADES were prepared using choline chloride as hydrogen bond acceptor (HBA). The hydrogen bond donor (HBD) is a sugar such as fructose, xylose, maltose, and glucose. The total phenolic content (TPC) was quantified and reported in mg gallic acid equivalent (GAE)/g extract. Kaempferia galanga exhibited the highest TPC among the rhizomes, reaching 32.21 mg GAE/g when extracted with ChCl–Fructose NADES. Curcuma longa and Zingiber officinale yield 20.05 mg GAE/g and 25.84 mg GAE/g under the same solvent conditions, respectively. When comparing the performance of the solvents, the following order was observed: ChCl–Fr > ChCl–Xyl > ChCl–Mal > ChCl–Glu. These findings suggest that solvent polarity and hydrogen bonding capacity significantly influence polyphenol solubility and extraction efficiency. NADES based on ChCl–Fructose showed the greatest potential for eco-friendly extraction of bioactive phenolics from medicinal rhizomes.

Keywords: extraction, NADES, spices, polyphenols
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1. INTRODUCTION

Indonesia is a country rich in herbs and spices; each plant has specific characteristics and properties. Consequently, the use of herbal plants for treatment has become a tradition in Indonesian society. Several studies have shown that rhizomes are underground stems, including ginger, turmeric, fingerroot, aromatic ginger, Java ginger, and black turmeric. These rhizomes are used as cooking spices, traditional medicine, and natural dyes. Turmeric extract is a natural remedy with the potential to be developed as an antibacterial, antioxidant, antiinflammatory, or other benefit that requires more research. Phytochemical studies using turmeric rhizomes have found the presence of medicinal compounds called curcuminoids, consisting of curcumin, desmethoxycurcumin, and bisdemethoxycurcumin (Permatananda, et al., 2021). Extraction of components from natural resources typically involves the use of several conventional organic solvents, such as ethanol, methanol, hexane, ethyl acetate, chloroform, and acetone. However, using these solvents must be reduced following the principles of green chemistry (Alanon, et al., 2020). Organic solvents are generally flammable, volatile, toxic, and not easily degraded, leading to increased risk of environment pollution (Bajkacz, et al., 2018). Extraction using these chemicals is also considered inefficient, requiring removal and purification stages to obtain natural material extracts. Several studies have been carried out to overcome the limitations to develop natural deep eutectic solvent (NADES) as alternatives. Eutectic solvents are a mixture of two or more components of hydrogen bond acceptors and donors in the form of solids or liquids. At a certain molar ratio, a decrease in the melting point is obtained, producing a liquid at room temperature (Alanon, et al., 2020). Unlike conventional solvents, NADES do not require separation from the extract after extraction. This property significantly reduces energy consumption and simplifies the purification process, making NADES-based extraction more environmentally friendly and economically efficient (Mbous et al., 2017). NADES has various beneficial properties, such as being easy to synthesize, not harmful to the environment, non-toxic, and can decompose naturally in nature. The most common NADES is produced from choline chloride (ChCl), carboxylic acids, and other hydrogen bond donors, such as sugars, citric acid, succinic acid, and amino acids found in living organism cells (Owczarck, at al., 2016).

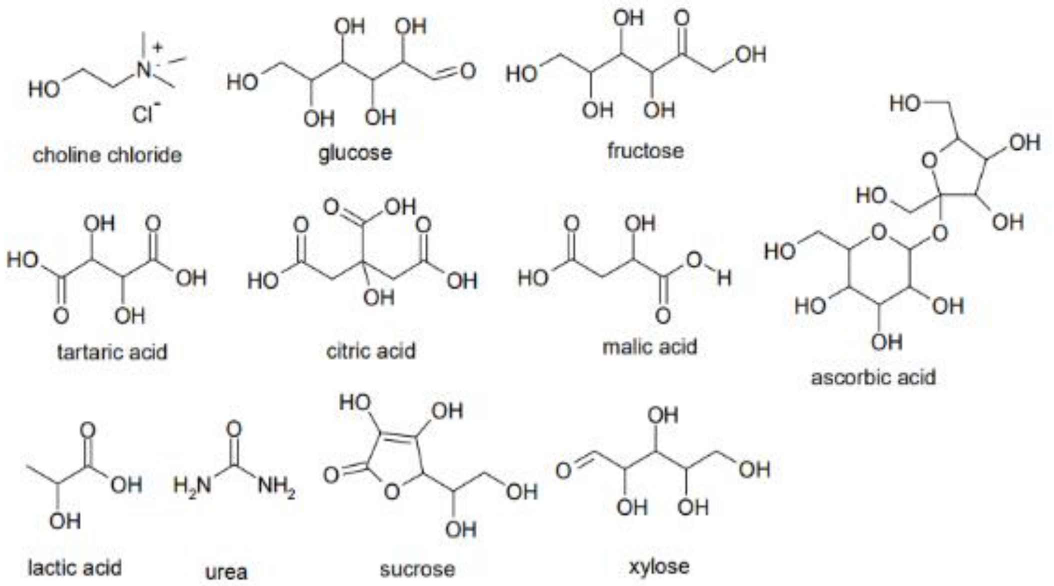

Figure 1. Structure of Component for NADES (Paiva, at al., 2018)

Several studies have been conducted using NADES as a solvent for the extraction of natural pigments (Xizhe Fu, at al., 2021), phenolics (Barbieri, et al., 2020), antioxidants (Alhakmani, et al., 2013), flavonoids (Bajkacz, et al., 2018), anthocyanin (Himawanti, et al., 2021), vanillin (Gonzales, et al., 2017), and quercetin (Dai, et al., 2019). However, the application of these solvents for the separation of active substances from typical Indonesian spices has not been widely developed. Due to their great potential, NADES can be applied to extract natural materials, specifically materials found in rhizomes. Therefore, this study aims to extract active compounds in turmeric (*Curcuma longa*), red ginger (*Zingiber officinale*), and aromatic ginger (*Kaempferia galanga* L) using NADES.

2. METHODS

2.1 Material

Rhizomes such as turmeric (*Curcuma longa*), red ginger (*Zingiber officinale*), and aromatic ginger (*Kaempferia galanga* L) were collected from Bogor, Indonesia, in August 2024. The reagents used in this study included choline chloride p.a. (Merck), fructose p.a. (Merck), glucose p.a. (Merck), maltose p.a. (Merck), xylose p.a. (Merck), Folin-Ciocalteu reagent p.a. (Merck), and gallic acid p.a. (Merck). The instrument used was a UV-Vis spectrophotometer (Shimadzu UV-1800).

2.2 Methods

a. NADES Synthesis

NADES was made from choline chloride (Ch-Cl) compounds as HBA and HBD compounds of glucose (Glu), fructose (Fr) with a mole ratio of 1:2, whereas maltose (Mal) and xylose (Xyl) with a mole ratio of 1:1. HBA and HBD compounds were mixed and heated at a temperature of 60 - 130ºC and stirred until clear (Dai, et al., 2013).

b. Extraction of Active Substances from Spices Using NADES

A total of 1 g dry rhizome sample with 20 mL of NADES was stirred at a speed of 150 rpm and room temperature for 30 minutes, the mixture was filtered to obtain the extraction supernatant (Xizhe Fu, at al., 2021).

c. Determination of Total Polyphenol Content

Total polyphenol content (TPC) was determined using gallic acid standards with concentrations of 10, 20, 30, 40, and 50 ppm. Separately, rhizome extract was dissolved in distilled water at a ratio of 1:20. A total of 1 mL of each standard solution and extract was added with 5 mL of 10% Folin-ciocalteu and 4 mL of 7.5% Na_2CO_3, the solution was stirred until homogeneous and left for 60 minutes in a dark room at room temperature. The absorbance of the solution was measured using a UV-VIS spectrophotometer at a wavelength of 765 nm. Furthermore, the total phenol content was calculated based on a gallic acid standard curve, and the results showed gallic acid equivalent (GAE)/gram dry weight (Koh, et al., 2023).

3. RESULTS AND DISCUSSION

a. NADES Synthesis

The NADES formulations were successfully synthesised using choline chloride (ChCl) as HBA and various sugars—fructose, glucose, maltose, and xylose—as HBD. The NADES that had choline chloride with fructose (1) and glucose (2) were made using a 1:2 ratio, and some water (20%) was added. The choline chloride-maltose (3) was produced with a 1:1 ratio, with the addition of 20% water. However, the sample comprising choline chloride-xylose (4) had a ratio of 1:1, with 30% water. The ratio was changed because the 1:2 choline chloride-maltose and chloride-xylose mixture were too viscous and made it hard to move around. The ratio composition of NADES in this study is shown in Table 1.

Table 1: The composition of NADES

NADES		Ratio	%Water
NADES 1	ChCl:Fr	1:2	20%
NADES 2	ChCl:Glu	1:2	20%
NADES 3	ChCl:Mal	1:1	20%
NADES 4	ChCl:Xyl	1:1	30%

More water was put in to make it easier to handle. Water helped reduce both the thickness and the weight of the solution (Mitar, et al., 2018), (Ivanović, et al., 2022), (Pires, et al., 2022). Several studies have shown that excess water can disrupt the bonding of the molecules (Dai, et al., 2019) also showed that adding water to NADES reduces the thickness and weakens the bonds between the molecules. Water levels below 50 has minimal effect (Che Zain, et al., 2021). In this study, the water added was not more than 20%. The efficacy of NADES in solubilizing substances can be modified by altering the proportions of its components (Bajkacz, et al., 2018), (Cao, et al., 2023). It was also noted that the efficacy of NADES in solubilizing substances can be enhanced by altering its composition, adjusting the proportions of its components, and varying the water content. The molar ratio of HBA and HBD affected the properties of the resulting solvent. Research about NADES with a 1:2 choline chloride - fructose ratio shows the optimum results and the addition of 20% water (Maimulyanti, 2023). Another study also employed a combination of choline chloride-glucose with a ratio of 1:2 (Islamčević, et al., 2020). The synthesised NADES is shown in Figure 2.

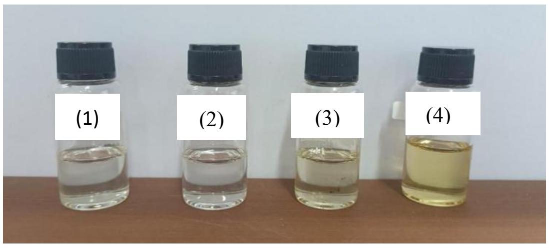

Figure 2. NADES (1) ChCl- Fr 1:2 (2) ChCl - Glu 1:2
(3) ChCl - Mal 1:1 (4) ChCl – Xyl 1:1

b. Extraction of Active Spice Substances Using NADES

These rhizomes were washed thoroughly before use, sliced into small pieces and dried at room temperature. The dried rhizomes are shown in Figure 3. The Polyphenol extract of the sample using NADES can be seen in Figure 4.

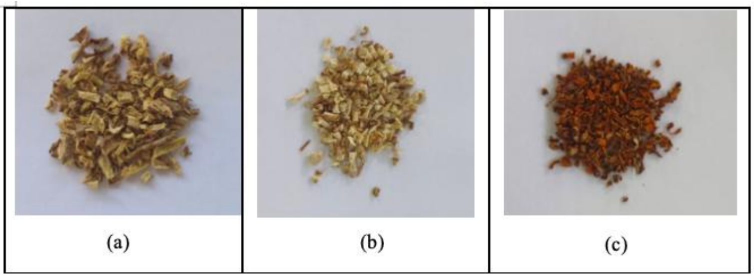

Figure 3. Dried rhizomes (a) *Zingiber officinale*, (b) *Kaempferia galanga* L, (c) *Curcuma longa*

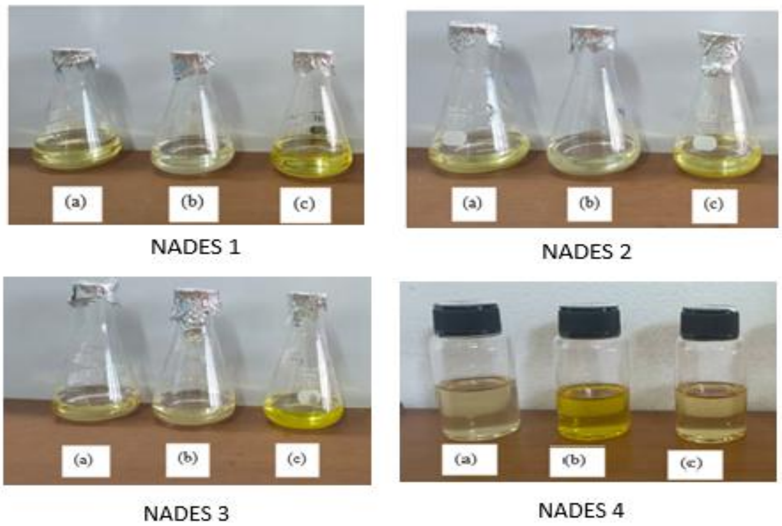

Figure 4. Polyphenol extracts using various NADES formulations from (a) *Kaempferia galanga* L., (b) *Curcuma longa*, and (c) *Zingiber officinale*.

NADES has the ability to extract active compounds from rhizomes. This can be seen from the extracted solution which is yellow in colour. Choline chloride combined with sugars such as fructose, glucose, maltose, and xylose can provide different compound concentration levels. The components in the extract can be analysed as phenolic acid.

Several previous studies have shown that the extraction of polyphenols from natural materials using NADES has proven to provide more effective results than conventional solvents. A study by Islamčević Razboršek et al. (2020) showed that choline chloride-based NADES has a higher polyphenol extraction capacity than conventional extraction using methanol.

c. Determination of Total Polyphenol Content

The total polyphenol content (TPC) in the rhizome extract was tested using the Folin-Ciocalteu method with gallic acid as the standard compound. The results of the extraction of polyphenol compounds from rhizome plants using the synthesised NADES is presented in Table 2.

Table 2: Polyphenols content in the extract of rizhome

NADES	TPC in extracts (mg/L)		
	Zingiber officinale	*Kaempferia galanga*	*Curcuma longa*
NADES 1	1292.14	1610.32	1002.59
NADES 2	308.55	334.37	294.29
NADES 3	381.25	454.17	328.13
NADES 4	1190.61	1027.78	930.39

In this study, it was concluded that NADES 1 is the best solvent in extracting polyphenols from rhizome plants. The maximum concentration has been found in *Kaempferia galanga* at 1610.32 mg/L. According to Chu et al. (2022), the efficiency of polyphenol extraction using NADES is 1.68 times greater than using 70% ethanol. Therefore, NADES has great potential for use in polyphenol extraction. The total polyphenol content obtained is reported as mg of gallic acid equivalents (GAE) per gram of dry extract is presented in Figure 5.

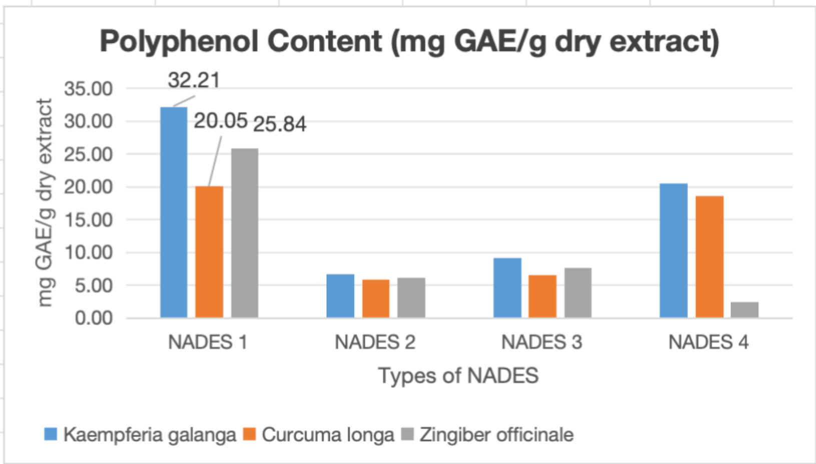

Figure 5. Polyphenol content in the extract of *Kaempferia galanga* L, *Curcuma longa* and *Zingiber officinale*

Polyphenol extraction in *Kaempferia galanga L* gave the best results using NADES 1 (ChCl-Fr) with a polyphenol content of 32.21 mg GAE/g extract. Under identical solvent conditions, *Curcuma longa* produced 20.05 mg GAE/g, and *Zingiber officinale* yielded 25.84 mg GAE/g.

The efficiency of polyphenol extraction in aromatic *Kaempferia galanga* L, *Curcuma longa* and *Zingiber officinale* was obtained from NADES with HBA and HBD arrangements, respectively, NADES 1> 4> 3> 2 or NADES based ChCl – Fr> ChCl- Xyl> ChCl – Mal> ChCl – Glu.

In contrast, NADES 2 and NADES 3 resulted in significantly lower TPC values, with all samples yielding less than 10 mg GAE/g. These findings indicate that the type of sugar used as the hydrogen bond donor (HBD) in the NADES formulation plays a crucial role in extraction efficiency. The ability of NADES in the polyphenol extraction process was influenced by several factors, such as polarity, viscosity, solubility, and physicochemical interactions, specifically hydrogen bonds between the solvent and the active compound (Rente, et al., 2023). Polyphenol is a secondary metabolite in plants with a phenol structure with an aromatic ring containing 1 or more hydroxyl groups (-OH) (Dheyab, et al., 2021). This study found the highest polyphenol content in rhizomes in NADES with the following composition ChCl-Fr > ChCl-Xyl > ChCl-Mal > ChCl-Glu. The factor influencing polyphenol content extracted from rhizomes by NADES was solubility. The "like dissolves like" theory stated that polar compounds dissolved well in polar solvents, and vice versa. In this case, polyphenol was a polar compound because it had hydroxyl groups. The order of HBD polarity used in this study included fructose> glucose> xylose> maltose. Therefore, ChCl-Fr was the most polar solvent, which aligned with the results that ChCl-Fr was the best solvent for extracting polyphenols from rhizome plants. However, the extraction of polyphenols from rhizomes using ChCl-Fr based NADES gave the highest polyphenol content.

These results align with the work of Dai et al. (2013), who found that NADES composed of ChCl and monosaccharides provided better solubility for phenolic acids than those made with disaccharides, due to their lower viscosity and stronger donor–acceptor interactions. Similarly, Bajkacz et al. (2018) reported that ChCl-based NADES with sugar components yielded higher phenolic recoveries from plant materials than conventional solvents like ethanol or methanol, particularly when the water content was maintained below 30%.

Furthermore, Barbieri et al. (2020) emphasised the role of viscosity as a limiting factor in extraction efficiency. Their findings demonstrated that using simpler sugars or adjusting water content to reduce viscosity improved phenolic yields. In line with these conclusions, the current study found that ChCl–Fr, a monosaccharide-based NADES, provided both low viscosity and strong hydrogen bonding capability, resulting in consistently higher TPC values.

4. CONCLUSION

In conclusion, NADES was an alternative solvent with great potential to be used to extract polyphenols in typical Indonesian rhizome plants. The overall order of extraction efficiency was found to be ChCl–Fru > ChCl–Xyl > ChCl–Mal > ChCl–Glu. Further studies related to the extraction of typical Indonesian rhizome compounds using NADES were needed to develop the potential application of NADES in food, medicine, and health.

5. ACKNOWLEDGMENT

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