Extraction of vitexin from binahong (*Anredera cordifolia* (Ten.) Steenis) leaves using betaine - 1,4 butanediol natural deep eutectic solvent (NADES)

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Extraction of Vitexin from Binahong (*Anredera cordifolia* (Ten.) Steenis*)* Leaves using Betaine - 1,4 Butanediol Natural Deep Eutectic Solvent (NADES)

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Abstract. The leaves of binahong (*Anredera cordifolia* (Ten) Steenis) contain flavonoids as bioactive substances that have efficacy to treat wounds and diseases caused by bacteria. One of the flavonoids contained in the leaves is 8-glucopyranosyl-4'5'7-trihydroxyflavone or vitexin. Conventional extraction of flavonoids from leaves of binahong has been developed and usually using non-friendly organic solvent. To overcome these problems, a Natural Deep Eutectic Solvent (NADES) is used to replace the conventional organic solvents, as it is an environmentally friendly, non-toxic and high boiling point solvent. In this study, a betaine-based NADES combined with 1,4-butanediol in 1:3 mole ratio was used as the extraction solvent. Vitexin in the extract was analyzed qualitatively and quantitatively using an HPLC. The extraction of vitexin from binahong leaves at room temperature (27 ^{o}C) for four hours give yield of 46 ppm, much lower than 200 ppm yield obtained after extraction at 55 ^{o}C for 90 minutes. This results showed that (a) NADES consisting of betaine and 1,4 butanediol is a promising green solvent for extraction of vitexin from binahong leaves, and, (b) the extraction can be performed above ambient temperature, as long as it does not exceed the degradation temperature of the bioactive compound extracted.

INTRODUCTION

Binahong (*Anredera cordifolia (Ten) Steenis*) is a medicinal plant from mainland China who can cure several diseases including kidney damage, diabetes, heart swelling, vomiting of blood, typhus, stroke, ulcer, gout and inflammation of the liver [1,2]. The phytochemical assays on the extract of binahong leaves showed that the leaves of binahong contain polyphenols, alkaloids, terpenoids, saponins and flavonoids. Binahong leaves ability to cure various types of diseases is closely related to these bioactive compounds [3]. The use of conventional solvents to extract flavonoids from medical plant such as binahong has been known widely. However, these conventional solvents sometimes are associated with the environmental problems as well as with health and safety issues [4]. Green solvents such as natural deep eutectic solvents (NADES) has emerged and developed to overcome the environmental problems, cost and toxicity issues of conventional solvents.

Deep eutectic solvents (DES) are mixtures of quaternary ammonium salts with a range of amides carboxylic acids and alcohols [5,6]. In Natural Deep Eutectic Solvent (NADES), choline derivatives as quaternary salts are mixed with common metabolites present in all types of cells and organisms such as sugars, sugar alcohols, amino acids, organic acids [7]. Quaternary salts such as choline chloride and betaine have roles as hydrogen bond acceptor (HBA) whereas organic acids or alcohols act as hydrogen bond donors (HBD) in this mixture. There is strong interaction between the protons of hydroxyl group from HBD with HBA. All molecules of NADES are arranged based on the hydrogen bonding and other physical intermolecular forces. The mixtures are liquids at room temperature, have lower melting than the melting point of each component [8]. Application of NADES as solvent extractant of biological compounds has been reported [8-11]. It was reported that many factors can affect the solubility of flavonoids from plants in NADES, such as HBA-HBD ratio, temperature and time of mixing.

The flavonoid glycoside compound in binahong leaves is known as vitexin (8-beta-D-Glucopyranosyl-apigenin) [12]. The application of NADES of betaine and 1,4 Butanediol in weight ratio of (1:3) as solvent extractant of binahong leaves has been investigated based on the the vitexin yield of extraction determined using

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high performance liquid chromatography (HPLC). The purpose of this study is to determine the effect of the extraction temperature on the yield of vitexin extracted from binahong leaves using NADES made of betaine and 1,4-butanediol.

MATERIAL AND METHODS

Plant material

The binahong cordifolia leaves were collected from the garden around Depok and identified by the Department of Botany, Indonesia Institute for Science. The sample leaves have been registered and identified as *Anredera cordifolia* leaves.

Chemicals

1,4-butanediol (>99.0%), ethanol (>99.0%), betaine (>98 %), were purchased from Sigma-Aldrich (Singapore). Vitexin (>99%) was purchased from Chengdu Biopurity Phytochemicals Ltd (China). All other solvents were high of performance liquid chromatography (HPLC) grade.

Preparation of Binahong Leaves Simplicia and NADES

Binahong leaves were cleaned and dried under shade in an airy room and at room temperature for seven days. Dry leaves were grinded and filtered using 65 mesh sieves. The simplicia of binahong leaves was stored in an airtight container and kept from contacting with sunlight. The betaine-alcohol based NADES were prepared by heating and stirring. The betaine and 1,4 butanediol were mixed in a mole ratio 1:3 of betaine to 1,4 Butanediol. The mixture was placed on a stirring hotplate at a temperature of 50 °C at 500 rpm, until a homogeneous liquid formed.

Extraction of Vitexin from Binahong Leaves

The extraction of vitexin from the simplicia of binahong was carried out by mixing NADES liquid with simplicia of binahong leaves with ratio of solid to liquid 1:10 (w / w) in sealed vials. The suspension was extracted by stirring at various times (1 hour, 2 hours, 4 hours, 6 hours and 8 hours) and at room temperature (25 °C). To investigate the effect of temperature, the temperature of extraction was raised to 55 °C, and the time of extraction was varied from 30 minutes until 240 minutes using a thermoshaker.

Quantitative and Qualitative Analysis using HPLC

Quantitative analysis of the yield obtained is very important to test the success of the yield. The method is applied to analyze the levels of flavonoids in leaf extract binahong in this study is HPLC. The suspensions obtained from each vials were cooled to room temperature and filtered through a filter paper. Each condition of extraction was repeated three times. Shimadzu LC-20AD HPLC Promienence UFLC Laboratory Department of Chemical Engineering FTUI used as analayzer. The column used was a C18 column and the eluent used was acetonitril. Standard solutions of vitexin were used to prepare the calibration curve for quantitative analysis of vitexin.

RESULT AND DISCUSSION

Preparation of NADES

A homogeneous liquid NADES formed from mixing betaine with 1,4-butanediol could takes as long as 10 days with regular slow mixing every 1-2 h. The liquid of NADES formation time is influenced by the interaction of two compounds to reach the eutectic point. The melting point of each compound affect the time required to obtain homogeneous liquid of NADES. High melting point of betaine which is 301 ^{o}C lead to longer time needed to mix with 1,4-butadienol to form a homogenous liquid. The formation of NADES of betaine and alcohol might have similar characteristic with NADES formed of choline and alcohols [7]. The proton of hydroxyl group of 1,4-butanediol may form a hydrogen bonding with betaine because of its simple and open structure allowing anion of betaine bonded to O-H groups of the alcohol from all directions (Fig 1).

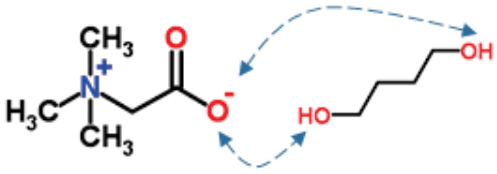

FIGURE 1. Interaction of betaine and 1,4-Butanediol forming a stable homogeneous liquid.

Effect of Time and Temperature on Yield of Extraction

The effect of extraction time and temperatures on the yield of vitexin obtained using ethanol and NADES (betaine and 1,4-butanediol in 1:3 mole ratio) is given in Table 1 and shown in Fig 2. Yields were determined as weight of vitexin extracted divided by weight of simplicia binahong leaves and reported in ppm. The obtained shows that the rate of extraction increased as the viscosity of solvent decreased. At room temperature the yield using ethanol was the much higher than that using NADES wheres the viscosity of the solvents are 1.1 and 25.0 cp, respectively. In addition, the kinetic energy from the stirring during extraction seemed not sufficient for NADES penetrating extensively into the matrix of simplicia leaves and bind the vitexin compound.

TABLE 1. Yield of vitexin as function of extraction temperature and duration at stirring rate of 500 rpm.

	Vitexin concentration in ppm			
	Ethanol	NADES		
Time	27 °C	27 °C	40 °C	55 °C
30	-	-	17.32	111.20
60	77.07	32.89	17.43	107.52
90	-	-	45.38	184.30
120	106.4	36.68	34.64	191.86
240	74.93	43.60	39.67	191.94

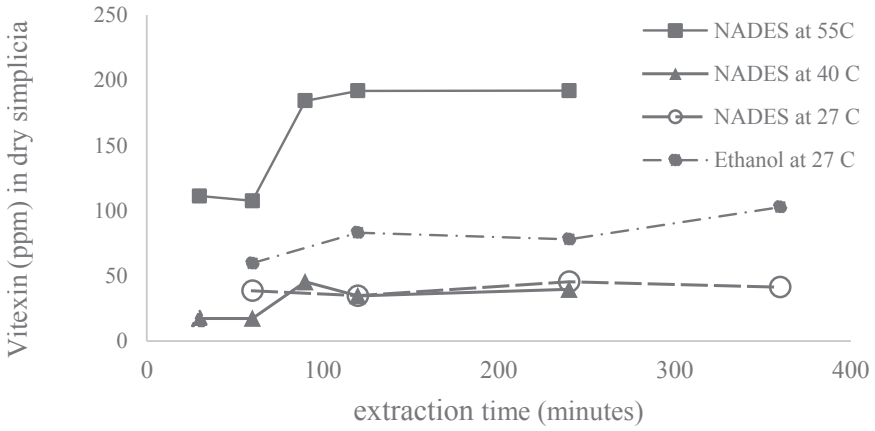

FIGURE 2. Effect of temperature and extraction time on vitexin yield using betaine-1,4-butanediol NADES and ethanol at stirring speed of 500 rpm.

In order to increase the yield of extraction, the temperature was increased to 40 and 55 °C and the time of extraction was varied from 30 minutes to four hours at stirring speed of 500 rpm. The results showed that increasing temperature of extraction from 40 to 55°C increased the amount of vitexin extracted almost five times, and, that extraction longer than 90 min at 55 °C will not increase the extraction yield significantly. The increase of extraction temperature on the extraction of binahong leaves using NADES of betaine and 1,4-

butanediol as extractant resulted in higher diffusion rate of solvent into the simplicia matrix increase as well as the solubility of vitexin in this solvent. One of the advantageous characteristics of NADES as the extracting solvent is their high boiling points compared to those of the conventional organic solvents such as ethanol. Extractions that uses ethanol cannot be performed at temperature higher than room temperature, due to the volatility of ethanol. When using NADES as solvent to extract bioactive compound from plant, the limiting upper temperature to be used is the degradation temperature of the bioactive compounds extracted.

CONCLUSION

Based on the results obtained in this research, vitexin could be extracted from the binahong leaves using NADES consisting of betaine and 1,4-butanediol, a non-toxic and environmentally friendly solvent. The extraction yield represented by the amount of vitexin in the NADES is affected by several factors, such as viscosity and solubility. At ambient temperature, extraction of vitexin from binahong leaves using this type of NADES does not give high yields. However, when the temperature of extraction is increased to 55 °C, the yield increases up to around five times of the yield obtained at ambient temperature of 27 °C. Hence, the extraction can be performed at temperature higher than ambient temperature, as long as it does not exceed the degradation temperature of bioactive compound extracted.

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