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Original Article

ANALYSIS OF GINGEROL AND SHOGAOL COMPOUNDS FROM RED GINGER (*ZINGIBER OFFICINALE* VAR. RUBRUM) EXTRACT USING SEVERAL COMBINATIONS OF NATURAL DEEP EUTECTIC SOLVENT

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ABSTRACT

Objective: The aim of this research was to analyze the levels of gingerol and shogaol in red ginger (*Zingiber officinale* var. rubrum) extracted with different combinations of Natural Deep Eutectic Solvent (NADES)

Methods: Red ginger was extracted using several combinations of NADES solvents, including Betaine: Citric Acid (1:2), Betaine: Malic Acid (1:1), Betaine: lactic Acid (1:2), Betaine: Tartaric Acid (1:1) and Betaine Oxalic Acid (1:1). The sieved ginger powder was added into 75 % v/v NADES in a solvent/solid ratio of 30/1. The mixture was ultrasonic extracted in an ultrasonic bath with an ultrasonic input power of 300 W and a frequency of 40 kHz under desired conditions. Analysis of the levels of gingerol and shogaol compounds in the extract was carried out using the validated High-Performance liquid Chromatography (HPLC) method. The method was validated by determining its specificity, linearity, accuracy, and precision.

Results: The results showed that the combination of NADES between betaine: lactic acid (1:2) is the best solvent that can extract the highest total gingerol and shogaol with a level of 15.09 mg/g.

Conclusion: The combination of NADES between betaine: lactic acid (1:2) can extract the highest total gingerol and shogaol compared to other NADES combinations.

Keywords: Red ginger (*Zingiber officinale* var. rubrum), NADES, Betaine, Organic acid, Gingerol and Shogaol

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INTRODUCTION

Zingiber officinale var. rubrum can be used as an alternative in the prevention of atherosclerosis. The results showed that ginger extract is effective for preventing the formation and development of coronary atherosclerosis due to its antioxidant and anti-inflammatory components [1]. The active compounds in ginger are gingerol and shogaol [2]. Gingerol and shogaol are compounds that are sensitive to heat and susceptible to dehydration at temperatures above 60 °C [3]. Gingerol and shogaol levels in the extract are influenced by the type and concentration of the extraction solvent. The most commonly used extraction solvents are organic solvents such as ethanol [4]. However, with improper handling, the use of organic solvents such as ethanol, hexane and ethyl acetate has a bad impact on the environment and ecosystems or even has an impact on humans so that it can trigger various diseases such as kidney damage, nerve disorders and even trigger cancer [5]. The use of conventional extraction methods such as maceration also requires a long time so it is less efficient when viewed from the factor of extraction time and extract yield [6].

Green extraction techniques continue to be developed to reduce energy consumption and the use of volatile solvents. Ultrasonic-Assisted Extraction (UAE) is a promising technology to be able to extract ingredients from plants without the need for high temperatures and does not require a long time. Research on the UAE method has been widely studied in the extraction of several plants [7, 8]. Green extraction is applied in addition to the extraction process by substituting the use of volatile solvents with green solvents. One of which is Natural Deep Eutectic Solvent (NADES). Ginger extraction using DES has been carried out; in this study 6-, 8-, 10-gingerol from ginger can be dissolved in DES better than using organic solvents. The best DES that can extract 6-, 8-, 10-gingerol is a combination of l-carnitine: 1,3-butanediol (1:4) with a water ratio of 25 % in UAE conditions with a solvent/solid ratio of 1:30 at 50 °C for 30 min resulted in a total gingerol yield of 4.41 mg/g [9]. However, there are still many NADES combinations that can be applied and researched so that it is possible to obtain better/higher levels of biomarkers (6-, 8-, 10-gingerol and 6-shogaol). The right combination of HBA and HBD will produce the appropriate polarity for the compound to be drawn as in the combination of betaine with organic acids [10].

MATERIALS AND METHODS

Plant material

The red ginger (*Zingiber officinale* var. rubrum) powder which was acquired from the Bogor Institute of Spice and Medicinal Research (BALITRO), Bogor, West Java, Indonesia. With the intention of macroscopically identifying the true plant to be utilized, the red ginger (*Zingiber officinale* var. rubrum) is determined by examining its leaves and rhizome. The Botanical Gardens Conservation Center in Bogor, West Java, Indonesia, was the site of the determination.

Chemical and reagent

The ingredients: 6-, 8-, 10-gingerol and 6-shogaol (Chromadex®, los Angeles, USA) as a standard Biomarker for the red ginger; choline chloride, betaine, l-carnitine (Qin Health Industry, Shaanxi, China), citric acid, malic acid, lactic acid, tartaric acid, oxalic acid (Merck, New Jersey, USA) as NADES solvent; ethanol 96 %; methanol pa (Merck, New Jersey, USA), Acetonitrile HPLC grade (Merck, New Jersey, USA), Aqua Pro Injection (Ika, Jakarta, Indonesia); distilled water from Research laboratory (Universitas Pakuan, Bogor, Indonesia).

Natural deep eutectic solvent (NADES) preparation

NADES is made with several types of mixture of Betaine components as Hydrogen Bond Acceptor (HBA) and acids in the form of Citric

Acid, Malic Acid, lactic Acid, Tartaric Acid and Oxalic Acid as Hydrogen Bond Donor (HBD) as shown in table 1. Mixture of HBA and HBD according to the mole ratio is placed on a hot plate stirrer at a temperature of 80 °C, stirred constantly using a magnetic stirrer at a speed of 200 rpm until a clear solution is formed for approximately 15 min. Next, 25 % water was added to reduce the viscosity of the NADES solution formed. The NADES liquid was left for 12 h at room temperature to ensure solution stability [9, 11, 12].

Table 1: NADES composition

Composition	NADES composition		
	HBA component	HBD component	Mole ratio
NADES 1	Betaine	Citric acid	1:2
NADES 2	Betaine	Malic acid	1:1
NADES 3	Betaine	Lactic acid	1:2
NADES 4	Betaine	Tartaric acid	1:1
NADES 5	Betaine	Oxalic acid	1:1

Preparation of extract

Simplicia red ginger powder was extracted using the UAE method with NADES solvent and 96 % ethanol as a comparison. A total of 0.5 g of simplicia and 15 ml of solvent (ratio 1:30), was extracted in a sonicate for 30 min at a temperature of 50 ℃. The extract formed was then transferred into a centrifuge tube and centrifuged at 2500 rpm for 25 min. The filtrate obtained was separated for assay using HPLC. Extraction was carried out three times in replication [9].

Extract analysis using HPLC

HPLC conditions

HPLC conditions for determining biomarker levels (6-, 8-, 10-gingerol and 6-shogaol) in red ginger are as follows [13]:

- HPLC system: HPLC Shimadzu® Prominence-I lC-2050C 3D
- Stationary phase: Column C18 Eclipse plus (150 mm x 4,6 mm; 5 µm)
- Mobile phase: Acetonitrile and water gradient as in the table 2
- Injection volume: 20 µl
- Flow rate: 1 ml/minute
- Detector: PDA (Photodiode Array Detector)
- Wavelength: 280 nm.

Table 2: Mobile phase composition for determining red ginger biomarker levels

Time (min)	Acetonitrile (%)	Water (%)
0	40	60
10.0	40	60
40.0	90	10
40.5	100	0
45	100	0
45.5	40	60
50	40	60

Preparation of mobile phase, standard solution and sample solution

The mobile phases used were acetonitrile and water. Each solution was filtered using 0.45 µm Whatman filter paper on a Buchner funnel with the help of a vacuum pump. Next, the solvent was aired using a sonicate for 30 min. The standard was prepared by a 1000 ppm Bio maker standard solution was prepared by weighing 1 mg each of the 6-gingerol, 8-gingerol, 6-shogaol, and 10-gingerol standards, then dissolving them in 1 ml of methanol. For testing, 100 µl of each solution was pipetted and diluted in a 5 ml flask, filled with methanol to the limit to obtain a concentration of 20 ppm. The solution was sonicated for 30 min, then left for 15 min, sonicated again for 30 min, then filtered using 0.45 µm Whatman filter paper. The sample preparation was done with diluted each extract, by pipetting 0.5 ml of the extract into 10 ml of methanol. The solution was sonicated for 30 min, then left for 15 min, and sonicated again for 30 min. The next step is filtering using 0.45 µm Whatman filter paper.

System suitability test

The test was carried out by injecting a standard solution of 6-, 8-, 10-gingerol and 6-shogaol with a concentration of 20 ppm each for 6 repetitions using the HPLC system according to point above. The data used are the area, retention time, number of theoretical plates and tailing factor of the chromatogram that appears, then the relative standard deviation value is calculated for each data [14].

Method validation

Before determining the levels of biomarkers (6-, 8-, 10-gingerol and 6-shogaol) in red ginger extract, it is necessary to validate the method, which is the process of assessing the action on a parameter carried out in the laboratory. The validation method parameters carried out are linearity, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy [15].

Linearity was carried out by preparing standard solutions of 6 different concentrations, namely 200; 150; 100; 50; 5 and 2.5 ppm. Each concentration was analyzed using HPLC by injecting 20 µl of solution. The calibration curve for the standard solution is obtained by plotting the peak area against the concentration of the standard solution to obtain the linear regression equation y = a+bx, which will also be used to calculate the compound levels in the sample.

LOD and LOQ

LOD and lOQ for biomarker compounds (6-, 8-, 10-gingerol and 6-shogaol) in red ginger can be calculated based on the calibration curve using the equation:

$$\text{LOD} = \frac{3 \text{ x SD}}{\text{b}}$$

$$\text{LOQ} = \frac{10 \text{ x SD}}{\text{b}}$$

Note: SD = standard deviation; b= b value in the regression equation

The precision tests carried out are repeatability (intra-day) and intermediate precision (inter-day). Precision was carried out on 6 samples. Each sample was weighed at 0.5 g, then extracted using 15 ml of NADES solvent and 96 % Ethanol with a ratio of 1:30 in an

ultrasonic bath for 30 min at a temperature of 50 °C. The extract formed was then transferred into a centrifuge tube and centrifuged at 2500 rpm for 25 min. Filtered using a 0.45 µm cellulose acetate filter, then diluted by pipetting 0.5 ml and adding methanol to 10 ml, then put into an autosampler vial. The sample was injected into the HPLC as much as 20 µl. Intra-day precision is carried out by analyzing all six samples on the same day, while inter-day precision is carried out by determining the levels on the following day. Precision data is obtained from the relative standard deviation (RSD) value.

Accuracy testing was carried out by determining recovery using the spike method. This method is carried out by analyzing the levels of biomarkers (6-, 8-, 10-gingerol and 6-shogaol) in the un-spike sample first, then adding spike or standard solution to the un-spike sample and then analyzing the levels again. Un-spike samples were made and analyzed the same as the precision test, whereas for spike samples first 3 standard solutions were made with different concentrations (80 %, 100 % and 120 %) then added to the un-spike samples. Samples with a concentration of 100 % were analyzed using HPLC 6 repetitions, while concentrations of 80 % and 120 % were analyzed 3 repetitions each. Percent recovery was calculated by subtracting the sample spike content from the un-spike content, divided by the added standard concentration and multiplying by 100 %.

Determination of biomarker compound levels in red ginger extract

The sample solution from the UAE red ginger extract using NADES solvent and the UAE using 96 % ethanol solvent was injected into the HPLC according to the HPLC system that had been tested. The data used are area and retention time. Calculation of biomarker compound levels was carried out using a calibration curve obtained from linearity calculations.

RESULTS AND DISCUSSION

NADES preparation and extraction result

NADES is a combination of Betaine and Acid that has been made to form a clear liquid with varying viscosity, so the addition of water is necessary to reduce the viscosity. The amount of water added is 25 %. The NADES that has been made shows that the liquid is quite stable, because it does not change again after 12 h of standing. NADES fluid can be seen in fig. 1.

Fig. 1: NADES fluid

Eutectic conditions can occur due to a decrease in the melting point during NADES preparation. This is due to charge delocalization through the interaction of HBD with anions from HBA so that when the two are mixed and heated it will produce a thick solution with high viscosity [16]. The high viscosity of NADES can affect mass transfer during extraction, thereby affecting the recovery of active substance levels [17]. To avoid this, it is necessary to reduce the viscosity level by adding water at the end of the manufacturing process.

Extraction of red ginger was carried out using NADES, which had been made and 96 % ethanol as a comparison. The extraction results can be seen in fig. 2.

Fig. 2: Extract NADES red ginger

UAE is an extraction method that uses ultrasonic waves to break down cell walls and then release the cell contents into the extraction medium, namely NADES [16]. The extraction process using UAE is very effective in extracting the active compounds contained in a material more effectively can be applied to those that are thermo labile, and uses less solvent and requires low energy [17].

System suitability test result

System suitability tests are carried out to verify that the parameters obtained meet the requirements so that the method can be used [15]. Based on the data obtained, it can be concluded that all parameters are within the range of acceptance requirements. This shows that the HPLC tool can be used to determine the levels of 6-, 8-, 10-gingerol and 6-shogaol compounds from red ginger. Acceptance requirements and system suitability test results are listed in table 3.

Method validation result

The validated HPLC method helps analysts produce consistent and accurate data. The following are the results of the method validation of the standard red ginger in table 4.

Table 3: System suitability test results for red ginger standard

Acceptance conditions	Condition value	Red ginger standard				Conclusion
		6-gingerol	8-gingerol	10-gingerol	6-shogaol	
Theoretical Plate (N)	N>2500	8814	62818	136285	73865	Acceptable
Tailing Factor (TF)	TF ≤ 2.0	1.09	1.11	1.12	1.10	Acceptable
% RSD Retention time	% RSD<1.0	0.15	0.09	0.07	0.08	Acceptable
% RSD Area	% RSD ≤ 2.0	0.29	0.29	0.32	0.28	Acceptable

Table 4: Method validation result for red ginger standard

Acceptance conditions	Condition value	Red ginger standard				Conclusion
		6-gingerol	8-gingerol	10-gingerol	6-shogaol	
Linearity	r>0.99	0.9998	0.9988	0.9986	0.9991	Acceptable
LOD	µg/ml	1.0876	1.002	1.0341	0.9495	Acceptable
LOQ	µg/ml	3.6253	3.3397	3.4469	3.1651	Acceptable
Accuracy	% Recovery 85-110	99.2991	93.5257	89.1526	91.0241	Acceptable
Precision	% RSD ≤ 2.0	1.43	1.39	1.41	1.49	Acceptable

Determination result of biomarker compound levels in red ginger extract

The results of HPLC analysis for the compounds 6-, 8-, 10, gingerol and 6-shogaol in red ginger showed that the retention time between the compounds in the sample and the standard compounds appeared at relatively the same time. The retention time of the 6-gingerol compound was 9.587±0.0015 min, 8-gingerol was 19.963±0.0176 min, 10-gingerol was 26.735±0.0203 min and 6-shogaol was 21.484±0.0170 min. The following are the results of standard chromatograms and samples of red ginger extract extracted using NADES, which can be seen in fig. 3.

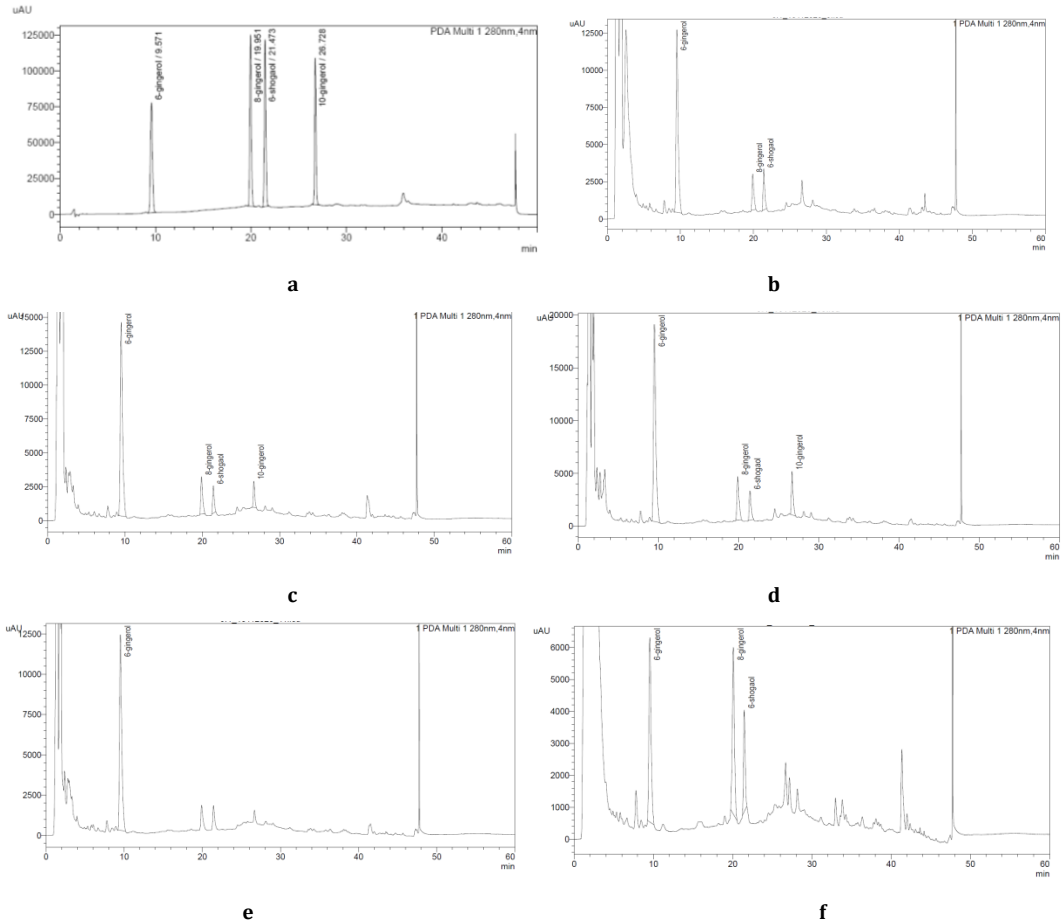

Fig. 3: Standard chromatogram and NADES extract of red ginger

Note: a) Standard Chromatogram of 6-, 8-, 10-gingerol and 6-shogaol, b) Chromatogram of NADES 1 extract red ginger, c) Chromatogram of NADES 2 extract red ginger, d) Chromatogram of NADES 3 extract red ginger, e) Chromatogram of NADES 4 extract red ginger, f) Chromatogram of NADES 5 extract red ginger.

Determination of levels is carried out based on the results of HPLC analysis. The results can be seen in table 5.

Based on the table above, the composition of NADES shows different results regarding the number of compounds extracted. NADES 3,

which is a combination of betaine: lactic acid (1:2), is a NADES combination that can extract the highest total compounds in red ginger of 15.09 mg/g. This can be influenced by various factors such as the level of polarity, surface tension, viscosity, and interactions between NADES and compounds [3].

The like-dissolves-like theory states that compounds will dissolve in solvents with the same properties, one of which is polarity. Solvents that are polar will more easily attract compounds that are porous, and vice versa. The polarity of a solvent can be measured from the number of hydroxyl groups it has. Based on its structure, lactic acid has fewer hydroxyl groups than other types of acid. Apart from polarity, the viscosity level of NADES is also an important factor in compound extraction. The ability of NADES in extraction will decrease with increasing viscosity because high viscosity will inhibit the mass transfer of compounds from the cell into the solvent [7, 8].

Table 5: Determination of 6-, 8-, 10-gingerol and 6-shogaol content in the extract

NADES	Active substances content mg/g				
	6-gingerol	8-gingerol	6-shogaol	10-gingerol	Total
1	6.41	1.58	1.11	0.00	9.11
2	7.51	1.67	0.85	1.15	11.18
3	9.81	2.16	1.15	1.96	15.09
4	6.41	0.00	0.00	0.00	6.41
5	3.07	0.29	1.19	0.00	4.55

CONCLUSION

The combination of NADES between betaine: lactic acid (1:2) is the best solvent that can extract the highest total gingerol and shogaol with a level of 15.09 mg/g compared to other NADES combinations.

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AUTHORS CONTRIBUTIONS

Yulianita: contributed to sample preparation, carried out the experiments, analyzed the data and administration of the research; Fadlina Chani Saputri: contributed to the interpretation of the results; Abdul Mun'im: conceived and planned the experiments, support the financial research; Arry Yanuar: supervised and verified the result; All authors provided critical feedback and helped shape the research, analysis and manuscript.

CONFLICT OF INTERESTS

The authors declare no conflict of interest.

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