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The Optimum Condition Design of Microwave-assisted Extraction Combined Lactic Acid-Sucrose Based Natural Deep Eutectic Solvent on Polyphenols Enrichment from *Eleutherine bulbosa* Mill. Urb. Bulbs

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ABSTRACT: The advantages of natural deep eutectic solvent (NADES) for bioactive extraction to create formulations for nutraceuticals and cosmetics include biodegradability, biocompatibility, secondary metabolite extractability, and food grade. This study aims to design the optimum condition of microwave-assisted extraction (MAE) combined lactic acid-sucrose (LA-Suc)-based NADES on polyphenolic enrichment from the bulbs of *Eleutherine bulbosa* Mill. Urb. using response surface methodology (RSM). In the present study, the condition of MAE-combined LA-Suc-based NADES parameters, including NADES (LA-Suc) ratio, microwave irradiation time, solid-liquid ratio, and microwave irradiation power, had been involved. RSM with four factors and three levels (Box-Behnken Design) was applied to design a predictive model and optimize the MAE-combined LA-Suc-based NADES condition. The response surface was calculated using the total polyphenols content (TPC) value as the dependent variable. According to the result, the optimum condition of MAE combined LA-Suc based NADES was recommended according to RSM analysis as follows: NADES (LA-Suc) ratio of 4:1 g/g, irradiation time of 10 min, the solid-liquid ratio of 1:10 g/mL, and irradiation power of 360 Watts. The scale-up confirmation test using a 50 g sample (ten times the number of the sample) was observed to be 82.434 ± 1.623 mg GAE/g sample.

KEYWORDS: *Eleutherin bulbosa* Mill. Urb.; Box-Behnken design; natural deep eutectic solvent; response surface methodology.

1. INTRODUCTION

The flora of Kalimantan (Indonesia) consists of abundant wild and cultivated endemic plants and many of which have been scientifically proven to possess biological activities [1]. Currently, global demand for natural products has been rising rapidly in the last decade, especially in the pharmaceutical, cosmetic and nutraceutical industries [2]. Plants are primary sources of bioactive natural products. The most commonly associated bioactive compounds from plants are polyphenols. Polyphenols have become a major concern in the search for natural raw materials since they have beneficial effects on human health such as antioxidant, antimicrobial, anticancer, anti-inflammatory, and chemoprotective potential. For this reason, many studies have been conducted to explore natural products containing polyphenols [3].

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Plant material undergoes various processes such as drying, extraction, separation and purification to obtain the desired secondary metabolites or enrichment extracts. The use of non-food grade organic solvents, the extraction process, the extraction method selection and the complexicity of secondary metabolite content during these bioprocess conditions directly affect extraction yield and extract quality. In addition, conventional extraction techniques for natural products have limitations, such as time and high energy consumption, low extraction capability, and toxic solvents. Therefore, it is needed to develop separation and extraction techniques for compounds of interest integrated with environmentally friendly practices with a green chemistry principles. One of the approaches is using green solvents and innovation and sustainable extraction technology [4,5].

Natural deep eutectic solvents (NADES) are mixtures of compounds that can remain liquid at room temperature. NADES has excellent biodegradability, biocompatibility, renewable, easy-to-make, secondary metabolite extractability, and food-grade [6,7]. One of the combinations of NADES composition that attracted our attention to study was the combination of lactic acid and sucrose. Several recent studies have succeeded in applying this composition for the enrichment of polyphenols from plants, including the extraction of polyphenols and caffeine from coffee beans [8], extraction of polyphenols from some plants, including *Foeniculum vulgare*, *Mentha spicata*, *Origanum dictamnus*, *Origanum majorana*, and *Salvia officinalis* [9], extraction of steroidal saponins from *Trillium govanianum* [10], and the bioactive metabolites extraction of *Humulus lupulus* L [11].

Microwave-assisted extraction (MAE) is one of the extraction technology innovation methods that is often used and combined with NADES [12,13]. This method is adequate, easy, and fast in extracting secondary metabolites from natural products. In addition, this method can be adjusted according to the target secondary metabolites [14]. Several MAE conditions that can be used as variables include extraction time, microwave power, and sample: solvent ratio. The design of optimization method can be done using the response surface methodology (RSM). RSM is a statistical tool that can predict the optimum condition of MAE according to the target secondary metabolite properties by examining the interaction between various MAE conditions on the response [15,16].

Eleutherine bulbosa (Mill.) Urb. (*E. Bulbosa*) is one of the native plants of Kalimantan, which has been used traditionally to treat various diseases such as hypertension, diabetes, prostate, gout, cancer/cysts, bronchitis, gastrointestinal disturbances, sexual disorders, and stamina [17]. *E. bulbosa* bulbs are rich in secondary metabolites such as terpenoids, polyphenols, and tannins [18,19]. Based on our best knowledge, some studies on *E. bulbosa* have been reported mainly related to the development of extraction methods, including the effect of extraction method on thin layer chromatography (TLC) profile and sunscreen activity [20], oral glucose tolerance activity [21], the effect of heat-assisted extraction on phenolic compound [22], and extraction polyphenols using NADES (citric acid-glucose) based MAE [23]. The results of previous studies on using citric acid-glucose as the composition of NADES showed promising results and prospects for continued development. Therefore, in this study, we focus on using lactic acid-sucrose (LA-Suc) as a constituent of NADES, considering that the design and optimization of this composition have not been reported.

Therefore, the main goal of our present study is to design the optimum condition of MAE combined LA-Suc based NADES on polyphenolic enrichment from *E. bulbosa* bulbs using RSM with four factors and three levels (Box-Behnken Design).

2. RESULTS AND DISCUSSION

2.1. Single factor screening

In this study, single-factor screening was performed using some different levels of each variable factor, including ratio of NADES (LA-Suc) ratio (2:1, 3:1, and 4:1 g/g), microwave irradiation time (10, 15, and 20 min), microwave irradiation power (180, 270, and 360 Watts), and solid-liquid ratio (1:10, 1:12, and 1:14 g/mL) are shown in Figure 1.

Figure 1A shows that the highest total polyphenols content (TPC) value was obtained at the NADES (LA-Suc) ratio of 3:1 g/g. Using lactic acid and sucrose combination with different ratios was selected as a NADES component according to some previous studies [8,10,11], and added 30% purified water was to adjust viscosity, and secondary metabolites target mainly polyphenols group [24]. Lactic acid is a NADES component with hydrogen bonding acceptor (HBA) properties, while sucrose has hydrogen bonding donor (HBD) properties [25–27]. Study on HBD-HBA combinations paves the way for tailoring the nature, physical properties, and phase behavior of eutectic solvents. In such combinations of NADES components, which are produced by mixing solid materials with high melting points, hydrogen bonding interactions are the primary cause of the eutectic phenomenon [11].

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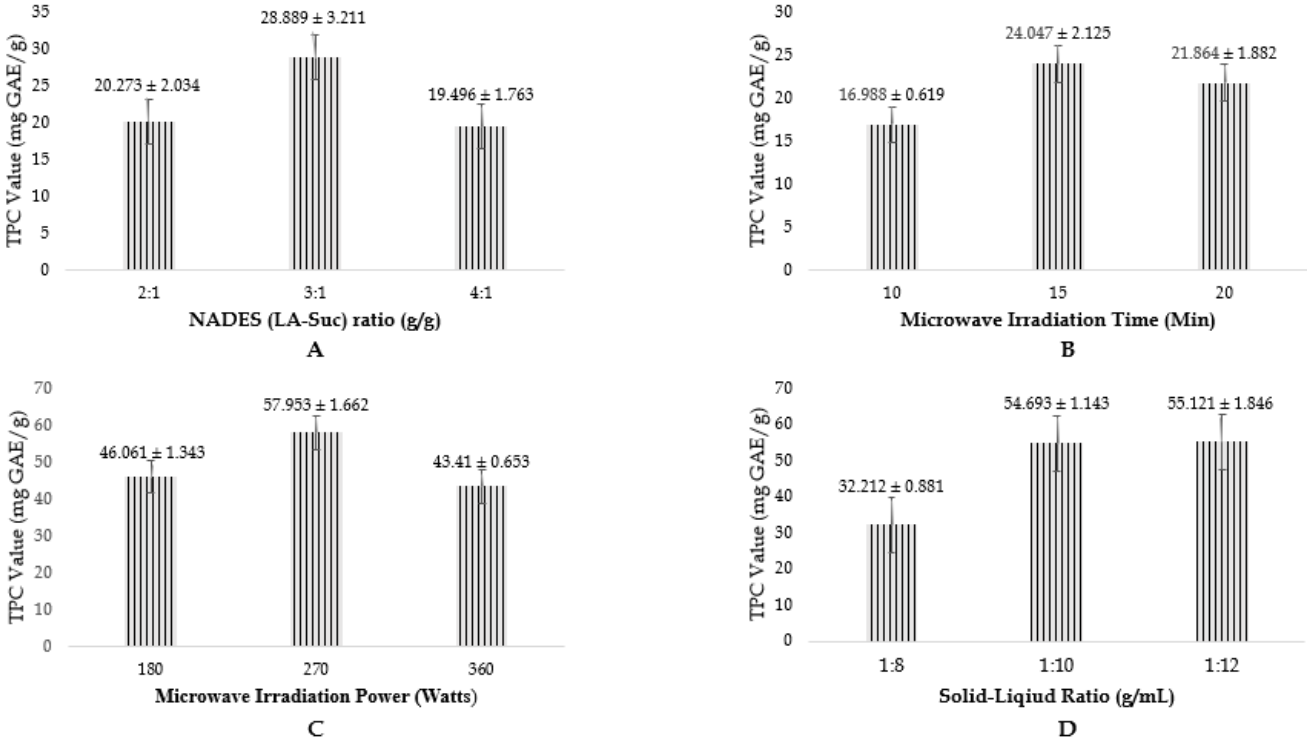

Figure 1. Single factor effect of condition of MAE combined NADES on TPC value (Mean ± SD) from *E. Bulbosa* bulbs performed in triplicate (n=3)

Figure 1B shows the effect of microwave irradiation time at 10 to 20 min. The TPC value increased from the 10th min to the 15th min and decreased at the 20th min. The maximum TPC value was obtained in 15 min of irradiation. This irradiation time indicates that the dissolution of the target secondary metabolites in the sample achieved a balance of target metabolite concentrations at the irradiation time of 15 minutes [28,29].

Figure 1C shows the impact of microwave radiation power on the TPC value. The maximum TPC value was obtained at 270 watts power of microwave irradiation. The increase in strength is directly proportional to the increase in temperature. Excessive increases in temperature can damage the surface of the sample cell wall and change the structure of polyphenols.

The solid-liquid (sample-solvent) ratio is another factor that significantly influences the extraction process, as shown in Figure 1D. The ratio variation in the range of 1:8 to 1:12 g/mL showed a tendency to increase the TPC value. However, increasing the ratio of the amount of solvent excessively can cause the extraction process to be ineffective (waste of solvent). In contrast, the use of a small amount of solvent causes the extraction process to be imperfect. The results of solid-liquid ratio screening showed that the ratio of 1:10 g/mL was more optimal than 1:8 g/mL and more effective than 1:12 g/mL.

2.2. The optimum condition design of MAE combined LA-Suc based NADES on TPC value

The efficiency of polyphenols extraction using the experimental design from RSM varied depending on the MAE combined LA-Suc based NADES conditions (Table 1). The result from 29 runs demonstrated extraction condition with the highest TPC value of 80.432 ± 2.121 mg/g sample (NADES ratio of 3:1 g/g, microwave irradiation time of 20 min, solid-liquid ratio of 1:12 g/mL, and microwave irradiation power of 360 watts). In comparison, the lowest TPC value was 25.311 ± 0.454 mg/g sample ((NADES ratio of 3:1 g/g, microwave irradiation time of 20 min, the solid-liquid ratio of 1:12 g/mL, and microwave irradiation power of 180 watts).

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Table 1. Experimental design results of TPC value from *E. bulbosa* using RSM with BBD

No	Variable Factor				Total Polyphenols Content (TPC)Value*
	NADES (LA-Suc) Ratio	Microwave Irradiation Time	Solid-Liquid Ratio	Microwave irradiation power	
	X_1 (g/g)	X_2 (Min)	X_3 (g/mL)	X_4 (Watt)	Mean ± SD (mg GAE/g)
1	3:1	20	1:10	270	43.413 ± 1.432
2	2:1	15	1:14	270	53.638 ± 1.356
3	4:1	15	1:12	180	49.551 ± 1.221
4	4:1	15	1:10	270	50.642 ± 2.154
5	4:1	15	1:12	360	60.713 ± 2.217
6	3:1	15	1:12	270	29.459 ± 0.656
7	3:1	15	1:12	270	34.454 ± 0.543
8	4:1	20	1:12	270	58.732 ± 2.198
9	3:1	15	1:14	360	30.534 ± 1.467
10	3:1	15	1:12	270	29.162 ± 0.521
11	3:1	20	1:12	180	25.311 ± 0.454
12	3:1	15	1:10	180	43.252 ± 1.244
13	3:1	10	1:12	360	65.174 ± 1.162
14	3:1	20	1:14	270	53.878 ± 1.133
15	2:1	15	1:10	270	43.579 ± 0.433
16	4:1	10	1:12	270	51.342 ± 1.326
17	2:1	15	1:12	180	49.744 ± 2.154
18	2:1	20	1:12	270	45.858 ± 1.278
19	4:1	15	1:14	270	60.612 ± 1.335
20	3:1	15	1:12	270	30.164 ± 1.352
21	3:1	10	1:14	270	35.122 ± 2.278
22	2:1	10	1:12	270	45.752 ± 3.187
23	3:1	10	1:10	270	58.321 ± 2.333
24	2:1	15	1:12	360	47.441 ± 1.376
25	3:1	15	1:10	360	60.889 ± 2.177
26	3:1	15	1:14	180	55.258 ± 1.197
27	3:1	15	1:12	270	35.704 ± 0.716
28	3:1	20	1:12	360	80.432 ± 2.121
29	3:1	10	1:12	180	61.433 ± 2.177

*each data was analyzed in triplicate (n=3)

The automatic Design Expert software system suggests a standard quadratic model based on the fit model summary statistic result (Table 2). The Fit Summary compiles the crucial statistics to choose the ideal model's initial condition. The Whitcomb Score is used to select the suggested model. The recommended model might not be the ideal one to employ. A negative coefficient correlation (R^2) value might suggest that the model needs to be simplified.

Table 2. Fit model summary statistic

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	Recommendation
Linear	13.37	0.1038	-0.0456	-0.2662	
Two-Factor Interaction	12.56	0.4068	0.0772	-0.3415	
Quadratic	9.05	0.7607	0.5215	-0.3454	Suggested
Cubic	6.00	0.9549	0.7897	-4.3773	Aliased

To better understand the relationship between both variables (independent and dependent), the results were modified to a second-order polynomial model. The analysis of variance (ANOVA) was used to estimate the statistical significance of the independent variables, their interactions, and predicted models. **Table 3** shows the best reduced quadratic model according to the ANOVA results. The model was remarkably significant, with an F-value of 18.10 and a p-value of 0.0001 for monitored responses. The model's suitability was further supported by the F-value of 2.43 and non-significant Lack of Fit (p-value>0.05). A large Lack of Fit F-value has a 20.32 percent chance of being caused by noise. A high R2 value for the response of TPC value indicates that the experimentally discovered and predicted values fit together well. In this study, X_1, X_3, X_2X_3, X_2X_4, X_3X_4, X_1^2, X_2^2, X_4^2, $X_1^2X_3$, $X_2^2X_4$, and $X_1^2X_3^2$ are significant model terms.

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Table 3. Response surface quadratic model with ANOVA

Source	Sum of Square	df	Mean Square	F-value	p-value
Model	4501	13	346.27	18.10	< 0.0001
X_1-NADES (LA-Suc) Ratio	173.05	1	173.05	9.04	0.0088
X_2-Irradiation Time	7.54	1	7.54	0.39	0.5397
X_3-Solid-Liquid Ratio	120.75	1	120.75	6.31	0.0239
X_4-Irradiation Power	0.39	1	0.39	0.020	0.8881
X_2X_3	283.42	1	283.42	14.81	0.0016
X_2X_4	659.98	1	659.98	34.49	< 0.0001
X_3X_4	448.80	1	448.80	23.46	0.0002
X_1^2	133.05	1	133.05	6.95	0.0187
X_2^2	966.20	1	966.20	50.50	< 0.0001
X_4^2	1060.72	1	1060.72	55.44	< 0.0001
$X_1^2X_3$	210.87	1	210.87	11.02	0.0047
$X_2^2X_4$	560.18	1	560.18	29.28	< 0.0001
$X_1^2X_3^2$	418.06	1	418.06	21.85	0.0003
Residual	298.99	15	19.13		
Lack of Fit	249.62	11	14.51	2.43	0.2032
Pure Error	37.37	4	9.34		
Cor Total	4788.55	28			

Table 4 shows the coefficient estimate of the regression equation that represents the expected change in the TPC (dependent variable) response value per unit change in the independent variable, where other factors are considered constant. The coefficient estimate is associated with the standard deviation. Confidence interval with 95% high and low shows a range of one positive limit and the other negative, indicating a significant or insignificant term in the coefficient that is considered correct [30]. The variance around the coefficient estimates that result from a lack of orthogonality in the design is measured using the variance inflation factor (VIF). The VIF is one factor that is orthogonal to every other factor in the model. Values over 10 signify that the variables are overly correlated with one another (they are not independent.) When working with a mixture and constrained response surface designs, VIFs are less significant [31,32].

Table 4. Coefficient estimate, degree of freedom, standard error, confidence interval, and variance inflation factor (VIF) from each factor

Factor	Coefficient Estimate	df	Standard Error	Confidence Interval		VIF
				95% Low	95% High	
Intercept	32.90	1	1.74	29.20	36.61	
X_1-NADES (LA-Suc) Ratio	3.80	1	1.26	1.11	6.49	1.00
X_2-Irradiation Time	-0.79	1	1.26	-3.48	1.90	1.00
X_3-Solid-Liquid Ratio	0.389	1	1.55	-7.18	-0.59	1.50
X_4-Irradiation Power	0.22	1	1.55	-3.07	3.52	1.50
X_2X_3	8.42	1	2.19	3.76	13.08	1.00
X_2X_4	12.85	1	2.19	8.18	17.51	1.00
X_3X_4	-10.59	1	2.19	-15.25	-5.93	1.00
X_1^2	4.95	1	1.88	0.95	8.95	1.30
X_2^2	12.98	1	1.83	9.09	16.87	1.23
X_4^2	12.60	1	1.83	9.71	17.49	1.23
$X_1^2X_3$	8.89	1	2.68	3.18	14.60	1.50
$X_2^2X_4$	14.49	1	2.68	8.78	20.20	1.50
$X_1^2X_3^2$	12.27	1	3.05	7.76	20.77	1.68

The equation formula obtained was $Y = 32.90 + 3.80X_1 - 0.79X_2 + 0.39X_3 + 0.22X_4 + 8.42X_2X_3 + 12.85X_2X_4 - 10.59X_3X_4 + 4.95X_1^2 + 12.98X_2^2 + X_4^2 + 8.89X_1^2X_3 + 14.49X_2^2X_4 + 12.27X_1^2X_3^2$, with R^2 of 0.9401. Where X_1 is the NADES (LA-Suc) ratio, X_2 is microwave irradiation time, X_3 is the solid-liquid ratio, X_4 is microwave irradiation power, and Y is the yield of TPC value. The R^2-predicted of 0.5318 is not as close to the R^2-adjusted of 0.8881 as one might typically expect. All empirical models should be tested using confirmation runs. Adeq-precision, also known as adequate precision, quantifies the signal-to-noise ratio. The ideal ratio is at least 4. An adeq-precision ratio of 18.137 indicates a good signal. Use this model to navigate the design space [30].

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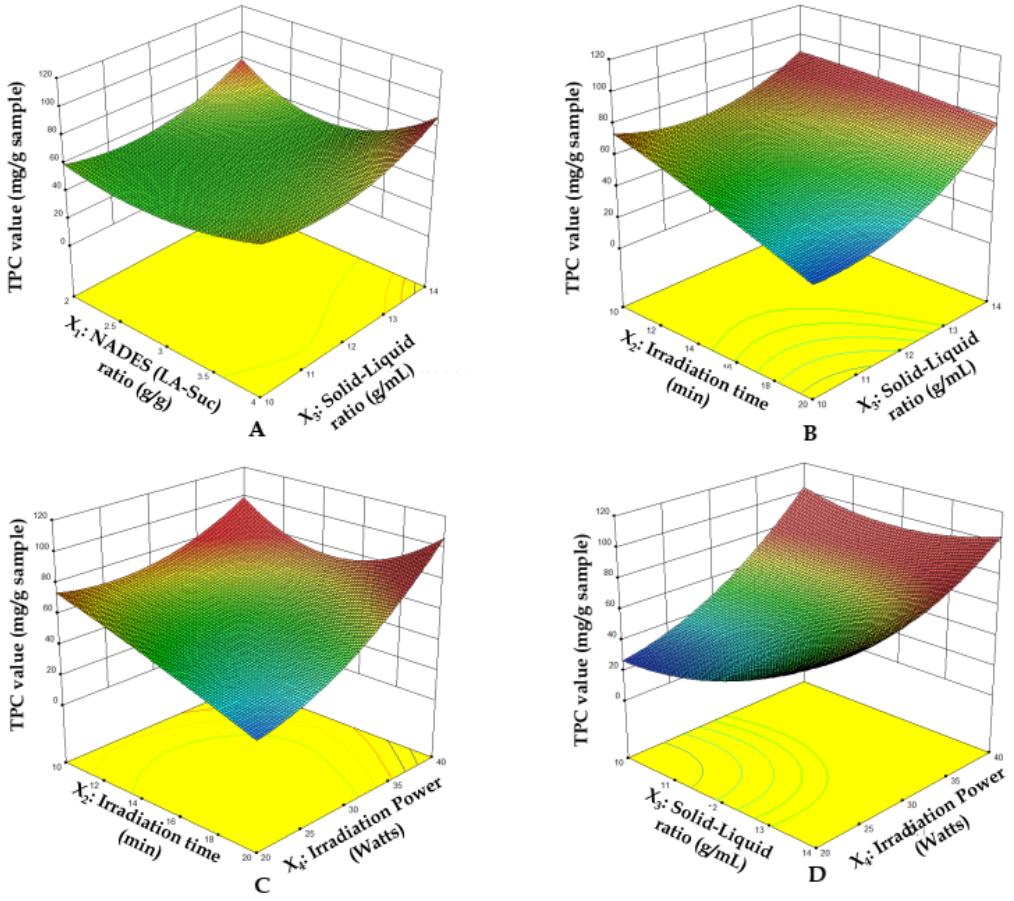

Figure 2. Three-dimensional contour plot interaction of factors and response

The optimum MAE combined LA-Suc based NADES condition was recommended according to the equation formula from RSM analysis as follows: NADES (LA-Suc) ratio of 4:1 g/g, microwave irradiation time of 10 min, the solid-liquid ratio of 1:10 g/mL, and microwave irradiation power of 360 Watts with predicted the TPC value of 90.312 ± 3.359 mg GAE/g sample. The scale-up confirmation test using a 50 g sample (ten times the number of the sample) was observed to be 82.434 ± 1.623 mg GAE/g sample. According to the results, MAE-combined LA-Suc-based NADES has a better extraction ability than NADES with citric acid and glucose composition in our previous study [23]. However, using NADES as an alternative solvent is very effective for extracting the target secondary metabolites from plants compared to conventional methods, as has been done by Munaeni et al. [33] and Shi et al. [34]. In addition, NADES is used not only as a solvent but also as an excipient to be formulated into the desired pharmaceutical product.

3. CONCLUSION

In the present study, the MAE-combined LA-Suc-based NADES was successfully used to extract polyphenols enrichment from *E. bulbosa* bulbs. In this regard, both the scientists and industry community are becoming more interested in NADES. Herein, lactic acid-based NADES were proposed for the firs time to tailor polyphenols extracts from *E. bulbosa* bulbs with the optimum condition including NADES (LA-Suc) ratio of 4:1 g/g, microwave irradiation time of 10 min, the solid-liquid ratio of 1:10 g/mL, and microwave irradiation power of 360 Watts. The results demonstrated that it was possible to enrich the polyphenol extracts by appropriately choosing the HBD/HBA pair. Comparing citric acid: glucose and conventional extraction with the organic solvent, lactic acid: sucrose demonstrated a significantly higher capacity to extract polyphenols. The extraction of phytoextracts that could be directly incorporated into food, dermatological, cosmetic, and other formulations was made possible by these simple, affordable, environmentally friendly, and effective solvents in addition to the conventional extraction process. Additional study is still needed to fully understand how the components of NADES interact with plant phytochemicals.

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4. MATERIALS AND METHODS

4.1. General equipment, chemical, and plant material

The equipments used include a Modena Microwave 900 watts (Buono-MV 3002, USA), Vortex mixer (Stuart, Germany), UV-VIS spectrophotometer double beam (Shimadzu, Japan), Magnetic Stirrer (Thermo Scientific, USA), food dehydrator (Wiratech, Indonesia), and licensed software of Design Expert V12 (Statease Inc. Minneapolis, MN, USA). The chemical materials used in this study were lactic acid and sucrose (food/pharmaceutical grade) purchased from CV. Chlorogreen, Bandung, Indonesia. Folin-Ciocalteau reagent, standard gallic acid, and sodium carbonate were purchased from PT. Elokarsa LLC, Indonesia (distributor of Sigma-Aldrich or Merck products). Ethanol p.a., methanol p.a., and purified water were purchased from PT. SmartLab, Tangerang, Indonesia. At the same time, *E. bulbosa* bulbs were collected from Kutai Kertanegara, Indonesia, and authenticated at the Laboratory of Dendrology, Universitas Mulawarman. The specimen (010/PTUP-LP/FFUNMUL/VI/2022) was stored at the Pharmaceutical Research and Development Laboratory of Universitas Mulawarman.

4.2. The preparation of NADES with LA-Suc composition

Lactic acid and sucrose were mixed to obtain LA-Suc-based NADES. LA-Suc-based NADES is made by heating a water bath while stirring continuously at 50^{o}C for 30-40 minutes until a homogeneous solution is obtained. Then, the solution was added to deionized water (30% of the total NADES). The liquid of LA-Suc-based NADES was stored at room temperature until ready for use.

4.3. The procedure of MAE combined LA-Suc based NADES

The MAE combined LA-Suc-based NADES was performed according to previous studies [23,35–37], with a slight adjustment. Briefly, a 5 g dried sample of *E. bulbosa* bulbs was added with a certain amount of LA-Suc-based NADES solution. The mixture was extracted using a certain condition of MAE. After completion of the extraction process, then the extract solution and residue were separated using a Buchner filter. Furthermore, it is stored in a food dehydrator until it is ready to be measured for its secondary metabolite content.

4.4. The determination of total polyphenols content

According to previous studies [23,38–40], the total polyphenols content (TPC) was determined using the Folin-Ciocalteu assay method, with gallic acid as a standard. A UV-VIS spectrophotometer was used to measure the absorbance of the sample and the standard at a wavelength of 761 nm (maximum wavelength) [23]. TPC values were examined using linear regression of Y= 0.001559X + 0.015 and coefficient correlation (R^2) value of 0.9977 [23]. Where: Y is the absorbance, and X is the TPC value.

4.5. Screening of single-factor

The effects of NADES (LA-Suc) ratio, microwave irradiation time, microwave irradiation power, and solid-liquid ratio on the TPC value were initially screened through a single-factor experiment. Single-factor screening aims to determine the maximum and minimum levels of each factor. The screening was performed by varying the investigated factor in three levels while three other factors were maintained constant during the experiment. In detail, the effects of NADES (LA-Suc) was screened at the ratio of 2:1, 3:1, and 4:1 g/g. The dried sample (5 gram) was placed in a round-bottom flask and mixed with NADES (LA-Suc) with 1:10 g/mL solid-liquid ratio. The microwave power was set at 450 Watts for 10 minutes of irradiation time. Later, the effect of microwave irradiation time was tested at minute of 10, 15, and 20 while the other factors were kept the same in each irradiation time variation (i.e. LA-Suc ratio of 3:1 g/g, 450 Watts of irradiation power, and 1:10 g/mL solid-liquid ratio). The microwave irradiation power was screened at 180, 270, and 360 Watts for 15 minutes of irradiation with NADES (LA-Suc) 3:1 g/g and 1:10 g/mL of solid-liquid ratio. Lastly, the effects of the solid-liquid ratio was examined by varying three different ratio of solid-liquid (1:8, 1:10, and 1:12 g/mL). The other factor were set the same during the screening process for the three different ratios (Lactic acid-Sucrose ratio of 3:1 g/g and 270 Watts microwave irradiation power for 15 min). Each level of factors were replicated three times.

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4.6. Design and optimization of MAE combined LA-Suc based NADES

After the single-factor screening, the best-selected factors were used to optimize the MAE-combined LA-Suc-based NADES condition. Design and optimization of MAE combined NADES (Lac-Suc) conditions were performed using the same equipment as in single-factor screening. The RSM with Box-Behnken Design (four factors and three levels) was applied to design and optimize the MAE combined LA-Suc based NADES process and examine the influence of MAE combined LA-Suc based NADES condition on the TPC value for polyphenols enrichment from *E. bulbosa* bulbs. The NADES (LA-Suc) ratio (X_1: 2:1, 3:1. 4:1 g/g), microwave irradiation time (X_2: 10, 15, 20 min), the solid-liquid ratio (X_3: 1:10, 1:12, 1:14 g/mL), and microwave irradiation power (X_4: 180, 270, 360 Watts) were examined as independent variables. The extraction efficiency of polyphenol enrichment with TPC value was monitored as a dependent variable.

A total of twenty-nine design experiments were performed in random order to alleviate bias. The polynomial equation formula (**Eq. (1)**) was used to explain the relationship between independent and dependent variables:

$$Y = \beta_o + \beta_{x1}X_1 + \beta_{x2}X_2 + \beta_{x3}X_3 + \beta_{x4}X_4 + \beta_{x1x2}X_1X_2 + \beta_{x1x3}X_1X_3 + \beta_{x1x4}X_1X_4 + \beta_{x2x3}X_2X_3 + \beta_{x2x4}X_2X_4 + \beta_{x3x4}X_3X_4 + \beta_{x1x1}X_1^2 + \beta_{x2x2}X_2^2 + \beta_{x3x3}X_3^2 + \beta_{X4x4}X_4^2 \quad (1)$$

Where Y is TPC value; X_1, X_2, X_3, and X_4 are examined MAE combined LA-Suc based NADES condition factors; β_o is intercepts coefficient; β_{x1}, β_{x2}, β_{x3}, and β_{x4} are linearity coefficients; β_{x1x2}, β_{x1x3}, β_{x1x4}, β_{x2x3}, β_{x2x4}, and β_{x3x4} are interaction coefficients; β_{x1x1}, β_{x2x2}, β_{x3x3}, and β_{X4x4} are quadratic regression coefficients.

Analysis of variance (ANOVA) was used to assess each term's influence quantitatively. The final models included terms necessary to preserve the hierarchy of model terms and those with a significance level with a p-value of <0.05. To develop the most accurate model, multiple linear regression analysis was used. The model's suitability was assessed through model F- and p-value, "Lack of Fit" F- and p-value, and coefficient correlation (R^2). To increase the extraction efficiency of all target compounds simultaneously, calculated optimal extraction conditions were used. The model's desirability was experimentally verified through three replicates of extracting under the forecasted ideal circumstances. The licensed software of Design Expert v12 (State-Ease, Minneapolis, USA) was used for optimization analysis.

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REFERENCES

[1] Ramadhan R, Phuwapraisirisan P, Kusuma IW, Amirta R. Ethnopharmacological evaluation of selected east kalimantan flora for diabetes therapy: The isolation of lupane triterpenoids as α-glucosidase inhibitors from *Ceriops tagal* (perr) c.b.robb. Rasayan J Chem. 2020;13(3):1727-1734. https://doi.org/10.31788/RJC.2020.1335559

[2] Ramadhan R, Phuwapraisirisan P, Amirta R, Darmawan MFB, Ul-Haq K, Kusuma IW, Suwito H, Abdulgani N, Mukhdlor A, Saparwadi. The potency of selected ethnomedicinal plants from East Kalimantan, Indonesia as antidiabetic agents and free-radical scavengers. Biodiversitas. 2022;23(4):2225-2230. https://doi.org/10.13057/biodiv/d230458

[3] Li AN, Li S, Zhang YJ, Xu XR, Chen YM, Li HB. Resources and biological activities of natural polyphenols. Nutrients. 2014;6(12):6020-6047. https://doi.org/10.3390/nu6126020

[4] Azmir J, Zaidul ISM, Rahman MM, Sharif KM, Mohamed A, Sahena F, Jahurul MHA, Ghafoor K, Norulaini NAN, Omar AKM. Techniques for extraction of bioactive compounds from plant materials: A review. J Food Eng. 2013;117(4):426-436. https://doi.org/10.1016/j.jfoodeng.2013.01.014

[5] Chemat F, Rombaut N, Meullemiestre A, Turk M, Perino S, Fabiano-Tixier AS, Abert-Vian M. Review of green food processing techniques. Preservation, transformation, and extraction. Innov Food Sci Emerging Technol. 2017;41:357-377. https://doi.org/10.1016/j.ifset.2017.04.016

[6] Florindo C, Lima F, Ribeiro BD, Marrucho IM. Deep eutectic solvents: overcoming 21st century challenges. Curr Opin Green Sustain Chem. 2019;18:31-36. https://doi.org/10.1016/j.cogsc.2018.12.003

[7] Bubalo MC, Vidović S, Redovniković IR, Jokić S. Green solvents for green technologies. J Chem Technol Biotechnol. 2015;90(9):1631-1639. https://doi.org/10.1002/jctb.4668

[8] Ahmad I, Pertiwi AS, Kembaren YH, Rahman A, Mun'im A. Application of natural deep eutectic solvent-based ultrasonic assisted extraction of total polyphenolic and caffeine content from coffe beans (Coffea Beans L.) for instant food products. J Appl Pharm Sci. 2018;8(8):138-143. https://doi.org/10.7324/JAPS.2018.8819
[9] Bakirtzi C, Triantafyllidou K, Makris DP. Novel lactic acid-based natural deep eutectic solvents: Efficiency in the ultrasound-assisted extraction of antioxidant polyphenols from common native Greek medicinal plants. J Appl Res Med Aromat Plants. 2016;3(3):120-127. https://doi.org/10.1016/j.jarmap.2016.03.003
[10] Suresh PS, Singh PP, Anmol, Kapoor S, Padwad YS, Sharma U. Lactic acid-based deep eutectic solvent: An efficient green media for the selective extraction of steroidal saponins from *Trillium govanianum*. Sep Purif Technol. 2022;294:121105. https://doi.org/10.1016/j.seppur.2022.121105
[11] Macchioni V, Carbone K, Cataldo A, Fraschini R, Bellucci S. Lactic acid-based deep natural eutectic solvents for the extraction of bioactive metabolites of *Humulus lupulus* L.: Supramolecular organization, phytochemical profiling and biological activity. Sep Purif Technol. 2021;264:118039. https://doi.org/10.1016/j.seppur.2020.118039
[12] Chemat F, Vian MA, Cravotto G. Green extraction of natural products: Concept and principles. Int J Mol Sci. 2012;13(7):8615-8627. https://doi.org/10.3390/ijms13078615
[13] Chemat F, Vian MA. Green Chemistry and Sustainable Technology: Alternative Solvents for Natural Products Extraction. Springer US; 2014.
[14] Ahmad I, Yanuar A, Mulia K, Mun'im A. Ionic liquid-based microwave-assisted extraction: Fast and green extraction method of secondary metabolites on medicinal plant. Pharmacogn Rev. 2018;12(23):20-26. https://doi.org/10.4103/phrev.phrev_40_17
[15] Yolmeh M, Jafari SM. Applications of response surface methodology in the food industry processes. Food Bioproc Technol. 2017;10(3):413-433. https://doi.org/10.1007/s11947-016-1855-2
[16] Riswanto FDO, Rohman A, Pramono S, Martono S. Application of response surface methodology as mathematical and statistical tools in natural product research. J Appl Pharm Sci. 2019;9(10):125-133. https://doi.org/10.7324/JAPS.2019.91018
[17] Naspiah N, Iskandar Y, Moelyono MW. Review article: Tiwai onion (*Eleutherine americana* Merr.), multifunction plant. Indo J Applied Sci. 2014;4(2):18-30.
[18] Kuntorini EM, Dewi M, Misrina M. Anatomical structure and antioxidant activity of red bulb plant (*Eleutherine americana*) on different plant age. Biodiversitas. 2016;17(1):229-233. https://doi.org/10.13057/biodiv/d170133
[19] Insanu M, Kusmardiyani S, Hartati R. Recent studies on phytochemicals and pharmacological effects of *Eleutherine americana* Merr. Procedia Chem. 2014;13:221-8. https://doi.org/10.1016/j.proche.2014.12.032
[20] Ahmad I, Arifuddin M, Rijai L. The effect of extraction methods of bawang Dayak (*Eleutherine palmifolia* L. MERR) against TLC profiles and sunscreen activities. Inter J PharmTech Res. 2016;9(9):428-436.
[21] Ahmad I, Ambarwati NSS, Indriyanti N, Sastyarina Y, Rijai L, Mun'im A. Oral glucose tolerance activity of bawang Dayak (*Eleutherine palmifolia* L. Merr.) bulbs extract based on the use of different extraction method. Pharmacogn J. 2018;10(1). :49-54. https://doi.org/10.5530/pj.2018.1.10
[22] Kamarudin AA, Mohd. Esa N, Saad N, Sayuti NH, Razak NAA. Heat assisted extraction of phenolic compounds from *Eleutherine bulbosa* (Mill.) bulb and its bioactive profiles using response surface methodology. Ind Crops Prod. 2020;144:112064. https://doi.org/10.1016/j.indcrop.2019.112064
[23] Yusuf B, Astati SJ, Ardana M, Herman H, Ibrahim A, Rijai L, Nainu F, Ahmad I. Optimizing natural deep eutectic solvent citric acid-glucose based microwave-assisted extraction of total polyphenols content from *Eleutherine bulbosa* (Mill.) bulb. Indo J Chem. 2021;21(4):797-805. https://doi.org/10.22146/ijc.58467
[24] Bubalo MC, Ćurko N, Tomašević M, Ganić KK, Redovnikovic IR. Green extraction of grape skin phenolics by using deep eutectic solvents. Food Chem. 2016;200:159-166. https://doi.org/10.1016/j.foodchem.2016.01.040
[25] Lu W, Alam MdA, Pan Y, Wu J, Wang Z, Yuan Z. A new approach of microalgal biomass pretreatment using deep eutectic solvents for enhanced lipid recovery for biodiesel production. Bioresour Technol. 2016;218:123-128. https://doi.org/10.1016/j.biortech.2016.05.120
[26] Fernández MdLA, Espino M, Gomez FJV, Silva MF. Novel approaches mediated by tailor-made green solvents for the extraction of phenolic compounds from agro-food industrial by-products. Food Chem. 2018;239:671-678. https://doi.org/10.1016/j.foodchem.2017.06.150
[27] Wang H, Ma X, Cheng Q, Wang L, Zhang L. Deep eutectic solvent-based ultrahigh pressure extraction of Baicalin from *Scutellaria baicalensis* Georgi. Molecules. 2018;23(12):3233. https://doi.org/10.3390/molecules23123233
[28] Gu H, Chen F, Zhang Q, Zang J. Application of ionic liquids in vacuum microwave-assisted extraction followed by macroporous resin isolation of three flavonoids rutin, hyperoside and hesperidin from *Sorbus tianschanica* leaves. J Chromatograp B. 2016;1014:45-55. https://doi.org/10.1016/j.jchromb.2016.01.045
[29] Ahmad I, Yanuar A, Mulia K, Mun'im A. Optimization of ionic liquid-based microwave-assisted extraction of polyphenolic content from *Peperomia pellucida* (L) Kunth using response surface methodology. Asian Pac J Trop Biomed. 2017;7(7):660-665. https://doi.org/10.1016/j.apjtb.2017.06.010
[30] de Gryze S, Langhans I, Vendebroek M. Using the correct interval for prediction: A tutorial on tolerance intervals for ordinary least-squares regression. Chemometr Intell Lab Systems. 2007;87(2):147-154. https://doi.org/10.1016/j.chemolab.2007.03.002
[31] Rao VK, Murthy PBGSN. Modeling and optimization of tool vibration and surface roughness in boring of steel using RSM, ANN and SVM. J Intell Manuf. 2018;29(7):1533-1543. https://doi.org/10.1007/s10845-016-1197-y

[32] Raissi S, Farsani RE. Statistical process optimization Through multi-response surface methodology. World Acad Sci Eng Technol. 2009;39(3):280-284. https://doi.org/10.5281/zenodo.1083451
[33] Munaeni W, Widanarni W, Yuhana M, Setiawati M, Wahyudi A. Phytochemical analysis and antibacterial activities of *Eleutherine bulbosa* (Mill.) Urb. extract against *Vibrio parahaemolyticus*. Asian Pac J Trop Biomed. 2019;9(9):397-404. https://doi.org/10.4103/2221-1691.267669
[34] Shi P, Du W, Wang Y, Teng X, Chen X, Ye L. Total phenolic, flavonoid content, and antioxidant activity of bulbs, leaves, and flowers made from *Eleutherine bulbosa* (Mill.) Urb. Food Sci Nutr. 2019;7(1):148-154. https://doi.org/10.1002/fsn3.834
[35] Souza OA, da Silva Ramalhão VG, de Melo Trentin L, Funari CS, Carneiro RL, da Silva Bolzani V, Rinaldo D. Combining natural deep eutectic solvent and microwave irradiation towards the eco-friendly and optimized extraction of bioactive phenolics from *Eugenia uniflora* L. Sustain Chem Pharm. 2022;26:100618. https://doi.org/10.1016/j.scp.2022.100618
[36] Gómez AV, Tadini CC, Biswas A, Buttrum M, Kim S, Boddu VM, Cheng HN. Microwave-assisted extraction of soluble sugars from banana puree with natural deep eutectic solvents (NADES). LWT - Food Sci Technol. 2019;107:79-88. https://doi.org/10.1016/j.lwt.2019.02.052
[37] Xie Y, Liu H, Lin L, Zhao M, Zhang L, Zhang Y, Wu Y. Application of natural deep eutectic solvents to extract ferulic acid from *Ligusticum chuanxiong* Hort with microwave assistance. RSC Adv. 2019;9(39):22677-22684. https://doi.org/10.1039/c9ra02665g
[38] Ainsworth EA, Gillespie KM. Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent. Nat Protoc. 2007;2(4):875-877. https://doi.org/10.1038/nprot.2007.102
[39] Bobo-García G, Davidov-Pardo G, Arroqui C, Vírseda P, Marín-Arroyo MR, Navarro M. Intra-laboratory validation of microplate methods for total phenolic content and antioxidant activity on polyphenolic extracts, and comparison with conventional spectrophotometric methods. J Sci Food Agric. 2014;95(1):204-209. https://doi.org/10.1002/jsfa.6706
[40] Sanchez-Rangel JC, Benavides J, Heredia JB, Cisneros-Zevallos L, Jacobo-Velazquez DA. The Folin-Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination. Anal Methods. 2013;5(21):5990-5999. https://doi.org/10.1039/c3ay41125g