Dear Islamudin Ahmad:

We have reached a decision regarding your submission to Indonesian Journal of Chemistry, "Response Surface Optimized of Natural Deep Eutectic Solvent Citric Acid-Glucose Based Microwave-Assisted Extraction on Total Polyphenols Content from Eleutherine bulbosa (Mill.) Bulb".

Our decision is to: Accept Submission

In accordance to the Journal policy, you are required to immediately pay the publication fee of USD 300 by transfer to the following bank account:
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Please send the proof of remittance by email to the editorial office of the Indonesian Journal of Chemistry (email: ijc@ugm.ac.id).

After payment, in a few days, you will receive an email for the further process, i.e. copy-editing, lay-outing, and proofreading.

Thank you for your valuable contribution to the journal.

Best regards,

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Phone +628157951198
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Reviewer A:

Additional Comment::
The authors studied the response surface optimized natural deep eutectic solvent citric acid-glucose based microwave-assisted extraction on total polyphenols content from E. bulbosa.

Major comments:
1) I recommend removing the last sentence "This finding is the development of an alternative green solvent to TPC enrichment effectively, quickly, efficiently, environmentally friendly, and edible." from the abstract.
And also remove the following sentences "This finding is the first step in various stages of our study into the development of a green solvent-based extraction method that is only limited to the TPC enrichment effectively, quickly, and efficiently, environmentally friendly, and edible. Therefore, it is necessary to develop an extraction method for target single or marker or active compounds from this plant in further study." from the conclusion part.
These sentences are not necessary when this study aims to optimize and not to investigate in detail from the green chemistry point of view.

2) Why the authors determined the absorbance at 791 nm on the TPC experiment? The monitored wavelength is too long.
Ref 18 and 19 used 765 nm while ref 20 used 750 nm. Please give a logical explanation.

3) The general mathematical models are linear, quadratic, and cubic. What is "2FI"? No explanation can be found in the text about the 2FI source.

Minor comments:
1) In the introduction part, "However, commercial use is limited. " this sentence is not clear.

2) Fix the typos:
~ Dried samples of E. bulbosa bulb was collected from Samarinda. Citric acid, glucose, and distilled water were obtained from CV. Chlorogreen, Bandung, Indonesia. Gallic acid, Folin-Ciocalteau reagent, and sodium carbonate were purchased from PT. Merck, Germany (throught PT. Elokarsa, Indonesia).
~ The instrumen used in this study includes a modified 900Watt domestic microwave (Modena, USA), UV-Vis Spectrophotometer (Shimadzu, Japan), micropipette, and licensed software of Design Expert v12 which was installed on the computer.

3) "%Watts" unit is not common, try to consider to change the unit to "Watt"

Reviewer B:

Additional Comment::
This manuscript reports the optimization study for Microwave-Assisted Extraction of Eleutherine

bulbosa (Mill.) Bulb using response surface methodology (Box Behnken Design). Authors used an NADES as solvent for extraction process. Others also reported the used of this solvents in various application. The manuscripts is at moderate quality and shows limited novelty. The novelty, however, need to be clearly addressed in the manuscript. The manuscript is recommended for publication after revision. The details comments are as follows:

1. Abstract: the statement provide d in the 1st sentence is contradict with the last sentence. The results reported didn't relate with the development of the NADES solvent. The solvent used was not developed in this work.

2. The title of the manuscript is quite long and confusing. Do authors want to optimize the TPC content or the solvent used? Pls consider to shorten the title to reflect the manuscript's content. Suggestion: Optimizing Natural Deep Eutectic Solvent Citric Acid-Glucose based Microwave-Assisted Extraction of Total Polyphenols Content from Eleutherine bulbosa (Mill.) Bulb

3. The introductory section can be further improved by adding the recent work done on bawang Dayak. The novelty of the work was not properly highlighted. The type of RSM method used and scope of the work should be properly written in the last paragraph of the introductory section.

4. The instrument section can be omitted, since it can be mentioned in the MAE or other analysis method whenever possible.

5. How the sample preparation was done prior to the extraction process? How the quality of the sample was controlled?

6. Did author bought the distillated water? It s quite confusing, since it can be prepared in laboratory.

7. The purity of the chemicals or analytical reagent used should be clearly stated.

8. Did authors purify or further treated the chemicals before used? Pls explained it in the manuscript.

9. The MAE steps should be explained in detail.

10. How the range of parameters used for optimization study were selected?

11. The method used for optimization should be clearly stated in the design experimental section. How many centre point was used?

12. Pls used standard SI unit for time.

13. Why authors used gallic acid as standard in this work?

14. Why the extract solution was stored at room temperature? Not in the refrigerator?

15. Hplc or lcms analysis should be performed to confirm the bioactive compounds present in the extract

16. Paragraph 1 and 2 under the result and discussion section can be removed. No need to restate the experimental work performed in this this section.

17. Table 3 shows that quadratic model is better to represent the result obtained in this work. However, the R2 value was not reported. The adjusted R2 value is quite reasonable. However, the predicted R2 is lower than adjusted R2. Pls justify the findings.

18. Table 4. P value for x2, x3 and x4 is larger than 0.01, which are insignificant to the results obtained. Pls provide reason for this matter.

19. The adjusted and predicted r2 values reported in paragraph 6th of the result and discussion section are different than those reported in table 3.

20. Why only 2 types of interactions were shown in Fig 1.? The labelling is blur. Pls improve it.

21. Fig 1 was not discussed in detailed. Reasons for the trend of the graph presented should be properly discussed and compared with the previous works whenever possible.

22. An optimum point was not observed in fig. 1

23. Several grammatical errors were spotted in the manuscript. Pls proof read.

~~Response Surface Optimized of~~Optimizing ~~-~~Natural Deep Eutectic Solvent Citric Acid-Glucose Based Microwave-Assisted Extraction o~~f~~n Total Polyphenols Content from *Eleutherine bulbosa* (Mill.) Bulb

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ABSTRACT

Application of natural deep eutectic solvent (NADES) citric acid-~~glucose~~ glucose-based microwave-assisted extraction (MAE) method for total polyphenol content (TPC) enrichment to increase the use of *Eleutherine bulbosa* (Mill.) bulb more widely and commercially. Therefore, this study aims to optimize ~~of~~ NADES citric acid-~~glucose~~ glucose-based MAE on the TPC from *E. bulbosa* bulbs using response surface methodology (RSM). In the present study, the dried sample of *E. bulbosa* bulb was extracted using the NADES based MAE. ~~The determination of TPC~~PC's Determination of TPC was conducted using Folin-Ciocalteau reagent and standard gallic acid, then measured using a UV-Vis spectrophotometer at 76~~9~~1 nm. Extraction condition optimization of the NADES based MAE method was performed using RSM with Box Behnken Design (four-factors-three-levels and 29 runs) on Design Expert v12. Based on the results study, the optimum ~~of~~ condition extraction on the TPC enrichment was obtained at the NADES ratio (citric acid: glucose) 1:1 g/g; solid-liquid ratio 1: 8 g/mL; extraction time of 15 min~~utes~~; and ~~30%~~270 Watt microwave power. The confirmation test and scale-up (50 g samples) were obtained with a 61.63 ± 2.23 mg GA/g sample. ~~This finding is the development of an alternative green solvent to TPC enrichment effectively, quickly, efficiently, environmentally friendly, and edible.~~

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Keywords: *Eleutherine bulbosa* Mill; Microwave-assisted extraction; Natural deep eutectic solvent; Response surface methodology; Total polyphenols content

INTRODUCTION

Eleutherina bulbosa Mill. (synonym *E. palmifolia*; *E., americana*), Indonesian people are known as "Bawang Dayak," traditional herbal medicine used by the Kalimantan community, especially its bulb part. *E. bulbosa* is a type of onion that grows wild in Kalimantan's interior forests that has the potential to be developed and identified compounds that are efficacious [1]. *E. bulbosa* bulbs' chemical content includes tannin, flavonoids, quinones, and triterpenoid compound [2]. Besides, some compounds have been successfully isolated, including anthraquinone, naphthoquinone groups (such as elecanacine, eleutherine, elutherole, eleutherine), and eleutosides A, B, C. *E* [3][4][5]. *E. bulbosa* bulbs are known to overcome various diseases such as cancer/cysts (breast/uterus), prostate, diabetes, gout, hypertension, gastrointestinal disturbances, cholesterol, goiter, bronchitis, stamina, and sexual disorders [6]. *E. bulbosa* bulb has enormous potential as a source of raw materials for natural-based medicines. This plant is abundant and easy to grow on Kalimantan island, but so far, commercial use is limited. Therefore, it is necessary to innovate for increasing the commercial use of this plant through the development of natural products with a green extraction approach.

On the other hand, some studies on *E. bulbosa* have been widely reported, including the effect extraction method of this plant against oral glucose tolerance activity [7], sunscreen activity, and TLC profile [8], heat-assisted extraction of phenolic compound [9], and effect of ethanolic extraction of *E. palmifolia* tuber on blood glucose and insulin level [10]. However, the extraction of target secondary metabolites from *E. bulbosa* using a green solvent approach based on non-conventional extraction methods has not been reported.

The development of non-conventional extraction methods is recently increasing, such as the microwave-assisted extraction method (MAE). This method has advantages over other conventional methods because it is expected to shorten the extraction process, minimize the use of solvents, and save energy use through microwave energy in the irradiation process [11].

The choice of solvent and extraction method are the main factors that must be considered to obtain the extraction efficiency of the target compound from natural products. The utilization of Natural Deep Eutectic Solvent (NADES) has increased in recent years. NADES has many advantages as a solvent, such as requires low cost, chemically inert, adjustable viscosity, readily biodegradable, acceptable toxicity, and sustainability [12][13][14]. NADES is a type of natural solvent in the form of a deep eutectic solvent, composed of a mixture component of primary metabolites (for example, sugar, amino acids, and organic acids). The right constituent

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of NADES will form a clear and stable liquid. In contrast, the inappropriate constituent components cause the liquid to be unstable, precipitable, and can return to solid form.

In this study, the selection of the composition of NADES in the form of citric acid and glucose is the right combination where glucose has hydrogen bonding acceptor (HBA) properties. Citric acid has hydrogen bonding donor (HBD) properties. Suppose both materials are fused at a specific temperature, forming a stable solution and ready to be used as a green solvent [15]. On the other hand, these two ingredients are pharmaceutical excipients that are safe for consumption, so it is hoped that the extract obtained can be readily consumed because not containing harmful organic solvents.

The combination of NADES with the non-conventional extraction method effectively extracts the desired target compound and minimizes the compound's undesirable extraction. Several studies have reported the success of NADES as an alternative solvent to replace conventional organic solvents, namely the experiment about extraction of flavonoid from *Radix Scutellariae* [16], extraction of phenol compounds from *Cajanus cajan* leaves [17], and extraction of polyphenols and caffeine from Robusta Coffee Beans [18][19].

The optimization process of various factors that influence non-conventional extraction based on NADES is carried out using response surface methodology (RSM).

RSM is a mathematical and statistical tool that has a crucial role in designing, formulating, developing, and analyzing natural product research, mainly in the extraction method optimization process [20]. RSM has the advantage of studying the interaction between various factors on response [21], [22]. In this study, box-Behnken design (BBD) with three-level fractional factorial was used to optimize the extraction condition of total polyphenol content (TPC) enrichment. The BBD is more economical and efficient than other factorial designs due to its ability to select points from a three-level factorial arrangement without center point or factorial points. This design requires fewer points than different methods. The BBD has been used extensively to optimize some extraction conditions such as extraction time, extraction temperature, solvent types, solvent concentration, sample-solvent ratio, dan lain-lain [22].

Therefore, this study aims to optimize the condition of the NADES citric-glucose acid-based MAE method for the enrichment of TPC from *E. bulbosa* bulbs using RSM.

EXPERIMENTAL

Materials

The samples of *E. bulbosa* bulb was collected from Samarinda, East Kalimantan, Indonesia. The specimen was authenticated and identified at Laboratory of Dendrology, Faculty of

Forestry, Universitas Mulawarman, Samarinda, Indonesia. The fresh bulb was washed and cut into small pieces and dried using a dryer cabinet at 50-60 °C. The citric acid (100% pure food grade non-GMO) and glucose (food grade) were obtained from CV. Chlorogreen, Bandung, Indonesia. Gallic acid (analytical purity), Folin-Ciocalteau reagent, and sodium carbonate were purchased from PT. Merck, Germany (through PT. Elokarsa, Indonesia). The quality test of the chemical purity was carried out prior to the experiment to ensure that the chemical quality was up to the specifications.

Procedure

The procedure of NADES based MAE Method

The extraction process was carried out using NADES based MAE by referring to some previous studies [18][23][24], with some modifications adjusting the extraction conditions. Firstly, the composition of NADES (citric acid and glucose) was melted at 50-80 °C until forming a concentrated solution. The NADES solution slowly added distilled water in a ratio of 1:1, 3:1, and 5:1 g/g. Next, 5 g of *E. bulbosa* bulbs were mixed with NADES in a round bottom flask. Secondly, the extraction process was conducted using the MAE method, operated under various conditions, as shown in **Table 1**. The extract solution and the residue were separated using a Buchner filter; The extract solution was kept in the refrigerator in a closed container until ready to analyze.

Determination of Total Polyphenols Content

Determination of total polyphenols content (TPC) was done by spectrophotometry using the Folin-Ciocalteau reagent regarding some literature [25][26][27], with slight modification. Briefly, a 1 mL of sample and standard solution was put into a test tube, added with 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu reagent, then allowed to react for 5 min. After that, 2 mL of sodium carbonate solution and 1.5 mL of distilled water was added. After incubating for 30 minutes at room temperature, absorbance was measured using a UV-Vis spectrophotometer at 761 nm (in this study, 761 nm was the maximum wavelength). The TPC in mg GAE/g samples were calculated using a linear regression equation obtained from gallic acid standard solutions at various concentrations (from 12.5 up to 200 µg/mL), namely, Y = 0.015 + 0.001559X, with an R^2 value of 0.9977, where Y is absorbance, and X is TPC value.

Design Experimental of NADES based MAE Optimization

Optimization of the NADES based MAE condition for the TPC enrichment was optimized by response surface methodology (RSM). The optimization process was estimated the interaction between variables and factors (independent parameters) on the TPC value (dependent variable). Box Behnken Design (with four-factor-three-level) was used for experimental design and requiring 29 experiments (with 1 block and 5 center points per block) for optimization extraction condition (in **Table 1**). A multilinear quadratic regression

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model was estimated according to the experimental data from different extraction conditions and TPC values using Design Expert v12 software licensed (Statease Inc. Minneapolis, MN, USA).

Table 1. Experimental design of response surface methodology with box Behnken design using NADES citric acid-glucose as a green solvent

No.	Independent Variables	Unit	Symbol	Factor Levels		
				Low (-1)	Medium (0)	High (+1)
1	NADES (citric acid-glucose) ratio	g/g	X_1	1:1	3:1	5:1
2	Liquid-Solid Ratio	mL/g	X_2	8:1	10:1	12:1
3	Extraction Time	mMinutes	X_3	5	10	15
4	Microwave Power	% Watts	X_4	1090	30270	50450

RESULTS AND DISCUSSION

In this research, the extraction process was carried out by optimizing the method of microwave-assisted extraction (MAE) based on the natural deep eutectic solvent (NADES) on total polyphenol contents (TPC) from *E. bulbosa* bulbs using response surface methodology (RSM). Box Behnken design (four-factors-three-levels) were optimized using RSM including NADES composition ratio of citric acid-glucose (1:1, 3:1, and 5:1 g/g) in 50% distilled water, solid-liquid ratio (1:8, 1:10, and 1:12 g/mL), extraction time (5, 10, and 15 minutes), and microwave power (10, 30, and 50% Watts).

The obtained experiment numbers based on the four factors were used as many as 29 times running. Each running has a point prediction of TPC value that has been predicted by Design-Expert software that functions to see the accuracy of the method used. Based on The study results from 29 runs obtained extraction conditions with the highest TPC of 85.251 mg GAE/g (NADES ratio of 5:1 g/g, the solid-liquid ratio of 1:12 g/mL, extraction time of 10 minutes and microwave power of 30%270 Watt) and the lowest TPC of 27.091 mg GAE/g (NADES ratio 3:1 g/g, a solid-liquid ratio of 1:8, extraction time of 5 minutes and 270 Watt of microwave power 30%), as shown in **Table 2**. The determination of TPC in extracts was carried out based on the standard gallic acid obtained. Gallic acid acts as a standard because it is a derivative of hydroxybenzoic acid, which is a simple phenol acid that is pure and stable [14,18,[18][25][26][27]19,20]. The concentration of gallic acid used is was at 12.5 to 200 ppm. Gallic acid analysis was performed using a UV-VIS spectrophotometer, which produced the equation Y = 0.015 + 0.001559X with a correlation coefficient (R^2) of 0.997, where Y is the absorbance. At the same time, the X value is the TPC value. The equation used to calculate sample levels with various pre-determined factor conditions using RSM.

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Table 2. Experimental values of TPC of the *E. bulbosa* bulb extract obtained by NADES-MAE at various conditions

Run	NADES Ratio (g/mL)	Liquid-Solid ratio (g/g)	Extraction Time (minute)	Microwave Power (Watt% Watts)	TPC (mg GAE/g sample)	
	X_1	X_2	X_3	X_4	Actual	Predicted
1	3:1 (0)	12:1 (+1)	10 (0)	45050 (+1)	38.70	43.37
2	5:1 (+1)	10:1 (0)	5 (-1)	27030 (0)	68.42	69.69
3	1:1 (-1)	12:1 (+1)	10 (0)	30 270 (0)	46.71	43.17
4	3:1 (0)	8:1 (-1)	15 (+1)	30 270 (0)	38.58	40.39
5	3:1 (0)	10:1 (0)	5 (-1)	50 450 (+1)	41.93	38.33
6	5:1 (+1)	10:1 (0)	15 (+1)	30 270 (0)	77.24	77.82
7	3:1 (0)	10:1 (0)	10 (0)	30 270 (0)	39.77	41.61
8	3:1 (0)	8:1 (-1)	10 (0)	50 450 (+1)	37.68	35.63
9	3:1 (0)	12:1 (+1)	5 (-1)	30 270 (0)	35.64	38.86
10	1:1 (-1)	10:1 (0)	15 (+1)	30 270 (0)	51.13	49.27
11	3:1 (0)	8:1 (-1)	10 (0)	10 90 (-1)	32.75	27.49
12	3:1 (0)	10:1 (0)	10 (0)	27030 (+1)	32.86	41.61
13	3:1 (0)	10:1 (0)	15 (+1)	45050 (+1)	41.28	42.62
14	5:1 (+1)	10:1 (0)	10 (0)	45050 (+1)	75.67	74.91
15	3:1 (0)	10:1 (0)	10 (0)	27030 (0)	49.50	41.61
16	1:1 (-1)	10:1 (0)	10 (0)	90 10 (-1)	31.28	37.08
17	1:1 (-1)	8:1 (-1)	10 (0)	27030 (0)	48.18	48.54
18	3:1 (0)	10:1 (0)	5 (-1)	9010 (-1)	31.00	25.21
19	3:1 (0)	12:1 (+1)	10 (0)	9010 (-1)	28.22	29.69
20	3:1 (0)	8:1 (-1)	5 (-1)	27030 (0)	27.09	33.15
21	3:1 (0)	12:1 (+1)	15 (+1)	27030 (0)	45.63	44.62
22	5:1 (+1)	10:1 (0)	10 (0)	1010 (-1)	57.54	62.18
23	1:1 (-1)	10:1 (0)	5 (-1)	27030 (0)	45.55	44.39
24	5:1 (+1)	8:1 (-1)	10 (0)	27030 (0)	66.04	65.12
25	3:1 (0)	10:1 (0)	15 (+1)	9010 (-1)	34.77	33.92
26	1:1 (-1)	10:1 (0)	10 (0)	45050 (+1)	45.76	46.16
27	5:1 (+1)	12:1 (+1)	10 (0)	27030 (0)	85.25	80.25
28	3:1 (0)	10:1 (0)	10 (0)	27030 (0)	44.62	41.61
29	3:1 (0)	10:1 (0)	10 (0)	27030 (0)	41.31	41.61

The absorbance data was measured using a spectrophotometer at 7691 nm. The TPC values were calculated using a regression equation formula from a standard calibration curve, then the TPC value obtained in mg GAE/g samples. All data were analyzed using the Design Expert 1210.0.3 licensed software to determine the optimum conditions for the enrichment of TPC in this study. In **Table 3**, the standard quadratic model was suitable for the response of TPCTPC's response with a value of p <0.0001 (<0.01%), which shows the probability of a model error of less than 5% or means the quadratic model has a significant effect response to the TPC. Next, an ANOVA analysis was performed on the chosen model. If the value of ""prob> F"" is lower than 0.05, then the relationship becomes significant, while for the value of ""prob> F"" higher than 0.1, then the relationship becomes not significant.

-The suggested standard quadratic model was based on the insignificant value in the lack of fit with the predicted R^2 value lower than the Adjusted R^2 value. However, with a predictive R^2

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of 0.7353, this is still in reasonable agreement with the adjusted R² of 0.9051. The predicted R^2 and the adjusted R^2 should be within 0.20 of each other. Otherwise, there may be a problem with either the data or the model. The regular R^2 can be artificially inflated by merely adding terms to the model, even if the terms are not statistically significant. The adjusted R^2 plateaus when insignificant terms are added to the model, and the predicted R^2 will decrease when there are too many insignificant terms. A rule of thumb is that the adjusted and predicted R^2 values should be within 0.2 of each other. For the optimal designs, the mixture polynomials can be reduced before point selection. Reducing the number of coefficients reduces the number of model points required and changes the variable's selection criterion [28],[29].

Table 3. The selection of mathematical model analysis for optimization

Source	Sequential p-value	Lack of Fit p-value	Adjusted R-Squared	Predicted R-Squared	Recommendation
Linear	0.0082	0.0714~~39347~~	0.3274~~364147~~	0.1163~~254413~~	
~~2FI~~Two-Factor Interaction	0.9948	0.0420~~196~~	0.1335~~41323~~	-0.7595~~20806~~	
Quadratic	< 0.0001	0.7209	0.8739	0.7353	Suggested
Cubic	0.8152	0.3899	0.8246	-1.0697	Aliased

Table 4~~,–~~ shows the best reduced quadratic model based on the results of the analysis of variance. T~~t~~he m~~M~~odel F-value of 34.39 and p-value of <0.0001 less than 0.050 indicate the model terms are significant. There is only a 0.01% chance of an F-value occurring due to interference. In this case, X_1, X_3, X_4, X_1X_2, X_1^2, X_4^2 are significant model terms. The ~~“~~"Lack of Fit~~”~~" F-value of 0.47 implies the ~~“~~"Lack of Fit~~”~~" is not significant relative to the pure error. There is an 87.43% chance that a ~~“~~"Lack of Fit~~”~~" F-value this large could occur due to noise. Non-significant ~~“~~"Lack of Fit~~”~~" is right. This value indicates that all four factors have a significant relationship with the response [30],[31]. ~~–~~The independent variable of X_1 and X_4 was less than 0.05, indicating statistically significant extraction results. Meanw~~W~~hile, the independent variables X_2 and X_3 were greater than 0.05, indicating that the variable was not significant to the extraction results. But overall, the interaction between variables influences response.

Table 4. Analysis of Variance~~ts~~

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	6016.31	8	752.04	34.39	< 0.0001	Significant
X_1~~-NADES ratio~~	2174.97	1	2174.97	99.46	< 0.0001	
X_2~~-Solid-liquid ratio~~	74.18	1	74.18	3.39	0.0804	
X_3~~-Extraction time~~	126.69	1	126.69	5.79	0.0259	
X_4~~-Power~~	357.00	1	357.00	16.33	0.0006	
X_1X_2	106.98	1	106.98	4.89	0.0388	
X_2X_4	7.69	1	7.69	0.3516	0.5598	

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X_1^2	2691.64	1	2691.64	123.09	< 0.0001	
X_4^2	210.73	1	210.73	9.64	0.0056	
Residual	437.36	20	21.87			
Lack of Fit	285.90	16	17.87	0.4719	0.8743	not significant
Pure Error	151.46	4	37.86			
Cor Total	6453.67	28				

In **Table 5**, the coefficient estimates represent the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal, the VIFs are 1; VIFs greater than 1 indicate multi-collinearity~~, t~~. The higher the VIF, the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable.

Table 5 Coefficient Estimate, Standard Error, Confidence interval, and VIF of the reduced quadratic model

Factor	Coefficient Estimate	df	Standard Error	95% Confidence Interval (CI) Low	95% Confidence Interval (CI) High	Variance Inflation Factor (VIF)
Intercept	40.32	1	1.41	37.38	43.27	
X_1 ~~(NADES ratio)~~	13.46	1	1.35	10.65	16.28	1.0000
X_2 ~~(Solid-liquid ratio)~~	2.49	1	1.35	-0.3296	5.30	1.0000
X_3 ~~(Extraction time)~~	3.25	1	1.35	0.4332	6.07	1.0000
X_4 ~~(Power)~~	5.45	1	1.35	2.64	8.27	1.0000
X_1X_2	5.17	1	2.34	0.2942	10.05	1.0000
X_2X_4	1.39	1	2.34	-3.49	6.26	1.0000
X_1^2	19.75	1	1.78	16.03	23.46	1.02
X_4^2	-5.53	1	1.78	-9.24	-1.81	1.02

The equation formula obtained was ~~obtained~~ Y~~-~~ = $40.32 + 13.46X_1 + 2.49X_2 + 3.25X_3 + 5.45X_3 + 5.17X_1X_2 + 1.39X_2X_4 + 19.75X_1^2 - 5.90X_4^2$ with R^2 ~~=~~value of 0.9322 with the ~~p~~Predicted R^2 of 0.8580 was in reasonable agreement with the ~~Adjusted~~ adjusted R^2 of 0.9051, the difference was less than 0.2. Adequate precision measures the signal to noise ratio. Greater than four ratios were desirable. The ratio of 21,150 indicates an adequate signal. This model can be used to navigate the design space. In this equation, Y is the TPC value, where X_1 is the NADES ratio (g/g), X_2 is the solid-liquid ratio (g/mL), X_3 is extraction time (min~~utes~~), and D is microwave power (Watt~~%~~). The equation in terms of actual factors can be used to make predictions about ~~the~~

each factor's given levels' response. Here, the levels should be specified in the original units for each factor. This equation should not determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor, and the intercept is not at the center of the design space [32].

From this equation, the optimum conditions were obtained according to the results of RSM analysis, including 1:1 g/g NADES ratio (citric acid: glucose), 1:8 g/mL solid-liquid ratio, extraction time of 15 min, and 270 Watt microwave power with predicted the TPC of 51.09 ± 4.68 mg GAE/g sample. The confirmation test and scale-up using ten times the number of samples (50 g) were obtained with a 61.63 ± 2.23 mg GAE/g sample, which showed that the TPC produced was within the tolerance interval (TI) range with 95% TI low and 95 % IT high. The TI means that the extraction process to obtain TPC's response compared to the extraction conditions predicted by the program is entirely consistent. Each parameter's value is determined by considering the efficiency of the results obtained in the extraction process using time, energy, and solvent consumption.

Based on the above findings, the use of NADES with the composition of citric acid

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CONCLUSION

The application of the green extraction approach in separating target secondary metabolites from natural products (mainly from plants) continues to experience a significant increase. The use of NADES combined with the MAE method has been successfully optimized using RSM for TPC enrichment. Optimum conditions obtained were 1:1 g/g NADES ratio (citric acid: glucose), 1:8 g/mL solid-liquid ratio, extraction time of 15 min, and 270 Watt microwave power, with the confirmed TPC value of 61.63 ± 2.23 mg GAE/g sample.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All author was involved in this study. BY, I.A, SJA, and MA conducted the experiment, He, AI, LR, and FN conducted the DFT calculations, BY, I.A and FN wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

REFERENCES

[1] Kuntorini, E.V.I.M., Dewi, M., 2016, Anatomical structure and antioxidant activity of red bulb plant (*Eleutherine americana*) on different plant age, *Biodiversitas*, 17(1), 229–233.

[2] Insanu, M., Kusmardiyani, S., Hartati, R., 2014, Recent studies on phytochemicals and pharmacological effects of *Eleutherine americana* Merr., *Procedia Chem.*, 13, 221–228.

[3] Shibuya, H., Fukushima, T., Ohashi, K., Nakamura, A., Riswan, S., Kitagawa, I., 1997, Chemical structures of eleuthosides A, B, and C, three new aromatic glucosides from the bulbs of *Eleutherine palmifolia* (Iridaceae)., *Chem. Pharm. Bull.*, 45(7), 1130–1134.

[4] Paramapojn, S., Ganzera, M., Gritsanapan, W. Stuppner, H., 2008, Analysis of naphthoquinone derivatives in the Asian medicinal plant *Eleutherine americana* by RP-HPLC and LC-MS., *J. Pharm. Biomed. Anal.*, 47(4–5), 990–993.

[5] Singab, A.N.B., Ayoub, I.M., El-Shazly, M., Korinek, M., Wu, T.Y., Cheng, Y.B., Chang, F.R., Wi, Y.C., 2016, Shedding the light on Iridaceae: Ethnobotany, phytochemistry and biological activity, *Ind. Crops. Prod.*, *92*, 308–335.

[6] Naspiah, N., Iskandar, Y., Moelyono, M.W., 2014, Review Article: Tiwai Onion (*Eleutherine americana* Merr.), multifunction plant, *Indones. J. Appl. Sci.*, 4(2),18–30.

[7] Ahmad, I., Ambarwati, N.S.S., Indriyanti, N., Sastyarina, Y., Rijai, L., Mun'im, A., 2018, Oral glucose tolerance activity of Bawang Dayak (*Eleutherine palmifolia* L. Merr.) bulbs extract based on the use of different extraction method, *Pharmacogn. J.*, 10(1), 49-54.

[8] Ahmad, I., Arifuddin, M., Rijai, L., 2016, The effect of extraction methods of Bawang Dayak (*Eleutherine palmifolia* L. MERR) against TLC profiles and sunscreen activities, *Int. J. PharmTech Res.*, 9(9), 428–436.

[9] Kamarudin, A.A., Mohd. Esa, N., Saad, N., Sayuti, N.H., Nor, N.A., 2019, Heat assisted extraction of phenolic compounds from *Eleutherine bulbosa* (Mill.) bulb and its bioactive profiles using response surface methodology, *Ind. Crops Prod*, 144, 112064 (1-8).

[10] Hasni, Y., Aminah, D., Tri, W., 2019, The effect of ethanolic extract of dayak onion (*Eleutherine palmifolia* (L) Merr) tuber on blood glucose and insulin level of strepzotocin-induced diabetic wistar rat, *Asian J. Pharm. Res. Dev.*, 7(4), 38–42.

[11] Ahmad, I., Yanuar, A., Mulia, K., Mun'im, A., 2018, Ionic liquid-based microwave-assisted

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extraction: Fast and green extraction method of secondary metabolites on medicinal plant, *Pharmacogn. Rev.*, 12(23), 20-26.

[12] Dai, Y., Witkamp, G.J., Verpoorte, R., Choi, Y.H., 2013. Natural deep eutectic solvents as a new extraction media for phenolic metabolites in *Carthamus tinctorius* L., *Anal. Chem.*, 85(13), 6272–6278.

[13] Dai, Y., Jin, R., Verpoorte, R., Lam, W., Cheng, Y.C., Xiao, Y., Xu, J., Zhang, L., Qin, X.M., Chen, S., 2020. Natural deep eutectic characteristics of honey improve the bioactivity and safety of traditional medicines, *J. Ethnopharmacol.*, *250*, 112460.

[14] Vanda, H., Dai, Y., Wilson, E.G., Verpoorte, R., Choi, Y.H., 2018. Green solvents from ionic liquids and deep eutectic solvents to natural deep eutectic solvents, *Comptes Rendus Chim.*, 2018;*21(6)*:628–38.

[15] Mulia, K., Fauzia, F., Krisanti, E.A., 2019. Polyalcohols as hydrogen-bonding donors in choline chloride-based deep eutectic solvents for extraction of xanthones from the pericarp of *Garcinia mangostana* L., *Molecules*, 24(3), 636–647.

[16] Wei, Z.F., Wang, X.Q., Peng, X., Wang, W., Zhao, C.J., Zu, Y.G., Fu, Y., 2015. Fast and green extraction and separation of main bioactive flavonoids from *Radix Scutellariae*, *Ind. Crops. Prod.*, *63*, 175–181.

[17] Wei, Z., Qi, X., Li, T., Luo, M., Wang, W., Zu, Y., Fu, Y., 2015. Application of natural deep eutectic solvents for extraction and determination of phenolics in *Cajanus cajan* leaves by ultra performance liquid chromatography, *Sep. Purif. Technol.*, *149*, 237–244.

[18] Ahmad, I., Pertiwi, A.S., Kembaren, Y.H., Rahman, A., Mun'im, A., 2018. 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.*, 8(8),138–143.

[19] Yuniarti, E., Saputri, F.C., Mun'im, A., 2019. Application of the natural deep eutectic solvent choline chloride-sorbitol to extract chlorogenic acid and caffeine from green coffee beans (*Coffea canephora*), *J. Appl. Pharm. Sci.*, 9(3), 82–90.

[20] Khuri, A.I., Mukhopadhyay, S., 2010. Response surface methodology. *Wiley Interdiscip. Rev. Comput. Stat.*, 2(2), 128–149.

[21] Yolmeh, M., Jafari, S.M., 2017. Applications of response surface methodology in the food industry processes. *Food. Bioprocess. Technol.*, 10(3), 413–433.

[22] Riswanto, F.D.O., Rohman, A., Pramono, S., Martono, S. 2019. Application of response surface methodology as mathematical and statistical tools in natural product research, *J. Appl. Pharm. Sci.*, 9(10), 125–133.

[23] Gomez, F.J.V., Espino, M., Fernández, M.A., Silva, M.F., 2018. A Greener Approach to Prepare Natural Deep Eutectic Solvents, *Chemistry Select*, 3(22),6122–6125.

[24] González, C.G., Mustafa, N.R., Wilson, E.G., Verpoorte, R., Choi, Y.H., 2018. Application

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of natural deep eutectic solvents for the “green”extraction of vanillin from vanilla pods, *Flavour. Fragr. J.*, 33(1), 91–96.

[25] Ainsworth, E., Gillespie, K., 2007, Estimation of total phenolic content and other oxidation substrates in plant tissues using Folin–Ciocalteu reagent, *Nat. Protoc.*, 2(4), 875–877.

[26] Sanchez-Rangel, J., Benavides, J., Heredia, J., Cisneros-Zevallos, L., Jacobo-Velazquez, D., 2013, The Folin-Ciocalteu assay revisited: improvement of its specificity for total phenolic content determination, *Anal. Methods.*, 5(21), 5990–5999.

[27] Bobo-García, G., Davidov-Pardo, G., Arroqui, C., Vírseda, P., Marín-Arroyo, M., Navarro, M., 2014, 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.*, 95(1), 204–209.

[28] Brusco, M.J., Steinley, D., Cradit, J.D., 2009, An exact algorithm for hierarchically well-formulated subsets in second-order polynomial regression, *Technometrics*, 51(3), 306–315.

[29] Piepel, G., Szychowski, J., Leoppky, J., 2002, Augmenting scheffe linear mixture models with squared and/or crossproduct terms, *J. Qual. Technol.*, 34(3), 297–314.

[30] Ba, D., Boyaci, I.H., 2007, Modeling and optimization I: Usability of response surface methodology, *J. Food. Eng.*, 78(3),836–845.

[31] Bezerra, M.A., Santelli, R.E., Oliveira, E.P., Villar, L.S., Escaleira, L.A., 2008, Response surface methodology (RSM) as a tool for optimization in analytical chemistry, *Talanta*, 76(5),965–977.

[32] Raissi, S., Farsani, R.E., 2009, Statistical process optimization through multi-response surface methodology, *World. Acad. Sci. Eng. Technol.*, 39(3), 280–284.

[33] Munaeni, W., Widanarni, W., Yuhana, M., Setiawati, M., Wahyudi, A., 2019, Phytochemical analysis and antibacterial activities of *Eleutherine bulbosa* (Mill.) Urb. extract against *Vibrio parahaemolyticus*, *Asian Pac. J. Trop. Biomed.*, 9(9), 397–404.

[34] Shi, P., Du, W., Wang, Y., Teng, X., Chen, X., Ye, L., 2019, Total phenolic, flavanoid content, and antioxidant activity of bulbs, leaves, and flowers made form *Eleutherine bulbosa* (Mill.) Urb., *Food Sci. Nutr., 7(1)*, 148-154.

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