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Microwave-assisted extraction of polyphenols from *Eleutherine bulbosa* Mill. Urb. bulbs using choline chloride-sorbitol based natural deep eutectic solvent

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ABSTRACT

Eleutherine bulbosa Mill. Urb. is a potential plant to be developed as a raw pharmaceutical active ingredient. This plant can be utilized as raw materials for herbal medicine production. A green solvent is one of the innovative approaches to developing and utilizing natural products as a source of raw materials. This study aims to design and optimize the microwave-assisted extraction (MAE) of polyphenols from *E. bulbosa* bulbs using choline chloride-sorbitol-based natural deep eutectic solvent (NADES). The MAE method with choline chloride and sorbitol as the deep eutectic solvent composition was used to extract polyphenols content from *E. bulbosa*. Experimental design with some independent variables was involved, including NADES (choline chloride-sorbitol) ratio, liquid-solid ratio, microwave power, and extraction time. Response surface methodology with the Box-Behnken Design (using Design-Expert v12 program) was used to optimize the extraction condition based on the total polyphenols content (TPC) as a dependent variable. TPC value was determined using the Folin–Ciocalteu reagent and measured using a UV–Vis spectrophotometer at 761 nm. According to the study results, the optimum MAE condition was obtained at 1:1 g/g NADES (choline chloride-sorbitol) ratio, 5 minutes extraction time, 20%W microwave power, and 12:1 ml/g liquid-solid ratio. A 50 g sample was extracted under the ideal MAE conditions for the scale-up confirmation test, yielding a TPC value of 39.88 mgGAE/g sample. The MAE of polyphenols with choline chloride-sorbitol-based NADES produced a higher TPC as a target secondary metabolite and was conducted efficiently, rapidly, and in an environment-friendly way.

INTRODUCTION

Eleutherine bulbosa Mill. Urb. belongs to the Iridaceae family, is a native plant of Kalimantan, and has been used for generations as traditional medicine by the Dayak tribe. This plant is known by the local name Bawang Dayak (Kalimantan), Bawang Tiwai (Sulawesi), or Bawang Sabrang (Java and Sumatra). *Eleutherine bulbosa* grows wild in the deep area of Kalimantan forests, although it has begun to be cultivated by local farmers because of its potential as a nutritious plant (Kuntorini and Dewi, 2016). This plant is believed to cure diseases such as diabetes mellitus, hypertension, gout, cholesterol, bronchitis, goiter, stamina, uterine cancer, breast cancer, prostate, cysts, and sexual disorders (Naspiah *et al*., 2014). The tubers of *E. bulbosa* contain secondary metabolites, including polyphenols, flavonoids, glycosides, quinones, tannins, and triterpenoids (Insanu *et al*.,

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2014). Some studies have reported the success of isolating and identifying the structure of secondary metabolites from this plant, mainly naphthoquinone groups (such as eleutherinone, eleuthero, and elenacine), eleutherosides (A, B, and C), and anthraquinones (Paramapojn *et al*., 2008; Shibuya *et al*., 1997; Singab *et al*., 2016). Therefore, this plant can be utilized as raw materials for herbal medicine production.

A green solvent is one of the innovative approaches to developing and utilizing natural products as raw materials for herbal medicine production. Deep eutectic solvents (DESs) or natural deep eutectic solvents (NADESs) have evolved dramatically in recent years as promising alternative solvents to replace conventional organic solvents (Cvjetko Bubalo *et al*., 2015). When compared to traditional organic solvents, the DES has advantages. NADES has a changeable viscosity and is non-toxic, non-flammable, non-volatile, biodegradable, pharmaceutically acceptable, and environment-friendly (Dai *et al*., 2013, 2015, and 2020). In addition, the most important thing is that NADES is food grade because it has a composition in the form of pharmaceutical excipients and includes components of daily food, which are safe, cheap, and sustainable (Ahmad et al., 2018; Wei *et al*., 2015).

In order to create an NADES, two or more solid components—among which one is a hydrogen bond donor (HBD) and the other is a hydrogen bond acceptor (HBA)—must be mixed in a specific molar ratio, with the solid components' self-association converting them into a liquid at room temperature (Abbott *et al*., 2017; Raja Sekharan *et al*., 2021). NADES is a mixture of some HBD and HBA compounds that have a eutectic point temperature below the temperature of an ideal liquid mixture at the proper molar ratio (Farias *et al*., 2020). Some examples of NADES compositions with typical HBA include choline chloride, ammonium acetate, betaine, and glycine. Meanwhile, NADES composition with typical HBD examples includes lactic acid, urea, glucose, ethylene glycol, and sorbitol (Liu *et al*., 2022).

NADES is very effective and selective in extracting target secondary metabolites, especially when combined with microwave-assisted extraction (MAE) (Hashim *et al*., 2020; Sagarika *et al*., 2017; Zhang *et al*., 2011). One of the exciting compositions of NADES that we developed in this study was the use of choline chloride and sorbitol. These two materials are widely used as NADES compositions to enrich target secondary metabolites in the extraction process (Ahmad *et al*., 2021; Mulia *et al*., 2019; Wang *et al*., 2018; Wei *et al*., 2015; Yuniarti *et al*., 2019a; Zhao *et al*., 2015).

Response surface methodology (RSM) was used to optimize the processes at three levels, analyze the effects of the extraction condition factors, and select the best model (Meeker *et al*., 2017). The Box–Behnken Design (BBD) is a response surface design introduced by Box and Behnken (1960). This procedure is specifically designed to require only three levels (coded as −1, 0, and 1). The BBD is formed by combining a two-level factorial design with an incomplete block design. This procedure results in a design with the desired statistical properties, with only a fraction of the experiments required for the three-level factorial design . This design is more efficient and economical than other three-level designs because it allows point selection from a three-level factorial arrangement (Bezerra *et al*., 2008; Riswanto *et al*., 2019).

This study aims to design the optimum extraction conditions of choline chloride-sorbitol-based NADES combined with the MAE method as green and optimum media for total polyphenols content (TPC) from *E. bulbosa* bulbs.

MATERIALS AND METHODS

Chemicals and equipment

The chemicals used in this work, including sodium carbonate, gallic acid standard, and Folin-Ciocalteu, were purchased from Sigma-Aldrich, Germany (through PT. Elokarsa LLC, Indonesia). Choline chloride (100% pure food grade) was purchased from Xi'an Sheerherb Biological Co., Ltd., China, and sorbitol (100% pure food grade) was purchased from CV. Clorogreen, Bandung, Indonesia. Chemical purity quality testing was performed prior to experimentation to ensure that chemical quality met specifications.

Plant material and sample preparation

The sample of *E. bulbosa* was obtained from Indonesia, East Kalimantan, Kutai Kartanegara District. The voucher specimen was recognized and verified as authentic at the Dendrology Laboratory, Forestry Faculty, Mulawarman University. The voucher specimen (010/PTUP-LP/FFUNMUL/VI/2022) was kept in the Pharmaceutical R&D Laboratory of FARMAKA TROPIS, Faculty of Pharmacy, Mulawarman University.

Choline chloride-sorbitol-based NADES preparation

Choline chloride and sorbitol were combined to create choline chloride-sorbitol-based NADES. Choline chloride-sorbitol-based NADESs are made by continuously stirring and heating both components (sorbitol is an HBD and choline chloride is an HBA) in a tube at 50°C for 40 minutes in order to create a homogeneous mixture. The mixture solution was then homogenized after adding deionized water (50% of the total solution). The NADES solutions based on choline chloride and sorbitol were stable in liquid form during storage at room temperature. The choline chloride-sorbitol-based NADES mixtures' viscosity was also measured before it was used.

The procedure of the MAE of polyphenols using choline chloride-sorbitol-based NADES

The MAE of polyphenols with choline chloride-sorbitol-based NADES was used following previous studies (Verónica *et al*., 2019; Xie *et al*., 2019; Yusuf *et al*., 2021). The dried powder sample (5 g) was mixed with various NADES compositions (choline chloride-sorbitol) before being extracted under some circumstances using MAE (modified domestic microwave 900W, Modena, USA). Using a Buchner filter, the extract solution and the residue were separated. The extract solution was dried in a food dehydrator for 24 hours to create a thick extract.

Calculation of TPC

According to literature (Ainsworth and Gillespie, 2007; Bobo-garcía et al., 2015; Sanchez-Rangel *et al*., 2013), the TPC was assessed using spectrophotometry and the Folin–Ciocalteu reagent with modification. In a nutshell, 5 ml of distilled water and 0.5 ml of the Folin–Ciocalteu reagent were added to a test tube containing 1 ml of the sample and the standard solution, and the mixture was allowed to react for 5 minutes. Then, add 2 ml of sodium carbonate solution and 1.5 ml of distilled water. Use a UV–Vis spectrophotometer set to measure absorbance at 761 nm (maximum wavelength) after 30 minutes of incubation at room temperature according to our previous study (Yusuf *et al*., 2021). A linear regression equation with an R^2 value of 0.9977 and gallic acid standard solutions at five concentrations (12.5–200 g/ml) was used to calculate the TPC. The equation used was $Y = 0.001559X + 0.015$ (where X is the TPC value and Y is absorbance).

Design and optimization of MAE condition

The MAE conditions for phenolic content extraction were designed and optimized using RSM. The impact of the TPC value (dependent variable) on various factors (independent parameters) was calculated by optimizing extraction conditions. In the experimental design, a BBD (four factors and three levels), a total of 29 experiment runs were performed randomly to reduce bias for the optimization MAE condition (Table 1). A multilinear quadratic regression model was estimated using experimental data from various factors and TPC values using licensed Design-Expert v12 software (Stat-Ease Inc., Minneapolis, MN). The second-order polynomial model was applied to describe the relationship between parameter factors (independent variable) and response as a dependent variable, as follows:

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_i X_i + \sum_{i=1}^{k} \beta_{ii} X_i^2 + \sum_{i=1}^{k-1} \sum_{j=2}^{k} \beta_{ij} X_i X_j \ , \qquad (1)$$

where Y is the TPC value as the response (dependent variable); X_1, X_2, …, and X_k are the independent variables affecting the response; β_0 is an intercept's coefficient; β_i (i = 1, 2, …, k) is linearity coefficients; β_{ii} (i = 1, 2, …, k) and β_{ij} (i = 1, 2, …k; j= 1, 2, ..., k) are quadratic regression coefficients ; k is the variables number (Silva *et al*., 2007).

RESULTS AND DISCUSSION

Single-factor effect analysis

This study used several extraction conditions as independent variables, including NADES (choline chloride-sorbitol) ratio, extraction time, microwave power, and liquid-solid ratio, as shown in Figure 1. The figure shows that the result of level selection for each factor is the maximum TPC value under certain conditions. The single-factor study aims to obtain an initial description of the effect of varying levels of each factor (Herman *et al*., 2021). The single-factor effect was analyzed by designing several levels on one factor and the other factors in a constant condition. The NADES ratio was used to see the effect of the solvent mixture between choline chloride and sorbitol on the levels of compounds extracted if the concentrations varied. The extraction time and microwave power are factors that influence each other. They are used to create suitable extraction conditions in the extraction process, so they must be carried out properly so that there is no excessive pressure in plant cells and they can cause degradation of active compounds. The solvent-sample ratio was used to determine the effect of the volume used on the desired strength of the phenolic compound.

Figure 1A shows the effect of NADES (choline chloride-sorbitol) ratio in the ranges of 1:1–1:3 g/g. The optimum TPC was obtained at 1:2 g/g NADES (choline chloride-sorbitol) ratio because the interaction effect of various factors and sample characteristics causes these conditions. Choline chloride (an HBA) and sorbitol (HBD) are a mixture of solids and liquids, which are components of NADES in a particular ratio that will form a eutectic system in liquid form at temperatures below 100°C (Duan *et al*., 2016; Farias *et al*., 2020; Leng and Suyin, 2019; Vian *et al*., 2017). The addition of water to NADES plays a role in reducing viscosity. In addition, adding a certain amount of water can increase the amount of the extracted target compound (Radošević *et al*., 2018). However, suppose the water percentage is too high. In that case, it will decrease the extraction yield because there is a decrease in the interaction between NADES and the target compound with an increase in polarity (Syakfanaya *et al*., 2019; Yuniarti *et al*., 2019a, 2019b).

Figure 1B demonstrated the effect of extraction time with the range of 3–7 minutes according to a previous study (Mandal *et al*., 2007). The maximum extraction time was obtained with the highest TPC from *E. bulbosa* bulbs at 5 minutes. The extraction time of 5 minutes shows that the solute dissolution process is in equilibrium (Şahin and Şamli, 2013) at that point.

Figure 1C shows the effect of microwave power in the range from 10% to 30%W. The maximum power of 20%W extracted TPC and gave a higher TPC value. Increasing the microwave power causes an increase in temperature. Too much increase in temperature can harm the sample matrix and alter the structure of the target compound (Ballard *et al*., 2010).

Figure 1D shows the liquid-solid ratio from 8:1 to 12:1 ml/g. The liquid-solid ratio affects the TPC value, increasing from 8:1 to 10:1 ml/g. However, there was a decrease in the TPC values at a ratio of 12:1 ml/g. A maximal ratio was obtained at 10:1 ml/g. Under these conditions, increasing the liquid-solid ratio maximizes direct contact between the target compound and the solvent in the sample matrix (Liu *et al*., 2019). A low ratio can lead

Table 1. Experimental design of factors (independent variable) using RSM with BBD.

No.	Factors	Units	Levels		
			Low (−1)	Medium (0)	High (1)
1	NADES (choline chloride-sorbitol) ratio	(g/g)	1:1	1:2	1:3
2	Extraction time	minutes	3	5	7
3	Microwave power	%W	10	20	30
4	Liquid-solid ratio	ml/g	8:1	10:1	12:1

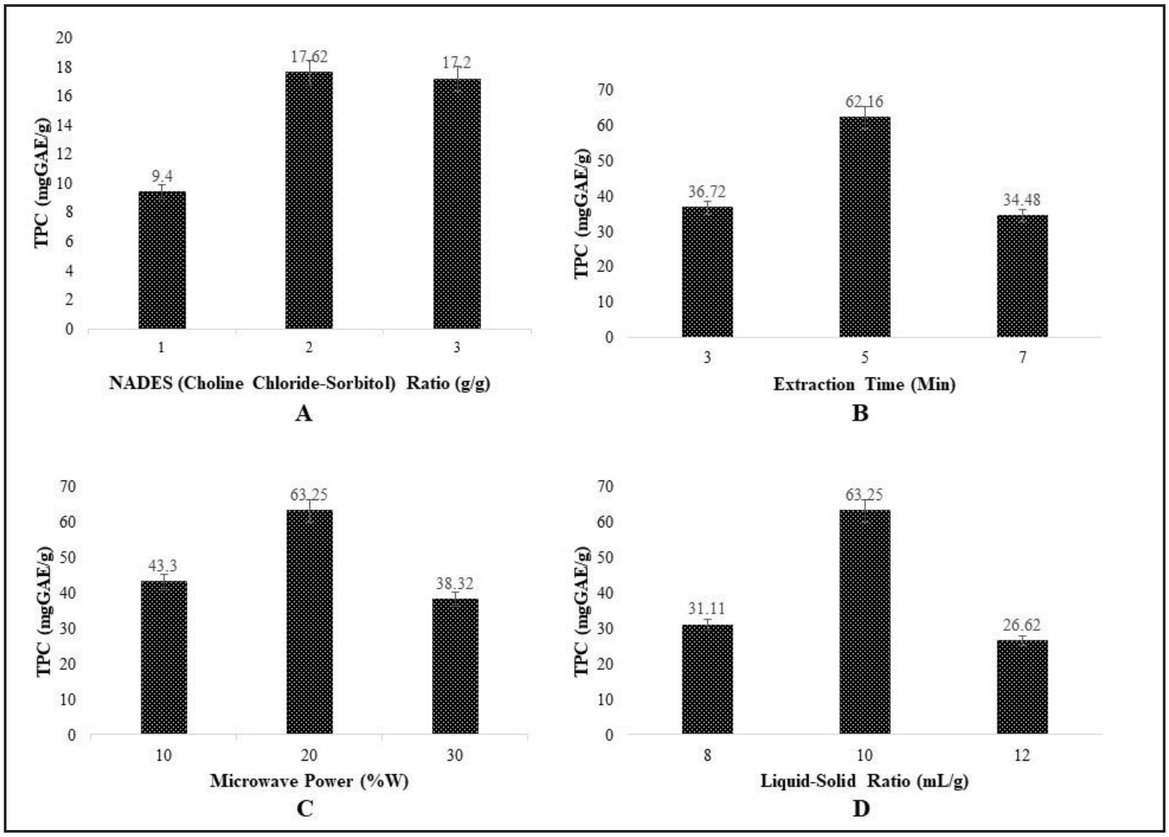

Figure 1. Single Factor Effect of some extraction conditions of MAE Method on TPC using choline chloride-sorbitol based NADES.

to a sub-optimal extraction process, and a high ratio can lead to an inefficient extraction process (Ozturk *et al*., 2018).

The results of single-factor analysis at each level are used to design and optimize MAE conditions. The basis for selecting these four parameters relates to their respective roles in the extraction process.

Design and optimization of MAE conditions using RSM

Design and optimization of MAE conditions with choline chloride-sorbitol-based NADES were conducted using RSM with BBD, which was operated with the licensed Design-Expert v12 software program. Four factors and three levels (Table 1) were developed to design and obtain optimum conditions. All factors are independent variables, and the TPC value is the dependent variable. Based on the results, the highest TPC value was obtained at 36.84 mgGAE/g with MAE condition (1:1 g/g NADES (choline chloride-sorbitol) ratio, 12:1 ml/g, and 20%W microwave power for 5 minutes), and the lowest TPC value was obtained at 9.96 mgGAE/g with extraction condition of 2:1 g/g NADES (choline chloride-sorbitol) ratio, the liquid-solid ratio of 8:1 ml/g, and microwave power of 10%W for 5 minutes. The extraction process results of a total of 29 trials are based on experimental design (the actual TPC value), as shown in Table 2.

Based on the experimental design results, 29 trials were analyzed with quadratic multivariate regression models. The calculation model's correlation coefficient (R^2), which is 0.8779, indicates that the model can explain a more significant percentage of the data than 87.79%. The adjusted R^2 of 0.7889 and the predicted R^2 of 0.6283 are reasonably in agreement, with the difference being less than 0.2. Before point selection, the mixture of polynomials can be reduced for optimal designs. Reduced model points are needed due to fewer coefficients, which alter the variable's selection criterion.

The multivariate regression reduced quadratic analysis was used to compute the obtained data. In the following equation, the regression model was used to obtain the TPC value from *E. bulbosa* bulbs; $Y = 22.26X_1 + 17.90X_2 + 16.98X_3 + 3.00X_4 - 0.99X_1X_2 - 2.31X_1X_3 - 0.39X_2X_3 - 0.10X_2X_4 - 0.99X_2^2 - 0.50X_3^2 - 0.05X_4^2 - 157.72$, where Y is the TPC value, X_1 is the NADES (choline chloride-sorbitol) ratio, X_2 is the extraction time, X_3 is the microwave power, and X_4 is the liquid-solid ratio. Using the equations associated with the actual factors, we can predict each factor's response at specific levels. The levels should be specified in the original units of the relevant factor. Because the intercept is not centered in design space and the coefficients are scaled to account for each factor's units, this equation cannot determine the relative importance of each factor (Raissi and Farsani, 2009).

Table 3 demonstrates the best model based on the reduced quadratic model selection from the ANOVA results. The model term is likely significant because of the model's F-value of 11.11 and p-value of <0.0001 (less than 0.050). X_1, X_3, X_1X_3, X_2^2, and X_4^2 are important model terms in this model. With an F-value of 0.53, the "lack of fit" indicates negligible compared to the absolute error. The p-value is significant, and the lack of fit value is not significant, indicating that the experimental data and the equation fit well. These values indicate that the independent variable significantly connects with the response (Ba and Boyaci, 2007; Bezerra *et al*., 2008). According to the ANOVA results, the independent variable of X_1 – NADES (choline chloride-sorbitol) ratio and X_3 – microwave power indicated statistically significant

Table 2. Experimental design of MAE condition by RSM using BBD of TPC as dependence variable.

Run	NADES (choline chloride-sorbitol) ratio (g/g)	Extraction time (minutes)	Microwave power (%W)	Liquid-solid ratio (ml/g)	TPC (mgGAE/g)	
	X_1	X_2	X_3	X_4	Y_{actual}	$Y_{prediction}$
1	1:2	5	20	10:1	22.51	24.54
2	1:2	3	20	8:1	18.90	16.02
3	1:2	3	30	10:1	21.22	20.90
4	1:2	7	10	10:1	13.51	13.44
5	1:2	5	20	10:1	21.93	24.54
6	1:2	7	20	12:1	14.35	17.94
7	1:2	5	20	10:1	20.90	24.54
8	1:3	5	20	8:1	18.41	20.64
9	1:3	7	20	12:1	12.19	12.93
10	1:3	7	20	10:1	14.95	13.02
11	1:2	5	30	12:1	23.50	21.91
12	1:2	7	20	8:1	19.83	19.55
13	1:1	5	10	10:1	19.79	21.04
14	1:3	5	10	10:1	11.51	9.55
15	1:2	7	30	10:1	15.56	17.43
16	1:1	3	20	10:1	22.48	24.12
17	1:2	5	10	12:1	14.54	14.04
18	1:1	7	20	10:1	30.62	28.47
19	1:1	5	20	12:1	36.84	33.65
20	1:1	5	20	8:1	24.60	22.91
21	1:2	3	20	12:1	19.67	20.66
22	1:3	3	20	10:1	14.71	16.57
23	1:1	5	30	10:1	25.88	28.91
24	1:2	5	30	8:1	20.31	20.40
25	1:2	3	10	10:1	10.43	9.17
26	1:2	5	20	10:1	27.65	24.54
27	1:3	5	30	10:1	19.45	17.42
28	1:2	5	20	10:1	28.57	24.54
29	1:2	5	10	8:1	9.96	12.53

extraction results based on the probability of an *F*-value less than 0.05.

Meanwhile, the independent variables X_2 – extraction time and X_4 – liquid-solid ratio were higher than 0.05, proving that the variable had no bearing on the extraction outcomes. Extraction time (X_2) was not significant. However, too long extraction time could lead to hydrolysis or oxidation of the polyphenolic compounds, possibly causing a decrease in the TPC value. The liquid-solid ratio (X_3) was not significant, and this similar result was also reported in previous literature (Ahmad *et al*., 2021). Overall, how the variables interact affects the response. Nevertheless, we kept all these factors in the model to obtain more accurate optimization parameters, considering that the four factors used mutually influence the significance of the other factors, as evidenced by the confirmation test.

The obtained optimization results by the software show the three dimensions (3D) of the response surface for reciprocal interactions between factor and process parameters. The 3D response graph of the TPC value to the variable factors is presented in Figure 2, which shows the relationship between the independent variable factors that become the extraction condition parameters. It includes a 3D graph of the response to plots of independently variable factors (A: liquid-solid ratio and extraction time, B: microwave power and extraction time, C: extraction time and NADES (choline chloride-sorbitol) ratio, and D: liquid-solid ratio and NADES ratio) on TPC value from *E. bulbosa* bulbs. The 3D response graph shows that the TPC value will be higher in the red area and lower in the light blue area, as seen in the contour plot after a cross section is made on the graph (Khuri and Mukhopadhyay, 2010).

The optimum condition was recommended based on the RSM analysis results, which included 12:1 ml/g liquid-solid ratio, 20%W microwave power, 5 minutes extraction time, and 1:1 g/g NADES (choline chloride-sorbitol) ratio with the TPC prediction of 33.65 ± 2.83 mgGAE/g sample. For the scale-up confirmation test, a 50 g sample was used to obtain the TPC value of 39.88 mgGAE/g sample using the optimum extraction condition of the MAE method with choline chloride-sorbitol-based NADES. The point prediction analysis determined that the TPC produced was within the 95% tolerance interval (TI) low

Table 3. Analysis of variance.

Source	Sum of squares	df	Mean square	*F*-value	*p*-value (Prob > *F*)
Model	976.89	11	88.81	11.11	<0.0001
X_1 – NADES (choline chloride-sorbitol) ratio	396.48	1	396.48	49.62	<0.0001
X_2 – Extraction time	0.49	1	0.49	0.061	0.8082
X_3 – Microwave power	185.47	1	185.47	23.21	0.0002
X_4 – Liquid-solid ratio	6.85	1	6.85	0.86	0.3674
X_1X_2	15.61	1	15.61	1.95	0.1802
X_1X_3	85.17	1	85.17	10.66	0.0046
X_2X_3	9.75	1	9.75	1.22	0.2846
X_2X_4	14.99	1	14.99	1.88	0.1887
X_2^2	107.29	1	107.29	13.43	0.0019
X_3^2	27.11	1	27.11	3.39	0.0830
X_4^2	189.93	1	189.93	23.77	0.0001
Residual	135.83	17	7.99	-	-
Lack of fit	85.99	13	6.61	0.53	0.8267
Pure error	49.85	4	12.46	-	-
Cor total	1112.73	28	-	-	-

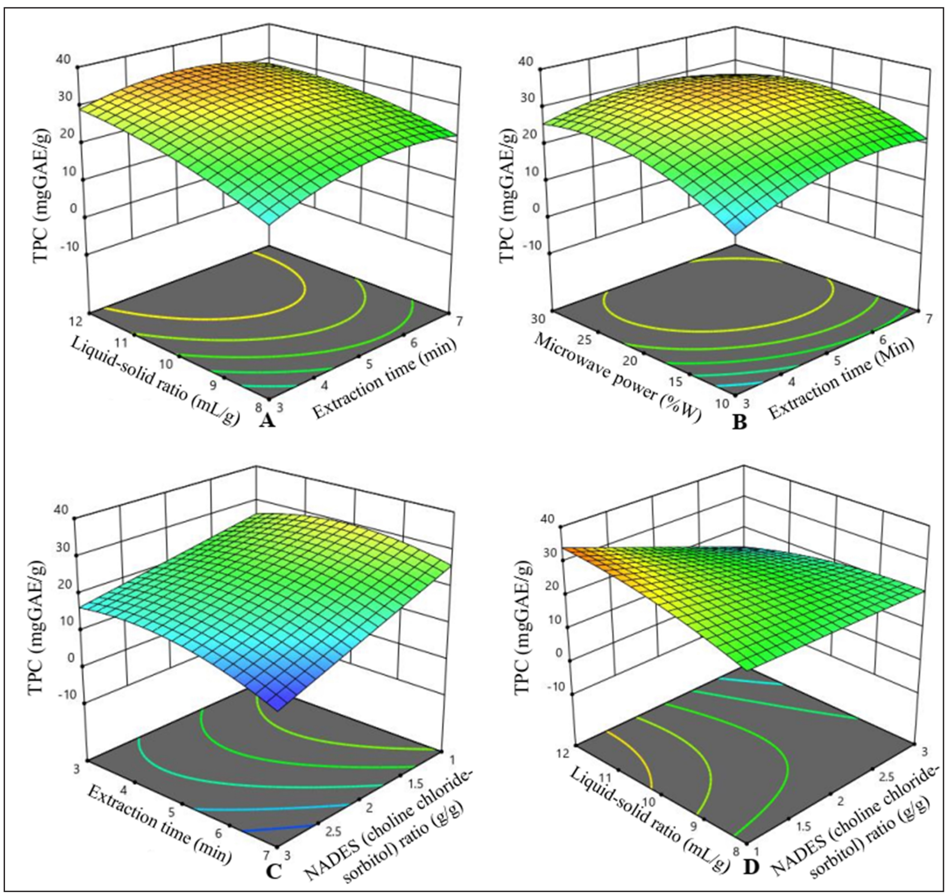

Figure 2. Three-Dimension Response Plots of (a) liquid-solid ratio and extraction time; (b) microwave power and extraction time; (c) extraction time and NADES (choline chloride-sorbitol) ratio; (d) liquid-solid ration and NADES (choline chloride-sorbitol) ratio againts TPC value.

and 95% TI high ranges. The TI indicates complete consistency between the extraction procedure used to obtain the TPC response and the extraction conditions predicted by the program. The effectiveness of the outcomes obtained during the extraction process, considering time, energy, and solvent consumption, is considered when determining the value of each parameter (Khuri and Mukhopadhyay, 2010; Raissi and Farsani, 2009). To sum up, the experimental results show that the predicted value has little difference from the experimental value, indicating that the model can be used for prediction.

Based on the findings mentioned above, using NADES containing choline chloride and sorbitol is much more efficient than the conventional approach employed by previous studies (Munaeni *et al*., 2019; Shi *et al*., 2019). Munaeni *et al*. (2019) reported a TPC value of 2.5 mgGAE/g from macerated ethanol extract, and Shi *et al*. (2019) reported a TPC value of 3.23 mgGAE/g with the extraction of combined phenols from *E. bulbosa* bulbs.

Choline chloride is the most commonly used HBA for NADES composition (Abbott *et al*., 2004; Liu *et al*., 2022; Zhao *et al*., 2015). Meanwhile, sorbitol was used as typical HBD for NADES composition because sorbitol is a sugar alcohol with a sweet taste that is slowly digested by the human body and is often used in diet food and drink products (Godswill, 2017). Therefore, sorbitol, as a typical HBD composition, is in line with the use of NADES-based *E. bulbosa* bulb extract as a raw material for herbal medicine in accordance with its efficacy as an antidiabetic.

These earlier extraction studies show that the MAE method with choline chloride-sorbitol-based NADES can be used in specific situations to quickly, efficiently, and in an environment-friendly way replace conventional solvents and extraction techniques.

CONCLUSION

Our findings in this study show that the MAE method with choline chloride-sorbitol-based NADES proved more efficient as a promising alternative solvent and could be developed to suit the characteristics of the specified target compound of *E. bulbosa*, especially as a raw material for herbal medicines. Therefore, the recommended extraction conditions based on the analysis results using RSM with BBD are 1:1 g/g NADES (choline chloride-sorbitol) ratio, 5 minutes extraction time, 20%W microwave power, and 12:1 ml/g liquid-solid ratio. The optimum condition of the MAE method with choline chloride-sorbitol-based NADES produced a higher extraction TPC from *E. bulbosa* bulbs. It was conducted efficiently, rapidly, and in an environment-friendly way.

AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the international committee of medical journal editors (ICMJE) requirements/guidelines.

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CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

ETHICAL APPROVALS

This study does not involve experiments on animals or human subjects.

DATA AVAILABILITY

All data generated and analyzed are included in this research article.

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