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ORIGINAL ARTICLE

Optimization of microwave-assisted extraction on polyphenol metabolite from *Eleutherine bulbosa* (Mill.) urb. bulbs using response surface methodology

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ABSTRACT

Eleutherine bulbosa bulbs, an endemic plant in Indonesia, have enormous potential as raw materials for pharmaceutical products. Therefore, it is necessary to strengthen and develop extraction methods that are easy, rapid, and efficient to enrich targeted secondary metabolites. This study aims to optimize the microwave-assisted extraction (MAE) method conditions for polyphenol metabolite from *E. bulbosa* bulbs. The MAE method (with different conditions) was applied to extract total polyphenol content (TPC) from *E. bulbosa* bulbs. TPC values were determined using a 96-well microplate reader spectrophotometry method and Folin–Ciocalteu reagent. The variables of MAE, as an experimental design-independent variable, were involved. The MAE method condition was optimized using response surface methodology (RSM) and Box–Behnken design based on the TPC value. The MAE condition was optimized with 60% ethanol, sample–solvent ratio of 1:10 g/mL, and 50% Watts of microwave power for 10 min. The quadratic regression analysis was achieved to predict the TPC value using the equation: TPC value = 28.63–5.545A +2.211B –0.741C +1.995D – 4.045AB +0.856AC –7.541BC +1.961CD –8.342A^2–0.071B^2 +1.840C^2–1.535D^2. For the scale-up confirmation test, a 50-g sample was used to prove the validity of the equation to predict the TPC value, yielding 35.33 ± 2.13 mg gallic acid equivalent/g samples. The optimum of the MAE condition recommended based on the results of RSM analysis can be applied directly to the enrichment of polyphenols metabolite constituent of *E. bulbosa* easily, cheaply, quickly, and efficiently.

Key words: *Eleutherine bulbosa* Mill. Urb, microwave-assisted extraction, polyphenol metabolite constituent, response surface methodology

INTRODUCTION

Eleutherine bulbosa (Mill.) Urb has been used as traditional medicine by the local community in Kalimantan, Indonesia, for generations. *E. bulbosa* bulbs contain metabolites that belong to constituent groups such as flavonoids, alkaloids, quinones, saponins, steroids, triterpenoids, monoterpenoids,

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sesquiterpenes, tannins, and polyphenols.[1] Previous research has shown that *E. bulbosa* bulbs have the potential to lower blood sugar levels and act as antioxidants,[2] antibacterials,[3] and anticholesterols,[4] which will not cause significant risk when used on humans.

The selection of the extraction method is one of the essential factors in determining the study's results. Recently, the extraction process has been widely used with a microwave as a heat source. Compared to traditional methods, the microwave-assisted extraction (MAE) method has some significant advantages for optimal, quick, and efficient extraction.[5,6] In other studies, MAE was also used to extract antioxidant phenolic compounds from peanuts and optimized using response surface methodology (RSM). The extraction of phenolic compounds from peanut shells under optimal conditions using a sample of 1.5 g, with 90% power, an extraction time of 30 s, and a total phenolic of 144 mg gallic acid equivalent (GAE)/g.[5]

The RSM continually optimizes the extraction process. RSM is a group of methods for developing, enhancing, and maximizing a process based on math and statistics. Hence, RSM is applied to be a reliable and precise tool in optimization by evaluating the effect of four-independent parameter factors. The value of this parameter can be used as a reference and experimental basis that shows the adequacy of the model to predict MAE conditions for optimal total polyphenol extraction.[7,8] The purpose of this study was to optimize MAE conditions and ethanol solvent on total polyphenol content (TPC) enrichment of *E. bulbosa* bulb using RSM with Box–Behnken design (BBD).

MATERIALS AND METHODS

Sample and chemical materials

The sample of *Eleutherine bulboba* (Mill.) Urb bulbs were obtained in April–May 2022 from Kutai–Kartanegara, East Kalimantan, Indonesia. *E. bulbosa* bulbs were identified and authenticated at the Laboratory of Dendrology, Mulawarman University. The sample specimen was kept in the Pharmaceutical R and D Laboratory of FARMAKA TROPIS (010/PTUP-LP/FFUNMUL/VI/2022). Sodium carbonate, Folin–Ciocalteau reagent, and gallic acid standard were purchased from Sigma-Aldrich, Germany (through PT. Elokarsa LLC, Indonesia). Ethanol and distilled water were purchased from CV. Chlorogreen, Indonesia.

Extraction process using microwave-assisted extraction method

According to previous studies,[8-10] the MAE method was applied to extract polyphenol content from *E. bulbosa*. Briefly, a 5-g dry sample was mixed with ethanol and water (with different concentrations) and extracted using the MAE method, which operated under some extraction conditions. The extraction results were then filtered through filter paper and centrifuged for 60 min. The extract solution was dehydrated in a food dehydrator for 24 h to create a dry extract.

Total polyphenol content determination

The TPC value was calculated in several previous studies.[11-17] Briefly, a sample solution of 20 µL (100 g/mL) and 100 µL of Folin–Ciocalteu solution were added to a 96-well microplate reader and allowed to stand for 4 min. Then, 75 µL of a sodium carbonate solution was added to the mixture, allowed to stand for a minute, and then incubated for 2 h. Next, absorbance was measured at a 750-nm wavelength using a 96-well microplate reader spectrophotometer (VersaMaxTM ELISA Microplate Reader, USA). The gallic acid used was made in various concentrations of 12.5–200 g/mL and resulted in a linear regression equation, $Y = 0.015 + 0.001559X$, with $R^2 = 0.997$.

Single-factor determination and optimization of microwave-assisted extraction method

In this study, we designed multiple levels on one factor and other factors in a stable condition, allowing for the analysis of the single-factor effect to examine the single factor. At the same time, RSM was used to optimize the MAE condition for polyphenols. By maximizing extraction conditions, the influence of the TPC value (dependent variable) on various factors (independent parameters) was calculated [Table 1]. Based on a design experiment from RSM using BBD, 29 trials were carried out, according to previous studies.[8,18] A multilinear quadratic regression model was used to optimize the extraction condition on TPC values using the licensed Design-Expert v12 software (Statease Inc. Minneapolis, MN, USA).

RESULTS

Single-factor determination

The effects of different levels of each parameter factor, such as ethanol concentration (50, 60, and 70%v/v), sample–solvent ratio (1:8, 1:10, and 1:12 g/mL), microwave power (30, 50, and 70%watts), and extraction time (10, 15, and 20 min), are shown in Figure 1.

Optimization of microwave-assisted extraction condition

According to the analysis carried out in the study from 29 running times, the condition with the highest TPC (16th run) was 45.817 mgGAE/g, and conditions with the lowest polyphenol content (26th run) were 12.564 mgGAE/g, as shown in Table 2.

The TPC value data [Table 2] were examined using multivariate regression analysis according to the experimental

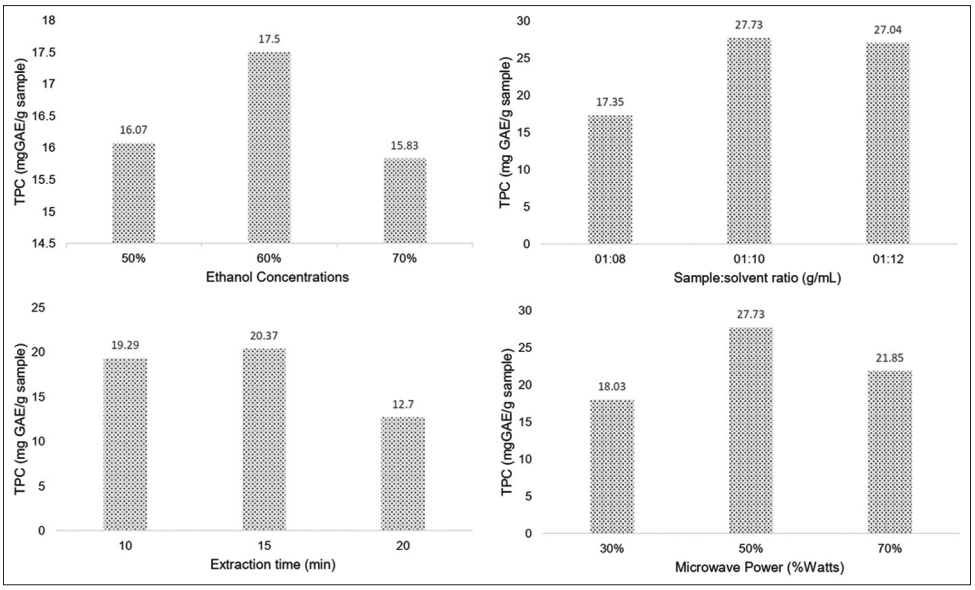

Figure 1: Single factor effect of MAE condition of polyphenol extraction from *E. bulbosa* bulb. MAE: Microwave-assisted extraction

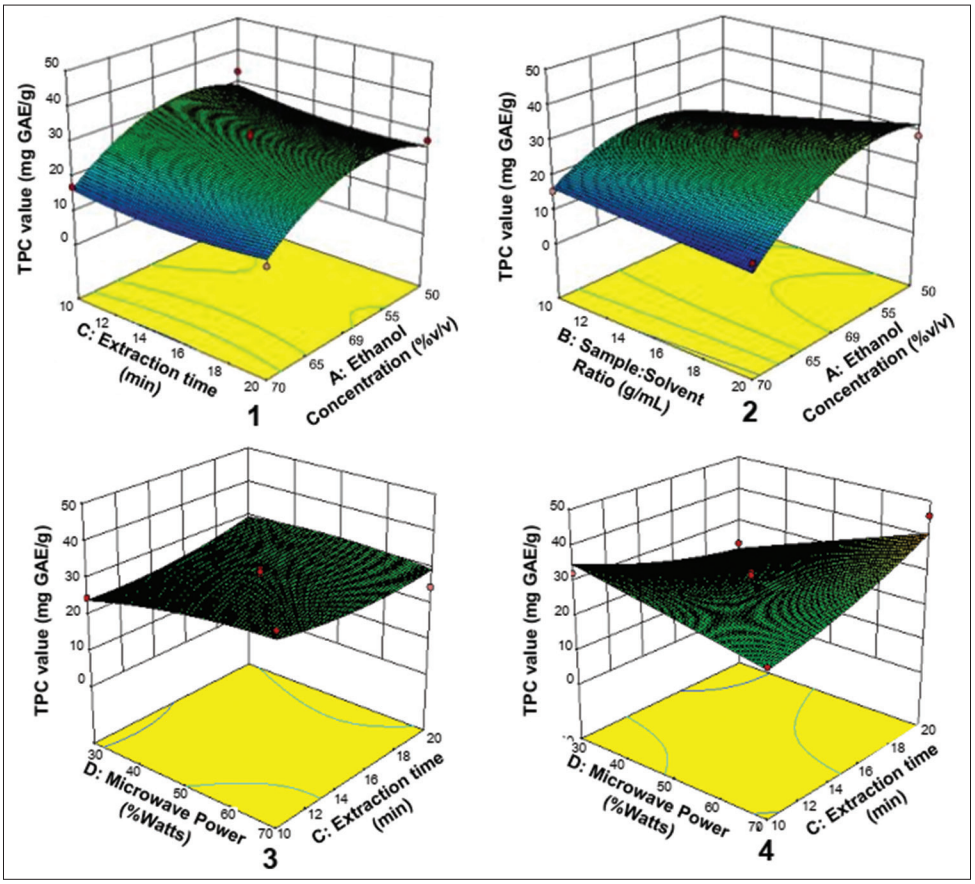

Figure 2: Contour plot three-dimension graph of extraction condition on TPC value. TPC: Total polyphenol content

design of RSM with BBD [Table 1]. The multivariate regression model was based on the analysis of variance results and was used to predict the TPC value from *E. bulbosa* with the equation: TPC value = 28.63–5.545A + 2.211B – 0.741C + 1.995D – 4.045AB + 0.856AC – 7.541BC + 1.961CD – 8.342A^2 – 0.071B^2 + 1.840C^2 – 1.535D^2, with R^2 = 0.8131.

The results are then adjusted to the model recommended automatically by the system in the Design-Expert software,

Table 1: Variable parameter factors of microwave-assisted extraction condition for experimental design

Parameter factors	Unit	Code	Levels		
			Low (−1)	Medium (0)	High (1)
Ethanol concentration	% v/v	A	50	60	70
Sample-solvent ratio	g/mL	B	1:8	1:10	1:12
Extraction time	min	C	10	15	20
Microwave power	% watts	D	30	50	70

Table 2: Total polyphenol content value based on design experimental of microwave-assisted extraction conditions

Run	Factors and levels				TPC-value (mg GAE/g)±SD
	A (% v/v)	B (g/mL)	C (min)	D (% watts)	
1	60 (0)	1:10 (0)	15 (0)	50 (0)	29.93±1.41
2	60 (0)	1:8 (−1)	15 (0)	30 (−1)	26.21±1.62
3	60 (0)	1:10 (0)	20 (1)	70 (1)	34.19±1.78
4	60 (0)	1:10 (1)	20 (1)	30 (−1)	24.76±1.23
5	60 (0)	1:8 (−1)	10 (−1)	50 (0)	23.01±1.65
6	70 (1)	1:8 (−1)	15 (0)	50 (0)	15.54±2.65
7	60 (0)	1:12 (1)	20 (1)	50 (0)	24.90±1.76
8	60 (0)	1:12 (1)	15 (0)	30 (−1)	24.24±1.75
9	50 (−1)	1:12 (1)	15 (0)	50 (0)	28.57±1.21
10	60 (0)	1:10 (0)	10 (−1)	30 (−1)	23.19±1.35
11	60 (0)	1:10 (0)	10 (−1)	70 (0)	24.77±1.68
12	60 (0)	1:10 (0)	15 (0)	50 (0)	30.45±1.88
13	60 (0)	1:10 (0)	15 (0)	50 (0)	27.52±1.67
14	70 (1)	1:10 (0)	15 (0)	30 (−1)	12.83±3.31
15	60 (0)	1:10 (0)	15 (0)	50 (0)	31.14±1.99
16	60 (0)	1:12 (1)	10 (−1)	50 (1)	45.82±1.05
17	60 (0)	1:10 (0)	15 (0)	50 (0)	24.10±1.93
18	70 (1)	1:10 (0)	20 (1)	50 (0)	14.71±2.77
19	70 (1)	1:10 (0)	15 (0)	70 (1)	13.36±2.43
20	50 (−1)	1:10 (0)	10 (−1)	50 (0)	33.47±1.55
21	70 (1)	1:11 (1)	15 (0)	50 (0)	15.36±2.44
22	60 (0)	1:8 (−1)	15 (0)	50 (0)	32.43±1.46
23	70 (1)	1:10 (0)	10 (−1)	50 (0)	17.11±1.97
24	60 (0)	1:12 (1)	15 (0)	70 (1)	29.63±1.63
25	50 (−1)	1:10 (0)	20 (1)	50 (0)	27.65±1.22
26	50 (−1)	1:8 (−1)	15 (0)	50 (0)	12.56±3.34
27	60 (0)	1:8 (−1)	20 (1)	50 (0)	32.25±2.12
28	50 (−1)	1:10 (0)	15 (0)	30 (−1)	26.19±1.87
29	50 (−1)	1:10 (0)	15 (0)	70 (1)	27.01±1.83

TPC: Total polyphenol content, SD: Standard deviation, GAE: Gallic acid equivalent

which explains the relationship between the extraction conditions as the independent variable and the TPC value as the dependent variable. The analysis of variance was used to estimate the statistical significance of the independent variables, their interactions, and the predicted models [Table 3]. Models were remarkably significant, with an F = 5.7996 and a P = 0.0001. In this instance, essential model terms include A, D, AB, BC, and A2. Statistically, a nonsignificant lack of fit ($P > 0.05$) further supported the models' suitability.

The optimization results obtained can be seen as a three-dimensional (3D) surface response graph. Figure 2 shows the 3D surface response graph for TPC.

DISCUSSION

Comparing the MAE method to other straightforward techniques reveals several advantages for optimal, quick, and efficient extraction. To obtain the TPC value of *E. bulbosa* bulb, this study used RSM to optimize the MAE extraction conditions. Compared to the full factorial or trial-and-error method, using an experimental design with RSM reduces the number of experiments conducted and the time needed to optimize the extraction conditions for MAE. The heat generated can facilitate the breakdown of the cell wall and penetration into the cell, increase the dissolution of the target compound in the extraction process, and shorten the extraction process.[19]

The TPC value of *E. bulbosa* bulb with various levels of each factor ranged from 12.564 to 45.817 mgGAE/g sample, according to the experimental results. Increasing microwave power can cause harmful effects. Therefore, for the highest rate of polyphenol extraction, the ideal balance between the extraction time and the ethanol concentration should be sought because the quadratic and linear effects were highly significant ($P < 0.01$) and increased the TPC yield for all microwave powers and extraction times, the ethanol concentration primarily influenced the response of TPC value. However, the sample matrix and solvent have more surface area in contact with one another, which boosts extraction yield.[20,21]

The combination of microwave power and extraction time can increase the target secondary metabolite compound's solubility and decrease the extraction solvents' viscosity, thereby accelerating the release of secondary metabolites. However, some phenolic compounds may also degrade due to high temperatures. The interaction between extraction time and microwave power seriously impacted the TPC value. Despite the synergistic effect being highly significant ($P < 0.01$), the linear and quadratic effects of these parameters (primarily factors B and C) were not significant ($P > 0.05$). The interaction effect of power and extraction time influenced how well the TPC value could be recovered using microwave energy. The contour plot demonstrates that the TPC results were affected ($P < 0.01$) in a quadratic and linear (modified) manner by extracting the sample–solvent ratio. A ratio of approximately 5 g/mL on various other operational factors can maximize TPC yields (microwave power and extraction time). Continuous

Table 3: Analysis of variance

Source	Sum of squares	df	Mean square	*F*	*P* Prob>*F*
Model	1332.4862	12	111.0405	5.7996	0.0007773
A-Ethanol concentration	368.9732	1	368.9732	19.2713	0.0004567
B-Sample-solvent ratio	6.5900	1	6.5900	0.3442	0.5656056
C-Extraction time	47.7720	1	47.7719	2.4951	0.1337654
D-Microwave Power	58.6419	1	58.6419	3.0628	0.099251
AB	65.4384	1	65.4384	3.4178	0.083062
AC	2.9337	1	2.9337	0.1532	0.7006364
BC	227.4562	1	227.4562	11.8799	0.0033164
CD	15.3891	1	15.3891	0.8038	0.3832608
A^2	451.4423	1	451.4423	23.5786	0.0001753
B^2	0.0329	1	0.0329	0.0017	0.9674432
C^2	21.9716	1	21.9716	1.1475	0.2999531
D^2	15.2864	1	15.2864	0.7984	0.384815
Residual	306.3406	16	19.1462		
Lack of fit	273.2857	12	22.7738	2.7559	0.1696197
Pure error	33.0549	4	8.2637		
Cor total	1638.8267	28			

temperature increases in the extraction system were caused by longer extraction time and higher microwave power.

One of the critical components of the MAE method that influences the release of polyphenols from different matrices by rupturing cell walls is microwave power. The microwave power may also alter the extraction equilibrium and mass transfer conditions. The extraction of polyphenols is accelerated by increased microwave power. The contour plot demonstrates that the total extraction yield of phenolic compounds gradually increases with an increase in power from 30% to 50%, followed by a decrease of 70% and an additional increase in the sample–solvent ratio. The trend that led to this TPC was a rise in the sample–solvent ratio, which slows mass transfer due to heating efficiency with lower microwave power and polyphenol solubility. The response of the TPC value was obtained after 10 min at a 1:10 g/mL ratio of sample–solvent, as shown by a response surface area plot generated for the TPC value with a varying extraction time and sample–solvent ratio.

Next, scale-up or verification of the recommended optimal MAE extraction method was carried out on a 50-g sample with a TPC value of 35.33 ± 2.13 mgGAE/g. The recommended optimum extraction conditions were 60% ethanol, 1:10 g/mL sample–solvent ratio, and 50% Watts microwave power for 10 min with the TPC value prediction of 31.21 ± 1.56 mgGAE/g.

CONCLUSION

This study found that the different extraction conditions used with the MAE method had a significant impact on the TPC value of *E. bulbosa* bulb. In addition, the recommended optimum conditions based on the results of the RSM analysis can be applied directly for TPC enrichment easily, cheaply, quickly, and efficiently.

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Conflicts of interest

There are no conflicts of interest.

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