

Response surface modeling to optimize sonication extraction with the maceration method for the phenolic content and antioxidant activity of *Justicia gendarussa* Burm f.

Waras Nurcholis^{1,2*}, Mega Safithri^{1,2}, Nelly Marliani¹, Muhammad Iqbal¹

¹Department of Biochemistry, Bogor Agricultural University, Bogor, Indonesia.

²Tropical Biopharmaca Research Center, Bogor Agricultural University, Bogor, Indonesia.

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ABSTRACT

The extraction of bioactive medicinal plants can be affected by solvent, temperature, and extraction time. This work describes the optimization of the extraction process of phenolic antioxidants from *Justicia gendarussa* Burm f. leaves based on response surface methodology. The independent variables used in the experimental design were ethanol concentration (10%, 50%, and 90%), temperature (30°C, 45°C, and 60°C), and time (60, 120, and 180 minutes). The total phenolic content (TPC) and antioxidant activity of 2,2-diphenyl-1-picrylhydrazyl were used as responses. The results for phenolic antioxidants were maximized, producing a desirability value of 0.918. The optimum formula for extracting *J. gendarussa* leaves was at an ethanol concentration of 30%, a temperature of 30°C, and an extraction time of 180 minutes, which resulted in a TPC content of 8.29 mg gallic acid equivalent/g dry weight (DW) and DPPH antioxidant activity of 4.010 μmol trolox equivalent/g DW. The predicted value is in accordance with the verification results for the best extraction conditions, as evidenced by the % percent residual standard error TPC and antioxidant activity, 4.465% and 7.082%, respectively. Sonication with the maceration extraction procedure can significantly increase the extraction of phenolic antioxidants from *J. gendarussa* leaves. This is a valuable method for extracting essential phenolic antioxidants from medicinal plants.

INTRODUCTION

Justicia gendarussa Burm f. is a quickly growing shrub with a pine scent. It is included in the Acanthaceae family (Subramanian *et al.*, 2012). *Justicia gendarussa* is shaped like a single lanceolate, pinnate, opposite with dark green leans and short stems. The edges of the leaves are flat and tapered. *Justicia gendarussa* provides several benefits, including anticancer, antibacterial, hepatoprotective antifungal, antioxidant, anthelmintic, and antiangiogenic activity (Kavitha *et al.*, 2014). This plant is used as part of traditional medicine in Indonesia. In the Papua region, it is used as a male contraceptive (Widiyanti *et al.*, 2016). The *J. gendarussa* plant contains ingredients that

can inhibit the function of hyaluronidase in the head of the chromosome during fertilization, so *J. gendarussa* is used as a male contraceptive (Utami *et al.*, 2013).

The medicinal activity of plants is influenced by their phytochemicals. In general, plants have two types of molecular metabolites, namely, primary and secondary, and these metabolites play a role in pharmacologic activity against various diseases (Vijayakumar *et al.*, 2019). According to Subramanian (2012), the ethanolic extract of the *J. gendarussa* stem contains glycosides, tannins, and phenolic components. The ethanolic extract of *J. gendarussa* leaves contains tannins, saponins, flavonoids, steroids, terpenoids, alkaloids, phenols, and glycosides (Nirmalaj and Perinbam, 2015).

Phenolic compounds are classified as primary antioxidants because they can inhibit the propagation stage with free radicals (Shahidi and Ambigaipalan, 2015). Antioxidants play a role in neutralizing free radicals, or reactive and unstable

*Corresponding Author
Waras Nurcholis, IPB University, Bogor, Indonesia.
E-mail: wnurcholis@apps.ipb.ac.id

molecules in the body. Free radicals play an important role in physiological conditions and have an impact on various diseases (Phaniendra *et al.*, 2015). They can cause cellular, tissue, and genetic damage. Tests of antioxidant activity can use DPPH methods. Interest in active compounds in plants is increasing, and many studies have been conducted on the subject. Secondary metabolites are divided into three groups based on chemical structure: terpenes, phenolic compounds, and nitrogen (Olivoto *et al.*, 2017). Various secondary metabolites in plants include alkaloids, steroids, tannins, glycosides, essential oils, resins, phenols, and flavonoids. Secondary metabolites are found in various plant parts, such as their leaves, stems, roots, flowers, seeds, fruits, and bark. Appropriate extraction methods are needed to identify and obtain secondary metabolites in plants. This must take into account the factors that affect the extraction, including time, temperature, type of solvent, ratio of material and solvents, and particle size (Chairunnisa *et al.*, 2019).

Response surface methodology (RSM) is a statistical and mathematical method used for optimal analysis under optimal conditions (Prabudi *et al.*, 2018). RSM is used to model and analyze the effects of an independent variable (x factor) on a response to optimize this response (Octaviani *et al.*, 2017). The advantages of RSM include reducing cost, time, and energy in research. In addition, RSM can reduce the number of experimental experiments, calculate complex interactions between independent variables, and analyze, optimize, and improve existing designs (Moradi *et al.*, 2016).

MATERIALS AND METHODS

Plant sample preparation

Justicia gendarussa Burm f. leaves were obtained in January 2022 from the Tropical Biopharmaca Research Center, Institut Pertanian Bogor, Bogor, Indonesia. The leaves were washed thoroughly with water. Then, they were cut into small pieces and dried in an oven for two days and one night at 45°C. The leaves were ground using a desk mill with an 80-mesh sieve to obtain *Simplicia*.

Experimental design and extraction

The experimental design drew on the procedure used by Uysal *et al.* (2016), applying some modifications. Design Expert software (version 13.0, Stat-Ease Inc., Minneapolis, MN) was used to design three numerical factors at the three levels used. The design consists of 17 *randomized runs* with 5 repeats at the center point. To normalize the parameters, each value of the created variables ranges from -1 to +1 (Table 1). The *Simplicia* in each treatment weighed 2 g and was dissolved in 10 ml ethanol. The

samples were homogenized using a sonicator for 30 minutes. They were macerated at different ethanol concentrations (10%, 50%, and 90%), at different temperatures (30°C, 45°C, and 60°C), and for different times (60, 120, and 180 minutes) using an oven. The sample was filtered using filter paper, and the resulting filtrate was a sample extract with a concentration of 0.2 g/ml.

Total phenolic compound (TPC)

For the determination of the total phenolic level, we referred to Yanuarti *et al.* (2017) with modifications. A 20 µl sample solution (0.2 g/ml; 10 ml) was mixed with 120 µl 10% Folin–Ciocalteu reagent and incubated for 5 minutes. Then, a 10% solution of Na₂CO₃ was added to the *microplate* and incubated for 30 minutes at room temperature. The absorbance was capped at 750 nm; 250 ppm standard stock solution C₇H₆O₅ was prepared by dissolving 0.025 g of C₇H₆O₅ into a 100 ml measuring flask with an ethanol solvent. The standard solution stock was then diluted to concentrations of 50, 75, 100, 150, 200, and 225 ppm. Blanks were made with ethanol solvent in place of the sample in the reaction mixture. The TPC is expressed as milligrams of gallic acid equivalent [mg gallic acid equivalent (GAE)/g DW of sample].

Determination of antioxidant activity using the DPPH radical scavenging method

For testing the antioxidant activity of the DPPH, we drew on Haeria *et al.* (2018) with modifications. A 100 µl sample was added to 100 µl DPPH 125 µM solution (in ethanol). The mixture in the *microplate* was then incubated in a dark room for 30 minutes. The absorbance was measured using a nanospectrophotometer at a wavelength of 515 nm. Ethanol was used as a blank. A calibration curve was then created between % inhibition and the standard solution stock of Trolox, diluted to concentrations of 20, 50, 70, 80, 90, and 100 µM. Blanks are made with ethanol solvent instead of the sample in the reaction mixture. The results are expressed as the Trolox equivalents [µmol trolox equivalent (TE)/g DW of sample].

Statistical analysis

Significance was analyzed for each TPC response and antioxidant activity based on a one-way analysis of variance (ANOVA) using Duncan's advanced test. The fitting model was carried out using ANOVA and determining the final solution of the optimum formula with the desirability value using the program Design Expert 13.0. These values were obtained in the range of 0, namely, the lowest level of confidence, to 1, complete confidence (Derringer and Suich, 1980). A second-order polynomial equation was used to predict the response of TPC and antioxidant activity, as follows:

$$Y_{\text{response}} = \beta_0 + \sum_{i=1}^3 \beta_i X_i + \sum_{i=1}^3 \beta_{ii} X_{ii}^2 + \sum_{ij, i < j} \beta_{ij} X_i X_j + e_i \quad (1)$$

where Y is the response of TPC or antioxidant activity (DPPH); X_1 is the ethanol concentration (%); X_2 is the extraction temperature (°C); X_3 is the extraction time (minute); e_i is the error term; β_0 is the intercept; β_i , β_{ii} , β_{ij} are the linear, quadratic, and interactive coefficients, respectively (Bas and Bayoci, 2007).

Model verification

The optimum formula (highest desirability) in relation to the determination of optimizing the extraction of *J. gendarussa*

Table 1. Symbol, variables, and code level of experimental extraction optimization of *J. gendarussa* leaf.

Symbol	Free variables	Code level		
		-1	0	1
A	Ethanol concentration (%)	10	50	90
B	Temperature (°C)	30	45	60
C	Time extraction (minute)	60	120	180

leaves was identified using method verification by conducting TPC testing and antioxidant activity. Next, it was analyzed by calculating the percent residual standard error (% RSE) using the following formula (Equation 2):

$$\%RSE = \frac{(\text{actual value} - \text{predicted value})}{\text{predicted value}} \times 100. \quad (2)$$

RESULTS AND DISCUSSION

RSM analysis

The RSM, in the Box–Behnken design, is used to determine the optimization point for the extraction conditions for TPC and antioxidant activity of *J. gendarussa* leaves. The average value of the total phenolic concentration and DPPH radical inhibitory activity is presented in Table 2. The two response variables showed a significant difference ($p < 0.05$) based on the test results using one-way ANOVA with Duncan's further test.

Fitting the model

Model selection can be a qualification for optimizing the evaluated variables. Drawing on data processing obtained from Design Expert 13.0, the equation for TPC (Y_1) and DPPH antioxidant activity (Y_2) was obtained. The optimization of phenolic content and antioxidant activity was performed using independent variables, including ethanol, temperature, and extraction time. Optimization has an important role to play in the efficiency and probability of industrial processes (Uysal *et al.*, 2016). The

experimental design used in this study was the RSM, used to see the effect of more than one independent variable. According to Azahar *et al.* (2010), the need to maximize or minimize various independent variables to evaluate the influence of several factors and the interaction of one or more variables at the same time can be met by the RSM method.

The optimization of the TPC of *J. gendarussa* leaf extract using the RSM method is described using the quadratic model equation (Table 3), with a coefficient of determination (R^2) obtained from the TPC quadratic equation of 0.9497, where values of the coefficient of determination of more than 0.80 or at more than 70% are preferable; this parameter describes the suitability of the regression model (Rasera *et al.*, 2019). In addition, the adjusted R^2 value of TPC was 0.8272. The quadratic TPC model was significant, $p < 0.05$. A nonsignificant lack of fit (0.8248 > 0.05) was expected, as this value indicates the adequacy of the installed model. For nonsignificant values or where the F statistic value is greater than the significance level, the installed model is considered to adequately explain the data in the experimental area. Conversely, if the F statistic of lack of fit is smaller than the significance level, then most of the residual errors are caused by the mismatch. The Adeq precision (AP) value of the TPC response model of 11.3541 is an adequate signal for the quadratic model. To confirm that the projected model could be used to navigate a design-defined space, AP values greater than 4 are preferred. The relationship between the TPC response for a certain level of each factor can be expressed through the following polynomial equation (Eq. 3):

Table 2. Box–Behnken experimental design with three independent variables and experimental data level of TPC and DPPH antioxidant activity.

Run	Independent variables			Dependent variables	
	Ethanol concentration (%)	Temperature extraction (°C)	Time extraction (minute)	TPC (mg GAE/g DW)	DPPH antioxidant (μmol TE/g DW)
1	10	30	120	8.868 ^a ± 0.24	3.422 ^{bcd} ± 0.27
2	90	60	120	2.764 ^g ± 0.24	3.796 ^{abcd} ± 0.21
3	50	60	60	6.053 ^e ± 0.07	4.207 ^a ± 0.42
4	50	60	180	7.962 ^b ± 0.12	3.681 ^{abcd} ± 0.34
5	90	45	60	2.311 ^h ± 0.21	3.447 ^{bcd} ± 0.08
6	50	45	120	7.305 ^c ± 0.17	3.254 ^{de} ± 0.31
7	50	45	120	7.251 ^c ± 0.40	2.970 ^e ± 0.34
8	50	30	180	6.720 ^d ± 0.29	3.970 ^{ab} ± 0.49
9	50	30	60	6.965 ^d ± 0.16	3.864 ^{abc} ± 0.36
10	90	45	180	2.336 ^h ± 0.03	2.983 ^e ± 0.06
11	50	45	120	6.683 ^d ± 0.38	3.403 ^{bcd} ± 0.08
12	90	30	120	2.770 ^g ± 0.07	3.655 ^{bcd} ± 0.27
13	50	45	120	6.802 ^d ± 0.11	3.307 ^{cde} ± 0.41
14	10	45	180	8.116 ^b ± 0.21	2.175 ^f ± 0.21
15	50	45	120	5.192 ^f ± 0.29	3.519 ^{bcd} ± 0.28
16	10	60	120	6.902 ^d ± 0.24	1.585 ^g ± 0.15
17	10	45	60	6.556 ^d ± 0.07	2.266 ^f ± 0.21

The analysis data listed are mean data ± standard error of the mean (SEM). GAE, gallic acid equivalent; TE, Trolox Equivalent; TPC, total phenolic content; DPPH, 2,2-diphenyl-picrylhydrazyl. Values with different letters in the same column differ significantly at p -value < 0.05.

$$Y_{\text{TPC}} = 13.91876 + 0.026808A - 0.275228B - 0.003916C + 0.000816AB - 0.000160AC + 0.000598BC - 0.001077A^2 + 0.001655B^2 - 0.000034C^2, \quad (3)$$

where Y is a response and A , B , and C are the coded variables for ethanol concentrations, extraction temperature, and extraction time, respectively.

For the installation of the model on the antioxidant activity of *J. gendarussa* leaf extract, which has a significant quadratic model (Table 4) ($p < 0.05$), the coefficient value R^2 is 0.9244, with adjusted R^2 being 0.8272, where the statistical results obtained by the ANOVA illustrate that the fitted model is reliable. The nonsignificant lack of fit value ($0.1424 > 0.05$) was also as expected. This model equation could maximize the yield of phenolic compounds with the antioxidant pharmacological activity of *J. gendarussa* leaf extract based on experiments from the RSM design used. In addition, the AP value was 10.4715; this value indicates an adequate signal for the model because it exceeds 4. Thus, the RSM model built to predict TPC and DPPH radical activity has a reasonable fit. The relationship of the DPPH activity responsible for a certain level of each factor can be expressed through the following polynomial equation (Eq. 4):

$$Y_{\text{DPPH}} = 8.19588 + 0.024860A - 0.241141B - 0.000281C + 0.000824AB - 0.000039AC - 0.000176BC - 0.000434A^2 + 0.002304B^2 + 0.000034C^2, \quad (4)$$

where Y is a response and A , B , and C are the coded variables for ethanol concentrations, extraction temperature, and extraction time, respectively.

Optimization of procedure

Effect of ethanol concentration, temperature, and time extraction on TPC

As shown in Table 2, the TPC yield of *J. gendarussa* leaves ranged from 2,311 to 8,868 mg GAE/g DW. The design of the 17-factor combinations in the *J. gendarussa* leaf extract sample for TPC resulted in the optimization point presented shown in Figure 1. The highest TPC yield was obtained at 8,868 mg GAE/g DW; this result was found at the optimum condition at ethanol concentrations of 10% and 30°C (Fig. 1a), 10% ethanol concentration at 120 minutes extraction time (Fig. 1b), and 30°C at 120 minutes extraction time (Fig. 1c). These results indicate that the optimum conditions for extracting *J. gendarussa* leaves are 10% ethanol concentration, 30°C, and an extraction time of 120 minutes. Variations in ethanol concentration, temperature, and extraction time significantly affected the phenolic content of *J. gendarussa* leaf extract. The effects of each parameter used are illustrated in a three-dimensional visualization, shown in Figure 1 (panels a–c). Different colors in the visualization indicate different TPC values. Greater TPC values are shown in red, green, and yellow, with values from higher to lower. The code equation is a predictive code for the response results of each factor, which is useful for identifying the relative impact of each factor by comparing its coefficients. Increased TPC is shown with a positive sign and a decrease with a negative sign (Eq. 5). The equation code for TPC provides a relatively high effect for extraction time (C), ethanol concentration and temperature (AB), and temperature and extraction time (BC). In addition, the square of temperature

Table 3. ANOVA for the TPC extraction of *J. gendarussa* fitted quadratic polynomial model.

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	68.68	9	7.63	14.68	0.0009	Significant
Residual	3.64	7	0.5199			
Lack of fit	0.6687	3	0.2229	0.3001	0.8248	Not significant
Pure error	2.97	4	0.7426			
Cor total	72.32	16				
R2 = 0.9497						
Adjusted R2 = 0.8850						
$p = 0.0009$ ($p < 0.005$)						
AP = 11.3541						

Table 4. ANOVA for the DPPH antioxidant of *J. gendarussa* fitted quadratic polynomial model.

Source	Sum of squares	df	Mean square	F-value	p-value	
Model	7.09	9	0.7879	9.51	0.0036	Significant
Residual	0.5800	7	0.0829			
Lack of fit	0.4112	3	0.1371	3.25	0.1424	Not significant
Pure error	0.1688	4	0.0422			
Cor total	7.67	16				
R2 = 0.9244						
Adjusted R2 = 0.8272						
$p = 0.0036$ ($p < 0.005$)						
AP = 10.4715						

(B^2) shows a relatively high effect with a decrease in ethanol concentration (A), extraction time (C), and extraction time squared (C^2). These terms can illustrate the way in which the interactive nature of each factor affects the increase in TPC.

$$\text{TPC} = 6.68 - 2.53A - 0.2052B + 0.4061C + 0.4898AB - 0.3836AC + 0.5385BC - 1.72A^2 + 0.3723B^2 - 0.1237C^2 \quad (5)$$

The solvent employed in extraction can affect the TPC (Ezez and Tefera, 2021). Overall, the TPC in the tested *J. gendarussa* leaf extract was strongly influenced by the ethanol solvent used (Fig. 1). This is due to the level of polarity, such that where ethanol has a high polarity but one that is not higher than water. Ethanol with a concentration of 10% is a very polar solvent, so it maximizes the content of polar compounds in the sample, as indicated by the high TPC content in these conditions. Polyphenol groups, such as phenolics, are often soluble in organic solvents that are less polar than water. However, their solubility depends on the polar nature of the polyphenol content itself, such that the selection of the type and concentration of the solvent for extraction is very important (Haminiuk *et al.*, 2014). The compound 6,8-di-C- α -L-arabinopyranosylapigenin, referred to as Geadarusin A, is the main component of *J. gendarussa* and belongs to the polyphenol group. This compound is widely extracted using polar solvents (Ratih *et al.*, 2019). The ideal extraction temperature is affected by the concentration of the solvent (Cacace and Mazza, 2003). In this study, the solvent concentration obtained at 10% was the lowest concentration available, with an extraction temperature of 30°C. Cacace and Mazza (2003), who studied blackcurrant extraction, found that its optimal results were shown at low temperatures and low ethanol concentrations. The temperature in the extraction process affects the solubility and stability of the components of phenolic compounds, and an optimum temperature produces the maximum TPC. In this case, the optimum temperature is 30°C. When the temperature exceeds the optimum limit, a decrease is seen in TPC levels (Ju and Howard, 2003). According to Lien *et al.* (2007), the acquisition of TPC from soybeans showed an extraction time of 120–240 minutes. The optimum extraction time for *J. gendarussa* leaf extract was 120 minutes.

Antioxidant activity using the DPPH method

Justicia gendarussa leaf extract showed optimum antioxidant activity using the RSM, namely, 4.20794 $\mu\text{mol TE/g DW}$; this result was indicated by the extraction conditions at 50% ethanol concentration at 60°C (Fig. 2a), 50% ethanol concentration at extraction time of 60 minutes (Fig. 2b), and at 60°C, with an extraction time of 60 minutes (Fig. 2c). This condition means, in determining antioxidant activity using the DPPH method on *J. gendarussa* leaves, optimum conditions were obtained at 60°C, 50% ethanol concentration, and 60 minutes of extraction time. The resulting code equation is presented in Equation 2. This presents a predictive code for the response results of each factor, which is useful for identifying the relative impact of the factors by comparing their coefficients. Based on Equation 6, DPPH can increase relative to the ethanol concentration (A), ethanol concentration and temperature (AB), and the square of temperature (B^2). In contrast, there was a decrease in temperature (B), extraction time (C), ethanol concentration and extraction time (AC), temperature and concentration time (BC), the square of the

ethanol concentration (A^2), and the square of the extraction time (C^2). These terms illustrate how the interactive nature of each factor increases the antioxidant activity of DPPH.

$$\text{DPPH} = 3.29 + 0.5543A - 0.2054B - 0.1220C + 0.4943AB - 0.0935AC - 0.1582BC - 0.6945A^2 + 0.5184B^2 - 0.1216C^2 \quad (6)$$

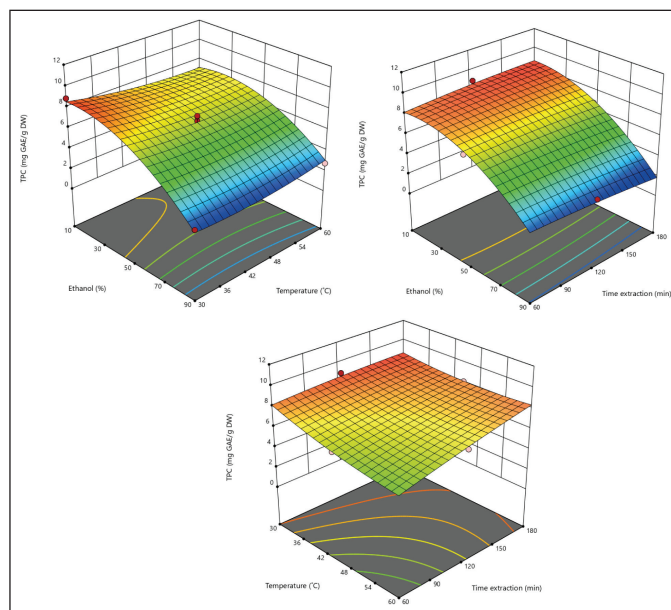


Figure 1. Response surface plots showing the interaction effect of ethanol concentration (%) with temperature (°C) (a), ethanol concentration (%) with extraction time (minute) (b), and temperature (°C) with extraction time (minute) (c) on the response of TPC (mg GAE/g DW).

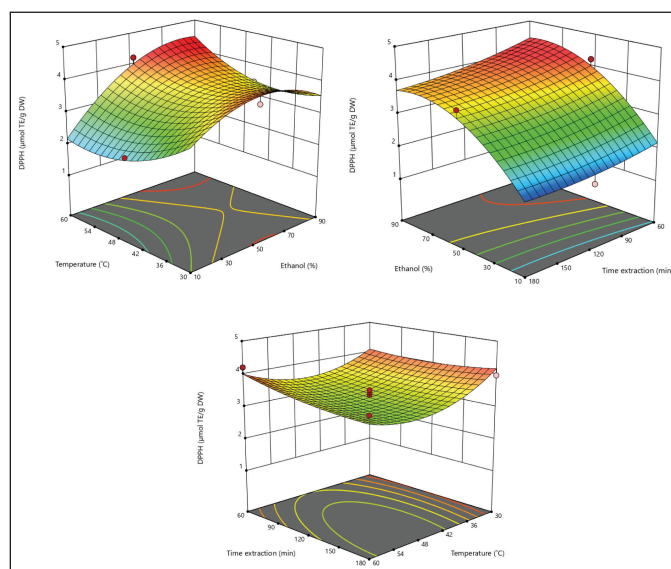


Figure 2. Response surface plots showing the interaction effect of ethanol concentration (%) with temperature (°C) (a), ethanol concentration (%) with extraction time (minute) (b), and temperature (°C) with extraction time (minute) (c) on the response of 2,2-diphenyl-picrylhydrazyl radical scavenging antioxidant activity ($\mu\text{mol TE/g DW}$).

Table 5. Verification of predicted and experimental values using conditions optimized for maximum TPC and DPPH antioxidant activity.

	Independent variables			Dependent variables		Desirability
	Ethanol (%)	Temperature (°C)	Time extraction (minute)	TPC (mg GAE/g DW)	DPPH (µmol TE/g DW)	
Prediction	30	30	180	8.286	4.010	0.918
Verification	30	30	180	9.382	4.294	
% RSE				4.465	7.082	

TPC, total phenolic content; RSE, residual standard error; DPPH, 2,2-diphenyl-picrylhydrazyl.

The DPPH method used in testing the antioxidant activity showed significantly different results ($p < 0.05$) across test treatments. These were influenced by solvent, temperature, and extraction time. Antioxidant compounds are usually derived from polyphenol group compounds. The solubility of polyphenolic compounds is mostly polar, and it is often preferred in organic solvents compared to those using only water. Hence, the choice of extraction solvent is very important. Kuber (2021) also used ethanol to extract *J. gendarussa* with the highest DPPH antioxidant activity. The solubility, structure, and polarity of antioxidant compounds are closely related to the antioxidant activity of these compounds. It is well known that plant extracts that have polyphenolic components have antioxidant properties due to their ability to absorb free radicals and act as hydrogen atoms or electron donors. The strength of the reaction depends on its ability to donate hydrogen from antioxidants (Bondet *et al.*, 1997).

Optimization procedure and verification

The TPC response and DPPH antioxidant activity showed an optimum point, with a desirability value of 0.918, as presented in Table 5. This desirability value is a parameter that is used to see the level of confidence in determining the optimization point based on the RSM from the Design Expert 13.0 program. The results obtained show that the resulting confidence level is 91.8%; therefore, we can determine the validity level of the procedure used. To maximize the acquisition of TPC with the antioxidant activity from *J. gendarussa* leaves, the extraction process can use a formulation that has an ethanol concentration of 30%, an extraction temperature of 30°C, and an extraction time of 180 minutes. Under the conditions of the formula, the levels of TPC and DPPH antioxidant activity were 8.29 mg GAE/g DW and 4.010 µmol TE/g DW, respectively. The verification of the formula of the method carried out is presented in Table 5, and it is analyzed on the basis of the RSE, namely, the standard deviation of the residuals. This value is used as an evaluation parameter for the level of accuracy of the model produced by comparing the predicted value and the actual value (verification) so that smaller values indicate better prediction. The %RSE TPC result was 4.465%, and the antioxidant activity of DPPH 7.082% showed a very good predictive value, meaning that the selected model was very good in determining the optimization of *J. gendarussa* leaf extraction.

CONCLUSION

This is the first study to optimize *J. gendarussa* leaves using RSM with the three variable factors of ethanol

concentration, temperature, and extraction time. The design of the RSM method effectively optimized the acquisition of TPC and DPPH antioxidant activity with a desirability value of 0.918, namely, by using the formula of 30% ethanol concentration, extraction temperature of 30°C, and extraction time of 180 minutes, through which the maximum TPC content and DPPH antioxidant activity were obtained, namely, 8.29 mg GAE/g DW and 4.010 µmol TE/g DW. A comparison between the predicted value and method verification produced a very valid response of the % RSE value of 4.465% (TPC) and 7.082% (DPPH antioxidant activity). We conclude that the RSM is a suitable approach for increasing the production of TPC and antioxidants from *J. gendarussa* for medical and other applications.

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AUTHOR CONTRIBUTIONS

All authors made substantial contributions to the 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.

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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