

Determination of Optimum Extraction Conditions for Antioxidant Capacity from White Turmeric Rhizome (*Curcuma zedoaria* (Christm.) Roscoe) Using Response Surface Methodology

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Abstract

*The role of free radicals in oxidative stress poses risks to cells and tissues. The effect of stress and tissue oxidation damage leads to loss of structure and, ultimately, tissue necrosis. Antioxidants quell free radicals and stabilize them. White turmeric (*Curcuma zedoaria* (Christm.) Roscoe) rhizome, another natural source used in traditional medicine, is said to have some potential as an antioxidant. The current study sought to investigate methods used in devising the optimization of antioxidant extraction from white turmeric using the decoction method. The main factors considered were temperature, extraction time, and sample-to-solvent ratio. The DPPH assay measured at 522 nm provided the readings for antioxidant activity. The initial OFAT approach used to devise the experiment tested and confirmed those optimal conditions of extraction (80 °C, 50 minutes, and 1:25 g/mL). These optimal conditions were tested in the central composite design within RSM. The confirmation of optimal conditions within RSM was (84 °C, 51 minutes, 1:25 g/mL). The extraction of 1.3963 mg AAE/g FW and the predicted value of 1.3450 mg AAE/g FW. These findings validate the extraction efficiency in RSM and demonstrate the antioxidant potential of white turmeric in the pharmaceutical industry.*

Keywords: *Curcuma zedoaria*, antioxidant activity, DPPH, extraction optimization, response surface methodology.

Introduction

Free radicals are unstable molecules containing one or more unpaired electrons, making them highly reactive toward other molecules^{[1][2]}. They can be generated through endogenous processes, such as mitochondrial respiration^[3], hemoglobin^[4] and myoglobin oxidation^[5], as well as exogenous sources including air pollutants, cigarette smoke, organic solvents, and drugs^{[6][7]}. Free radicals can be generated. Excessive production of free radicals leads to oxidative stress, which is associated with aging and various degenerative diseases^{[8][9]}. Therefore, antioxidant compounds are required to neutralize free radicals by donating electrons and preventing oxidative damage.

Natural antioxidants are widely found in spices, herbs, fruits, and vegetables, particularly those rich in phenolic and flavonoid compounds^[10]. One of the potential herbal sources is white turmeric (*Curcuma zedoaria* (Christm.) Roscoe), a member of the Zingiberaceae family. Traditionally, white turmeric has been used to treat digestive disorders, circulatory problems, and toothache^{[11][12]}. The rhizome contains various bioactive compounds, including curcuminoids, flavonoids, and sesquiterpenes such as curzerenone and germacrone, which exhibit antioxidant, anticancer, anti-inflammatory, and antimicrobial activities^{[13][14]}. These compounds contribute to the ability of white turmeric to scavenge free radicals and reduce oxidative damage^{[13][15]}.

The antioxidant activity of plant extracts is commonly evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, which measures the radical scavenging ability of antioxidant compounds based on hydrogen atom donation. The results are typically expressed as antioxidant capacity in terms of ascorbic acid equivalent (AAE). Previous studies have reported that the antioxidant activity of white turmeric varies depending on extraction conditions and solvent types^[8]. However, most studies focus on conventional extraction methods without systematic optimization, and quantitative comparisons of antioxidant capacity values remain limited.

Extraction is a crucial step in obtaining bioactive compounds from plant materials. Among various extraction techniques, the decoction method is widely used due to its simplicity, cost-effectiveness, and suitability for heat-stable compounds^[16]. Several factors, including temperature, extraction time, and sample-to-solvent ratio, significantly influence extraction efficiency and antioxidant yield^[17]. Therefore, optimization of these parameters is essential to maximize the extraction of antioxidant compounds.

Response Surface Methodology (RSM) is a statistical and mathematical tool that can be used to optimize extraction processes by evaluating the effects of multiple variables and their interactions^[18]. Compared to the One Factor at a Time (OFAT) approach, RSM provides a more efficient and systematic method for determining optimal conditions. While OFAT can provide preliminary insights, it does not account for interactions between variables, which may lead to suboptimal conditions^[19].

Despite the recognized potential of white turmeric as a source of natural antioxidants, studies applying RSM to optimize decoction extraction of *Curcuma zedoaria* are still limited. Furthermore, quantitative comparisons of antioxidant capacity with previous studies are rarely discussed. Therefore, this study aims to optimize extraction conditions (temperature, time, and sample-to-solvent ratio) using RSM to

obtain maximum antioxidant capacity from white turmeric rhizomes.

Experimental

Materials

White turmeric rhizomes (*Curcuma zedoaria* (Christm.) Roscoe) were obtained from Anduring, Padang City, West Sumatra, Indonesia. The analytical reagents used were ascorbic acid (Merck), methanol (Merck), DPPH (Sigma-Aldrich), distilled water obtained from the Environmental Laboratory, Department of Chemistry, Andalas University, and aluminum foil.

Instruments

The instruments used in this study included a hot plate (DLAB MS-H280 Pro), an analytical balance (Mettler 200), a UV-Vis spectrophotometer (Thermo Scientific Genesys 20), and standard laboratory glassware (Pyrex). The experimental design and statistical analysis were performed using Design Expert version 13 software (Stat-Ease, Inc., Minneapolis, MN, USA).

Methods

Sampling and Sample Identification

White turmeric was obtained from Anduring, Padang City, West Sumatra, Indonesia. The plant material was taxonomically identified at the Herbarium of the Department of Biology, Faculty of Mathematics and Natural Sciences, University of Andalas, Indonesia.

Sample Preparation

The white turmeric rhizomes were washed and cleaned under running water, and subsequently rinsed and drained. Afterward, the rhizomes were peeled, and the flesh was separated from the skin. To facilitate extraction, the flesh of the rhizomes was cut into small 1 cm pieces and weighed to 5.000 g.

Calibration Curve Preparation of Ascorbic Acid Standard Solution

Standard solutions of ascorbic acid were prepared at concentrations of 3, 6, 9, 12, and 15 mg/L. For each concentration, 1 mL was taken and placed in a test tube. Then, 2.5 mL of 0.1 mM DPPH solution was added, and the mixture was kept in the dark for 30 min. After this, the absorbance was measured at 522 nm using a UV-Vis spectrophotometer. A blank solution was prepared by mixing 1 mL of methanol with 2.5 mL of a 0.1 mM DPPH solution. The calibration curve was constructed based on these measurements.

Determination of Antioxidant Capacity in Samples

In a volumetric flask, 1 mL of the sample extract was diluted to 10 mL with distilled water. Then, 1 mL of this solution was mixed with 2.5 mL of 0.1 mM DPPH solution and incubated in the dark for 30 minutes. The absorbance was measured at 522 nm. All measurements were performed in triplicate ($n = 3$).

Determination of Optimum Conditions for Extraction by the OFAT Method

The OFAT method was used to evaluate the effect of individual variables while keeping other variables constant. The variables studied were temperature (60, 70, 80, 90, and 100 °C), time (30, 40, 50, 60, and 70 min), and sample-to-solvent ratio (1:10, 1:15, 1:20, 1:25, and 1:30 g/mL). The extraction process was carried out using the decoction method as described in Table 1. The results obtained from the OFAT method were used to determine preliminary optimum conditions, which were subsequently applied as the basis for the RSM experimental design.

Determination of Optimum Extraction Conditions by the RSM Method

Design-Expert software V.13 was used to create an experimental matrix based on the central composite design (CCD) methodology. The independent variables were temperature (A), time (B), and sample-to-solvent ratio (C). A total of 20 experimental runs were conducted, including 6 center points. The variables and their coded levels are presented in Table 2, where each factor was studied at five levels (α , -1, 0, +1, and $+\alpha$). The experimental design matrix generated using the CCD approach is shown in Table 3. The response variable was antioxidant capacity (mg AAE/g FW).

Table 1. Experimental design for OFAT optimization of extraction parameters

Variation Type	Temperature (°C)	Time (min)	Sample-to-Solvent Ratio (g/mL)
a. Temperature Effects	60	30	1:20
	70	30	1:20
	80	30	1:20
	90	30	1:20
	100	30	1:20
b. Time Effects	80	30	1:20
	80	40	1:20
	80	50	1:20
	80	60	1:20
	80	70	1:20
c. Ratio Effects	80	50	1:10
	80	50	1:15
	80	50	1:20
	80	50	1:25
	80	50	1:30

Table 2. Symbols, variables, and code levels of white turmeric rhizome experimental extraction optimization

Symbol	Independent Variable	Variable Level Code				
		$-\alpha$	-1	0	+1	$+\alpha$
A	Temperature (°C)	63	70	80	90	97
B	Time (min)	33	40	50	60	67
C	Sample-to-solvent ratio (g/mL)	1:17	1:20	1:25	1:30	1:33

Description: ($-\alpha$): Lower axial point; (-1): Lower limit; (0): Center point; (+1): Upper limit; ($+\alpha$): Upper axial point.

Table 3. Experimental design matrix using the RSM-CCD method

Run	Temperature (°C)	Time (min)	Sample-to-Solvent Ratio (g/mL)
1	70	30	1:15
2	63	40	1:20
3	80	40	1:20
4	80	40	1:20
5	80	40	1:12
6	80	40	1:20
7	70	50	1:15
8	80	23	1:20
9	97	40	1:20
10	90	30	1:15
11	80	40	1:28
12	90	30	1:25
13	80	40	1:20
14	70	30	1:25
15	90	50	1:15
16	80	40	1:20
17	90	50	1:25
18	80	57	1:20
19	70	50	1:25
20	80	40	1:20

Statistical Analysis

Analysis of Variance (ANOVA) was used to evaluate the significance of the model and the effects of individual variables. A second-order polynomial model was used to describe the relationship between variables and response^[20].

$$Y = \beta_0 + \sum_{i=1}^k \beta_i X_i + \sum_{i=1}^k \beta_{ii} X_i^2 + \sum_{i < j} \beta_{ij} X_i X_j + \epsilon$$

Y represents the antioxidant capacity. X_1 is temperature (°C), X_2 is time (min), and X_3 is the sample-to-solvent ratio (g/mL). β_0 is the intercept; β_i are linear coefficients; β_{ii} are quadratic coefficients; β_{ij} are interaction coefficients; and ϵ is the error term.

Results and Discussion

Sample Identification

The white turmeric plant used in this study are shown in Figure 1. Based on the identification document (035/K-ID/ANDA/I/2025), the plant are classified as follows:

Family : Zingiberaceae

Genus : *Curcuma*

Species : *Curcuma zedoaria* (Christm.) Roscoe.

Calibration Curve of Ascorbic Acid Standards by the DPPH Method

A calibration curve of ascorbic acid was generated to evaluate the relationship between concentration (x) and absorbance (y) using the

DPPH assay. Absorbance was measured at 522 nm using a UV-Vis spectrophotometer. In this assay, antioxidants reduce the purple DPPH radical to a yellow-colored DPPH-H, resulting in a decrease in absorbance^[21]. Ascorbic acid has a strong record of effectively neutralizing DPPH free radicals as a result of its hydrogen atom-donating properties. The calibration curve (Figure 2) shows a strong linear relationship between concentration and absorbance, with a regression equation of $y = -0.0324x + 0.7302$ and a coefficient of determination (R^2) of 0.9932. This indicates excellent linearity of the model, confirming its suitability for quantifying antioxidant capacity. The obtained regression equation was used to calculate the antioxidant capacity of the sample extracts based on their absorbance values.



Figure 1. White turmeric plant and white turmeric rhizome

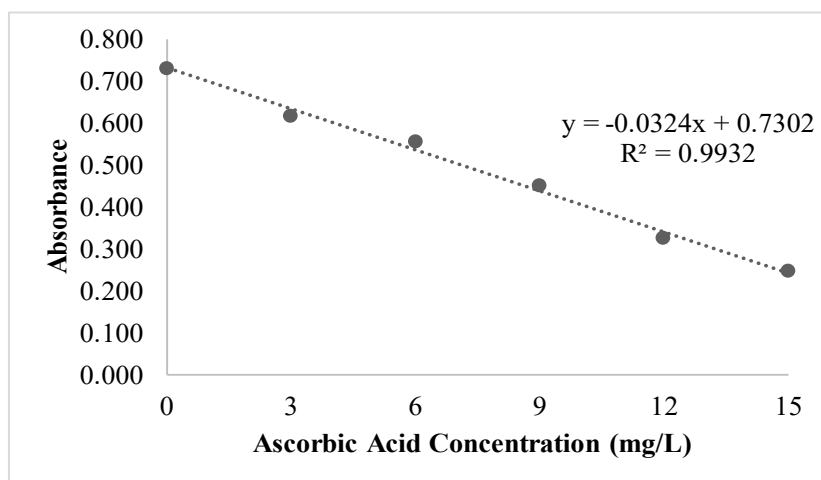


Figure 2. Ascorbic Acid Standard Calibration Curve

Optimum Conditions for Extraction of White Turmeric Rhizome by the OFAT Method

The effect of temperature, extraction time, and sample-to-solvent ratio on the antioxidant capacity of white turmeric rhizome was evaluated using the OFAT method. one variable was varied while the other variables were kept constant^[22]. This approach was used to determine the appropriate range of conditions for subsequent optimization using RSM-CCD^[23]. To ensure the reliability of the results, all experiments were conducted in triplicate (n = 3), and the data were evaluated for consistency.

Temperature Variation

Temperature is a key factor influencing the extraction efficiency of antioxidant compounds from white turmeric rhizomes. Increasing temperature enhances the solubility of bioactive compounds and improves mass transfer by facilitating solvent penetration into the plant matrix^[24]. As shown in Figure 3, antioxidant capacity increased with temperature from 60 °C to 80 °C, reaching a maximum value of 1.2902 mg AAE/g FW at 80 °C. This increase can be attributed to the enhanced diffusion of phenolic and curcuminoid compounds into the solvent, as well as the disruption of plant cell walls during heating. These effects improve the release of antioxidant compounds and increase extraction efficiency.

However, further increases in temperature to 90 °C and 100 °C resulted in a decrease in antioxidant capacity to 1.2041 mg AAE/g FW and 1.1754 mg AAE/g FW, respectively. This decline is likely due to the thermal degradation of heat-sensitive compounds, particularly curcuminoids and phenolic constituents. At elevated temperatures, these compounds may undergo structural degradation, reducing their ability to donate hydrogen atoms and scavenge free radicals, thereby decreasing antioxidant activity^[25].

Similar trends have been reported in previous studies, where excessive temperature led to degradation of phenolic compounds and reduced antioxidant activity. For example, extraction of bioactive compounds from *Zingiber officinale* var. *rubrum* showed that optimal extraction at 80 °C resulted in maximum antioxidant activity, while higher temperatures led to decreased activity due to thermal degradation^[26]. Based on these results, 80 °C was selected as the optimum temperature and used as the center point (0) in the RSM optimization.

Time Variation

Extraction time is a crucial factor influencing the efficiency of antioxidant compound recovery from white turmeric rhizomes. Antioxidant capacity refers to the ability of an extract to neutralize free radicals by donating electrons or hydrogen atoms.

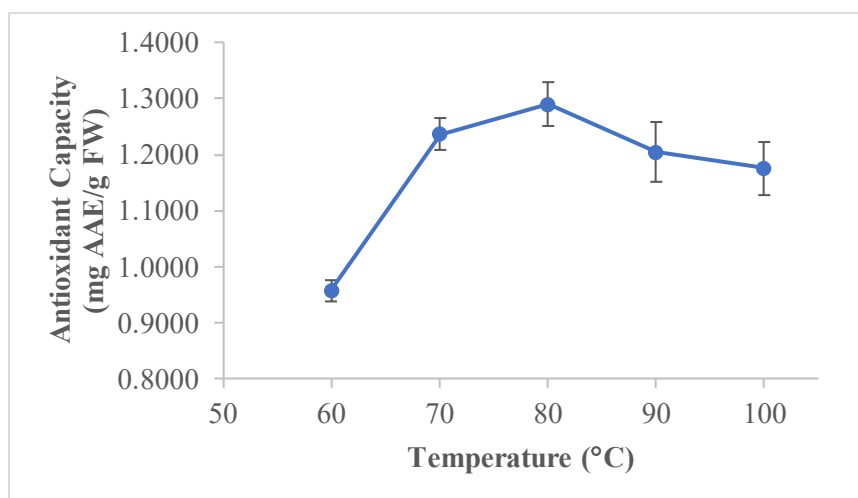


Figure 3. Effect of Extraction Temperature on the Antioxidant Capacity of White Turmeric Rhizome by the OFAT Method

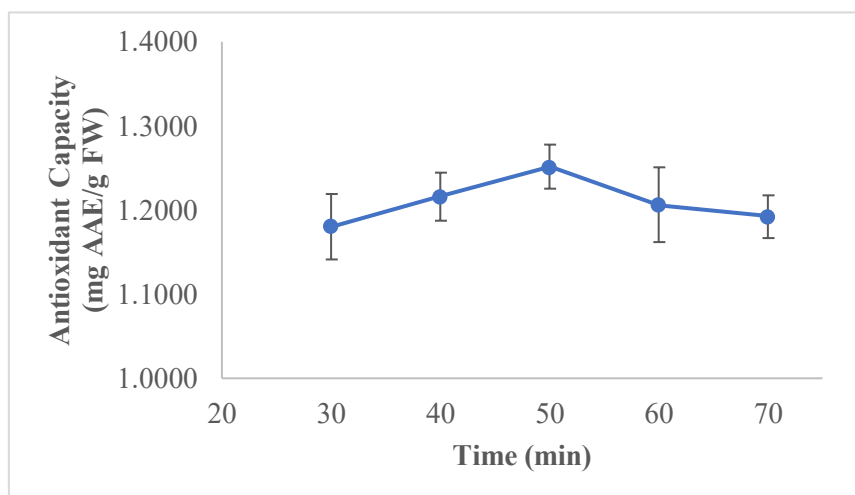


Figure 4. Effect of Extraction Time on the Antioxidant Capacity of White Turmeric Rhizome by the OFAT Method.

In this study, antioxidant capacity was determined using the DPPH assay and expressed as mg AAE/g FW. As shown in Figure 4, antioxidant capacity increased with extraction time and reached a maximum value of 1.2518 mg AAE/g FW at 50 minutes. This indicates that sufficient extraction time enhances solvent penetration and promotes the diffusion of phenolic and curcuminoid compounds into the solvent, thereby increasing extraction efficiency^[25].

However, further increase in extraction time to 60 and 70 minutes resulted in a decrease in antioxidant capacity to 1.2063 and 1.1926 mg AAE/g FW, respectively. This decline is likely due to prolonged thermal exposure, which can induce degradation of heat-sensitive antioxidant compounds, leading to reduced hydrogen-donating ability and lower radical scavenging activity^[27].

Similar findings have been reported in previous studies on turmeric, where the extraction time was varied up to 60 minutes. For example, ultrasonic-assisted extraction (UAE) of *Curcuma longa* showed that antioxidant-related compounds increased with extraction time up to 40 minutes, but slightly decreased at 60 minutes due to degradation of bioactive compounds^[28]. Based on these results, 50 minutes was selected as the optimum extraction time and used as the center point (0) in the RSM optimization.

Sample-to-Solvent Ratio Variation

In the extraction process, the sample-to-solvent ratio is one of the main determinants of the rate of dissolution of bioactive compounds in the material matrix, and thus, the solvent volume is critical. An adequate solvent volume enhances mass transfer by improving solvent penetration into the plant matrix and facilitating the dissolution of antioxidant compounds^[29]. As shown in Figure 5, antioxidant capacity increased with the sample-to-solvent ratio, rising from 0.9311 mg AAE/g FW at a ratio of 1:10 g/mL to a maximum value of 1.3097 mg AAE/g FW at a ratio of 1:25 g/mL. The x-axis values shown in Figure 5 (10–30) correspond to sample-to-solvent ratios of 1:10 to 1:30 (g/mL), respectively. This increase suggests that an adequate solvent volume improves penetration through the plant cell wall, facilitating the dissolution of phenolic compounds and polar antioxidants, including curcuminoids and flavonoids^[16].

However, at a ratio of 1:30 g/mL, antioxidant capacity decreased to 1.1371 mg AAE/g FW. This reduction can be attributed to the dilution effect, where excessive solvent volume reduces the concentration gradient and limits the effective interaction between the solvent and the plant matrix^[30]. Therefore, the ratio of 1:25 g/mL was chosen as the optimum condition in this experiment because it gave a high antioxidant capacity value, and was used as the midpoint (0) in the RSM in the next optimization stage.

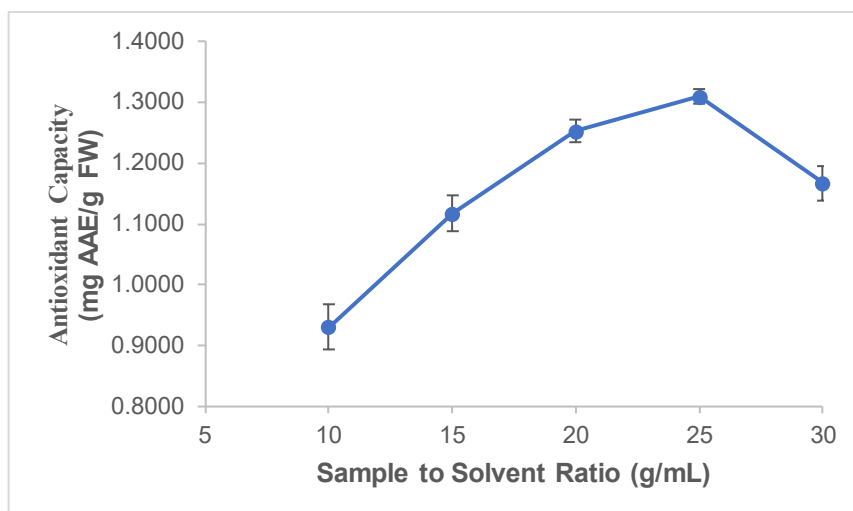


Figure 5. Effect of Sample-to-Solvent Ratio on Antioxidant Capacity by OFAT Method

Optimum Conditions for Extraction of White Turmeric Rhizomes Using the RSM Method

In this study, the optimization of total antioxidant extraction from white turmeric rhizomes was carried out using RSM with a CCD approach. The parameters that varied as independent variables included temperature (63-97 °C), time (33-67 minutes), and the sample-to-solvent ratio (1:17-1:33 g/mL). The experimental design consisted of 20 runs, including factorial points, axial points, and center points, to evaluate both the individual and interactive effects of the variables on antioxidant capacity. The results from the 20 experimental runs carried out according to the RSM-CCD design are presented in Table 3.

The mathematical model used to predict the antioxidant capacity of white turmeric rhizomes follows a second-order polynomial equation, as shown below:

$$\text{Antioxidant capacity} = 1.33 + 0.477A + 0.0220B + 0.0119C + 0.0320AB - 0.0327AC + 0.0152BC - 0.0576A^2 - 0.0149B^2 - 0.1251C^2$$

In this model, A, B, and C represent temperature (°C), extraction time (min), and sample-to-solvent ratio (g/mL), respectively. The positive coefficients of A, B, and C indicate a positive contribution to antioxidant capacity within the studied range. However, their statistical significance differs. The significance of the

model and its terms was evaluated using ANOVA, as presented in Table 4. The overall model was highly significant ($p < 0.0001$) with an F-value of 34.14, indicating that the model adequately describes the relationship between the independent variables and antioxidant capacity. The coefficient of determination ($R^2 = 0.9685$) and adjusted R^2 (0.9401) indicate that 96.85% of the variation in antioxidant capacity can be explained by the model^[31].

Among the linear terms, only temperature (A) showed a significant effect on antioxidant capacity ($p = 0.0022$), while time (B) and sample-to-solvent ratio (C) were not statistically significant ($p = 0.0888$ and 0.3336 , respectively). Although B and C have positive coefficients, their lack of statistical significance indicates that their linear contributions are weak within the studied range. In contrast, the quadratic terms A^2 , B^2 , and C^2 were highly significant ($p < 0.001$), indicating the presence of strong nonlinear effects. In particular, the quadratic term of time (B^2) exhibited a very high F-value (172.16), confirming a pronounced curvature effect of extraction time on antioxidant capacity. Although the regression coefficient of B^2 (-0.0149) appears relatively small, this does not contradict its statistical significance, as coefficient magnitude depends on variable scaling, while the F-value reflects the importance of the term in explaining response variability.

Table 3. Results of Antioxidant Capacity Using the RSM-CCD Method

Run	Temperature (°C)	Time (min)	Sample-to-solvent ratio (g/mL)	Antioxidant capacity (mg AAE/g FW)
1	80	33	1:25	0.8727
2	80	50	1:25	1.3205
3	80	50	1:33	1.0530
4	70	40	1:20	0.9327
5	97	50	1:25	1.2507
6	80	50	1:25	1.2967
7	90	40	1:30	0.9177
8	80	50	1:25	1.3282
9	70	40	1:30	0.9909
10	63	50	1:25	1.0963
11	70	60	1:20	0.9198
12	90	60	1:30	1.0934
13	90	40	1:20	1.0740
14	90	60	1:20	1.1054
15	80	50	1:25	1.3891
16	80	67	1:25	0.9572
17	80	50	1:25	1.3036
18	70	60	1:30	0.9550
19	80	50	1:25	1.3583
20	80	50	1:17	0.9122

The obtained antioxidant capacity ranged from 0.8727 to 1.3891 mg AAE/g FW. The highest value (1.3891 mg AAE/g FW) was obtained in run 15 at a temperature of 80 °C, an extraction time of 50 minutes, and a sample-to-solvent ratio of 1:25 (g/mL). In contrast, the lowest value (0.8727 mg AAE/g FW) was observed in run 1 at 80 °C for 33 minutes with the same sample-to-solvent ratio.

Table 4. ANOVA Results of the Quadratic Model for Extracting Antioxidant Capacity from White Turmeric Rhizomes

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	0.5714	9	0.0635	34.14	< 0.0001	Significant
A-Temperature	0.0311	1	0.0311	16.73	0.0022	
B-Time	0.0066	1	0.0066	3.55	0.0888	
C-Rasio	0.0019	1	0.0019	1.03	0.3336	
AB	0.0082	1	0.0082	4.40	0.0623	
AC	0.0086	1	0.0086	4.60	0.00575	
BC	0.0018	1	0.0018	0.9891	0.3434	
A ²	0.0479	1	0.0479	25.74	0.0005	
B ²	0.3201	1	0.3201	172.16	< 0.0001	
C ²	0.2256	1	0.2256	121.34	< 0.0001	
Residual	0.0186	10	0.0019			
Lack of Fit	0.0124	5	0.0025	2.02	0.2287	not significant
Pure Error	0.0061	5	0.0012			
Cor Total	0.5900	19				
R ² = 0.9685	Adjusted R ² = 0.9401				C.V. % = 3.90	

The interaction terms (AB, AC, and BC) were not statistically significant ($p > 0.05$), suggesting that the combined effects of the variables are less influential compared to their quadratic effects. Furthermore, the lack-of-fit test was not significant ($p = 0.2287$), indicating that the model fits the experimental data well and is suitable for predicting antioxidant capacity within the studied range^[20].

The interaction effects between the independent variables were evaluated using three-dimensional (3D) response surface plots, as shown in Figure 6. These plots provide a visual representation of the combined effects of variables on antioxidant capacity and help identify the optimum extraction conditions^[20]. The interaction effects between the independent variables were evaluated using three-dimensional (3D) response surface plots, as shown in Figure 6. These plots provide a visual

representation of the combined effects of variables on antioxidant capacity and help identify the optimum extraction conditions for antioxidant compounds^[29]. Figure 6(b) illustrates the interaction between temperature and sample-to-solvent ratio. The antioxidant capacity increased with both variables up to an optimum region around 85 °C and a ratio of approximately 1:25 g/mL. Further increases resulted in a decline in response, which may be attributed to compound degradation and dilution effects. Figure 6(c) presents the interaction between extraction time and sample-to-solvent ratio. The highest antioxidant capacity was observed at approximately 50 minutes and a ratio of 1:25 g/mL. Outside this optimum region, the response decreased, indicating that excessive extraction time and solvent volume reduce extraction efficiency.

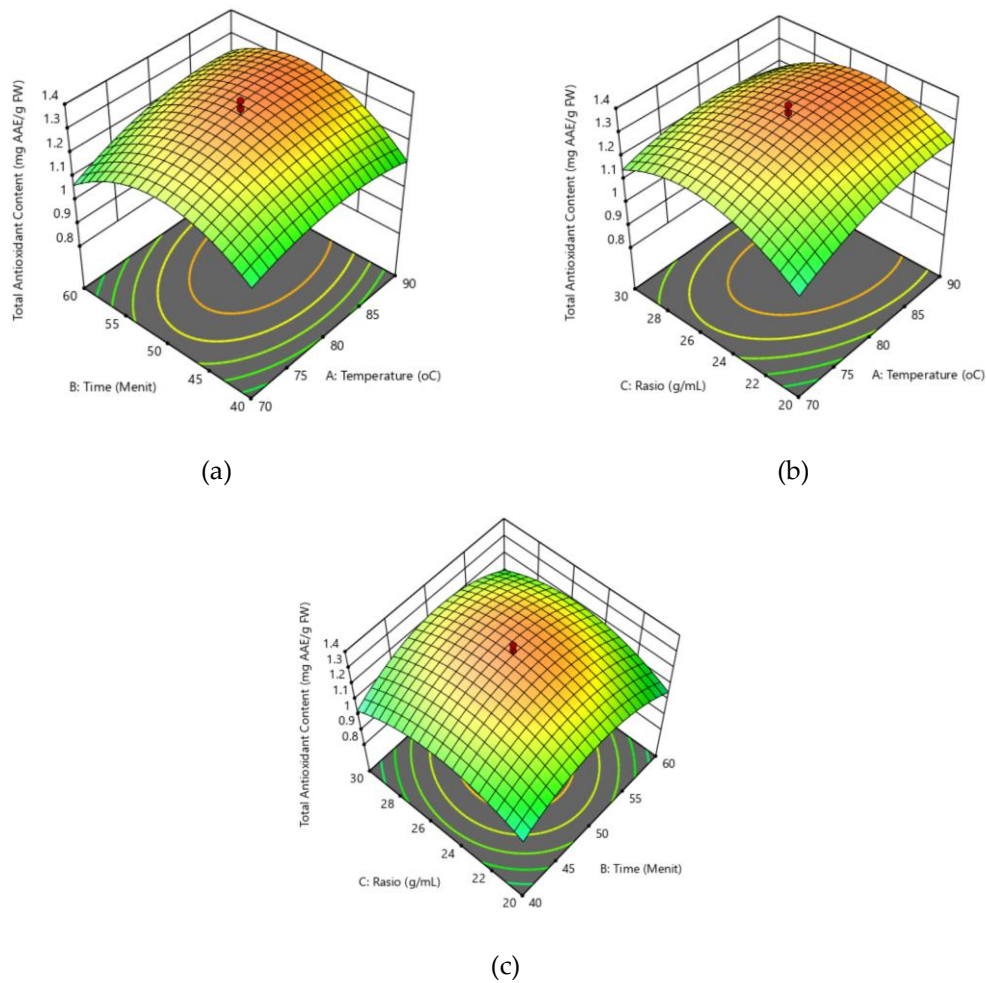


Figure 6. 3D surface graph of interaction between variables on antioxidant capacity: (a) effect of temperature and time, (b) effect of temperature and S/P ratio, (c) effect of time and S/P ratio.

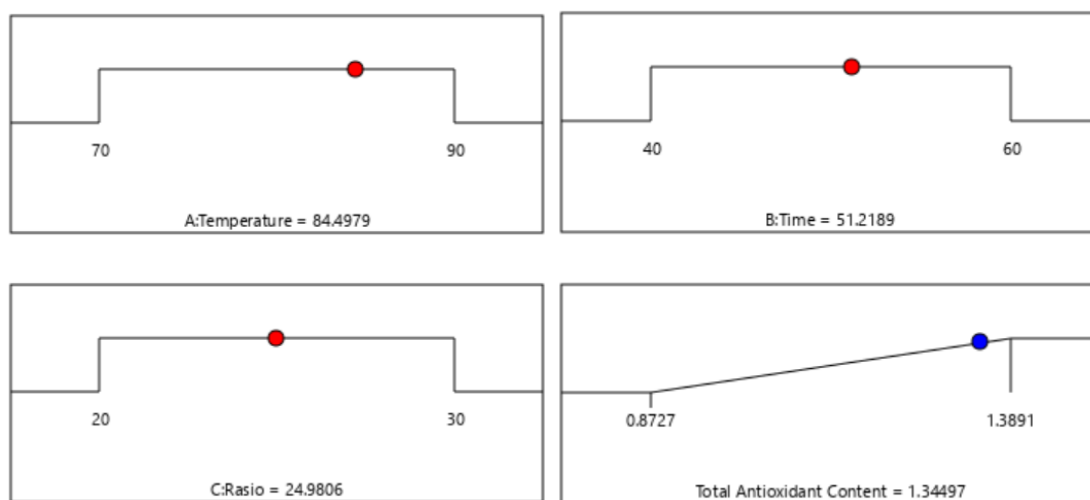


Figure 7. Optimum Conditions for Antioxidant Capacity

Optimization results using RSM indicated that the optimal extraction parameters were: 84 °C for temperature, 51 minutes for extraction time, and a sample-to-solvent ratio of 1:25 g/mL. Under these conditions, the model predicted an antioxidant capacity of 1.34497 mg AAE/g FW. The desirability value of 0.915 indicates that this parameter combination is close to the expected optimum condition, with a desirability value close to 1 indicating a very good model fit^[20].

The precision of the model was evaluated by conducting validation experiments under the optimum conditions. The experimental antioxidant capacity was 1.3963 mg AAE/g FW, while the predicted value obtained from the model was 1.34497 mg AAE/g FW. The deviation between the predicted and experimental values was calculated to be 3.82%, indicating good agreement between the model and experimental results. This small deviation confirms the reliability of the quadratic model in predicting antioxidant capacity^[29].

In earlier research, RSM was effectively applied to optimize TPC and DPPH antioxidant activity extraction from *Justicia gendarussa*, achieving a desirability score of 0.918. The method produced results where predicted values closely matched experimental verifications, reflected by low %RSE values of 7.082% for DPPH activity and 4.465% for TPC. This shows that RSM is very good at getting the most TPC and antioxidant yields from *Justicia gendarussa*^[20].

Conclusions

The extraction optimization of antioxidant compounds from white turmeric rhizomes was done using the OFAT method and RSM-CCD methods. From the OFAT results, the first optimum conditions were established at 80 °C, 50 minutes, and a sample-to-solvent ratio of 1:25 g/mL. These parameters were then used as the central point in the RSM design. According to the RSM analysis, the optimal extraction conditions changed to 84 °C, 51 minutes, and a sample-to-solvent ratio of 1:25 g/mL with an antioxidant capacity of 1.3963 mg AAE/g FW. This indicates the efficiency of RSM in extraction as compared to the OFAT method and indicates

that the developed model is reliable in predicting extraction results accurately.

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