New Insights of Response Surface Methodology Approach in Optimizing Total Phenolic Content of *Zanthoxylum acanthopodium* DC. Fruit Extracted Using Microwave-Assisted Extraction and the Impact to Antioxidant Activity

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Abstract: Zanthoxylum acanthopodium *DC., a unique spice plant from North Sumatra, is rich in beneficial secondary metabolites, particularly phenolic compounds. This study utilized the microwave-assisted extraction method to enhance the extraction of these bioactive compounds. The goal was to determine the optimal extraction conditions, including solvent concentration (X_1), microwave power (X_2), and extraction time (X_3) to maximize the total phenolic content (TPC) of* Z. acanthopodium *fruit. A Box-Behnken design, part of response surface methodology, was used with three factors at three levels: X_1 (50%, 75%, and 100% ethanol in water), X_2 (180, 300, and 450 W), and X_3 (3, 8.5, and 14 min). The phenolic compounds in the optimized extract were identified using LC-HRMS, and its antioxidant activity was measured using radical scavenging activity assays. The statistical analysis indicated a significant quadratic model (p-value < 0.05), with a high R^2 of 86.25%. Optimal conditions for maximum TPC (159.637 ± 5.72 mg GAE/g) were achieved with 50% ethanol, 450 W, and 8.5 min, outperforming conventional extraction methods. Compared to the non-optimized extract, the optimized extract also exhibited strong antioxidant activity, particularly in DPPH radical inhibition. This method successfully optimized TPC in* Z. acanthopodium *fruit, enhancing its antioxidant properties.*

Keywords: Zanthoxylum acanthopodium *DC.; phenol; response surface methodology; microwave-assisted extraction; antioxidant*

■ INTRODUCTION

Phenolics are an essential group of secondary metabolites and bioactive compounds in plants, a source of natural antioxidants that are often used in the herbal medicine industry [1]. Phenolic compounds have become the main focus in the search for natural raw materials on account of their pharmacological effects, such as antioxidant, antimicrobial, anticancer, anti-inflammatory, and chemoprotective potential [2]. Andaliman (*Zanthoxylum acanthopodium* DC.) is a

spice plant that is often used to produce herbal medicines. Known for its antioxidant effects, *Z. acanthopodium* is proven to contain lots of metabolite compounds, including phenols, alkaloids, glycosides, carbohydrates, tannins, flavonoids, steroids, oils, and fats [3].

Microwave-assisted extraction (MAE) is an extraction technique that exerts microwave radiation to heat solvents quickly and efficiently [4]. MAE is one of the new methods that has received a lot of attention recently due to its shorter irradiation time with higher quality results and reduced solvent consumption [5]. Several studies have reported the efficiency of MAE in extracting metabolite compounds. Wong et al. [6] carried out a comparison of secondary metabolites produced from extraction using conventional methods (maceration, reflux, and soxhlation) and non-conventional methods (MAE and Ultrasound-assisted extraction (UAE)). The results obtained in conventional extraction methods, soxhlation produced the most secondary metabolites, but the time required for extraction was longer. Meanwhile, in non-conventional extraction methods, the results show that MAE was the method with the highest results with the shortest extraction time. Alara et al. [7] conducted a comparison of the soxhlation and MAE methods. It was obtained that MAE produced more yield than the soxhlation method.

The efficiency of microwave-assisted extraction is influenced by factors such as microwave power, extraction time, temperature, solvent-to-sample ratio, and solvent concentration, and there are also interactions between these parameters [8]. To obtain *Z. acanthopodium* extract with high phenolic content, the parameters of the optimum extraction conditions need to be carefully investigated. This study aims to determine the extraction conditions, specifically solvent concentration (X_1), microwave power (X_2), and extraction time (X_3) which produce optimum total phenolic content (TPC). Many parameters need to be studied when producing the optimum extract, conventional experimental designs for optimizing all parameters are thus costly and time-consuming [9]. Alternatively, numerical methods such as response surface methodology (RSM) can minimize conventional experimentation difficulties [10]. Therefore, optimization was conducted using RSM. This method integrates mathematical and statistical techniques used to analyze problems regarding independent variables that influence the dependent variables or responses to optimize the response [11].

Presently, RSM has been widely applied for the industrial optimization and manufacturing of various functional products in the field of biotechnological and biochemical applications [12]. The application of RSM in the optimization of *Z. acanthopodium* fruit extraction conditions to obtain optimum TPC also has good prospects [13]. Optimum conditions are obtainable using complex experimental designs including three-level full factorial design, Central Composite designs (CCD), Box-Behnken design (BBD), or Doehlert matrix (DM) [14]. BBD is one of the multivariate optimization techniques based on three-level incomplete factorial designs which were utilized in the second-order response surface model [15]. Ferreira et al. [16] stated that BBD is preferable to other response surface designs for the quadratic model (e.g., three-level full factorial design, CCD, and DM) due to lower experimentation costs and efficient experiment design. Abd-El-Aziz et al. [17] optimized the phenolic content of *Leontodon hispidulus* extraction using high-performance liquid chromatography by performing RSM using BBD. This method could provide the optimum condition of ethanol to water ratio, extraction time, and the ratio of material to solvent to obtain the extract with optimal TPC in relation to its antioxidant, anti-inflammatory, and cytotoxic activities. Elgudayem et al. [18] found that RSM can explain the effect of extraction temperature, extraction time, and liquid-solid ratio on its phenolic content and DPPH scavenging activity.

Subsequently, a study by Zeković et al. [19] showed the difference in MAE conditions, including time extraction, microwave power, and solvent concentration, has a significant impact on the phenolic content of *Coriandrum sativum*. On the other hand, it is similar to the report by Woumbo et al. [20], who revealed these three factors in MAE significantly affect polyphenol extraction of *Glycine max*. Lastly, Filip et al. [21] successfully optimized the phenolic content of *Ocimum*

basilicum using RSM under MAE conditions, which include solvent concentration, microwave power, and extraction time. However, these previous studies exhibited that the MAE factors were crucial in phenolic extraction. Unfortunately, the optimization of the phenolic content of *Z. acanthopodium* has not been reported. Therefore, the main purpose of this study was to optimize the phenolic content of *Z. acanthopodium* fruit under MAE, and the optimization will be carried out using the BBD.

■ EXPERIMENTAL SECTION

Materials

The main materials in this experiment included *Zanthoxylum acanthopodium* DC fruit collected from the local village Ria-Ria, in Pollung district, North Sumatra province, Indonesia, that is fresh or ripe and green in color. At the same time, those that are too old or rotten are disposed of. The sample was identified as *Z. acanthopodium* fruit by Prof. Dr. Etti Sartina Siregar (Botanical expert) in Herbarium Medanese, Universitas Sumatera Utara (Voucher ID: 1470/MEDA/2023). The chemical reagents, including gallic acid, Folin-Ciocalteu, methanol, and ethanol (pro analysis), were purchased from Sigma Aldrich.

Instrumentation

The equipments used in this experiment are a Phillips grinder, a Samsung-ME731K microwave, Microlit micropipettes, a Shimadzu UV-1800 UV-vis spectrophotometer, and a Thermo Scientific liquid chromatography-high resonance mass spectroscopy (LC-HRMS) system.

Procedure

The preparation of Z. acanthopodium *fruit*

The *Z. acanthopodium* fruit was washed and dried at 45 °C until it reached a constant weight and then weighed. The dry sample was dry sorted, ground using a grinder, and stored at room temperature before being used [22].

The extraction of Z. acanthopodium *fruit using MAE*

The dried sample was extracted by microwave, following 17 differing conditions designed using Design Expert®13 software. RSM with BBD of three factors and their combination was used to determine the extraction conditions that provide the most optimal TPC. All independent variables, including X_1, X_2, and X_3, were created at three levels in BBD (see Table 1) for each code value (−1, 0, and 1). The extraction of *Z. acanthopodium* fruit was done by dissolving 30 g of the sample in 300 mL of solvent, with ethanol to water concentration according to Table 2, in a 500 mL flask and then placing it in the MAE device [23]. Extraction was conducted in 17 runs with conditions according to Table 2.

Total phenolic content determination of Z. acanthopodium *fruit extract*

All extracts' total TPC was determined colorimetrically using the Folin-Ciocalteu reagent and gallic acid as a standard. The sample solution was prepared at 200 μg/mL and dissolved in the appropriate

Table 1. Actual level of factors used in the Box-Behnken design

Factors	Symbol	Actual levels		
		−1	0	1
Solvent concentration (X_1, %)	X_1	50	75	100
Microwave power (X_2, W)	X_2	180	300	450
Extraction time (X_3, min)	X_3	3	8.5	14

Table 2. The design of extraction conditions after plotting the factors into software

Run	X_1 (%)	X_2 (W)	X_3 (min)
1	100	300	3
2	100	450	8.5
3	50	180	8.5
4	75	450	3
5	50	300	14
6	75	300	8.5
7	100	300	14
8	75	300	8.5
9	75	300	8.5
10	50	300	3
11	75	300	8.5
12	100	180	8.5
13	75	180	14
14	75	180	3
15	75	300	8.5
16	75	450	14
17	50	450	8.5

solvent, distilled water. The Folin-Ciocalteu reagent was diluted with distilled water with a 1:10 ratio before testing. The sample solution was pipetted and mixed with 0.5 mL of Folin-Ciocalteu reagent. Then, sodium carbonate 10% w/v (1 mL) was added. Afterward, the mixture was incubated for 35 min at room temperature. The absorbance was measured at wavelength 742 nm by a UV-vis spectrophotometer. TPC was calculated and reported in terms of gallic acid equivalents (mg GAE/g extract) [24].

Data analysis for optimization of total phenolic content by the application of RSM

Based on the responses obtained, a contour plot was created. Data from the analysis of TPC were tested for significance and model suitability using analysis of variance (ANOVA). The ANOVA model used can be selected according to what is suggested by the program, namely the model that has the highest level and produces a significant ANOVA value. The data obtained will go through three stages of model selection. The first is selection based on the sum of squares of the model sequence (sequential model sum of squares) with a probability value (p-value) ≤ the degree of significance (< 0.05). Second, model selection is based on testing to assess model suitability (lack of fit) if the p-value ≥ 0.05. Third, model selection is based on a summary of statistical models (summary of statistics) with an R^2 value close to 1.00 to get the optimum point. The model that provides significance in ANOVA and non-significance in lack of fit is chosen to analyze the variables. Apart from that, the Design Expert®13 program also provides a normal plot of the residual, indicating whether the residual (the difference between the actual response and the predicted response value) follows the normal line (straight line). Data points that are close to the normal line indicate data that is normally distributed, which means the actual results will be close to the normal line shows data that are normally distributed, which means the actual results will be close to the results predicted by the Design Expert®13 program [25-28].

The correlation between trial data and response is determined using the correlation value of determination (R^2), adequacy precision, and accuracy of the coefficient of determination (R^2 adjusted). Thus, the R^2 value must be comparable to the adjusted R^2 for a good statistical model. The R^2 value obtained is not much different from the adjusted R^2. Additionally, the significant adequacy of the model was verified at a probability level of 0.0001% with R^2, adjusted R^2, and predicted R^2 greater than 90% [29-31].

The analysis of phenolic compound using LC-HRMS

The examination of phenolic compounds from the optimized extract of *Z. acanthopodium* fruit was obtained using LC-HRMS. The analysis was conducted using TSQ Exactive (Thermo) (LSIH, Brawijaya University), and 0.1% formic acid in water was conducted as mobile phase A, whereas 0.1% formic acid in acetonitrile was used as mobile phase B. The gradient system was applied in this experiment. The Hypersil GOLD aQ column, measuring 50 × 1 mm × 1.9 μm, was subjected to a flow rate of 40 μL/min during the analysis, which lasted for 70 min. The outcomes were scrutinized through the utilization of Compound Discoverer software, employing mzCloud [32].

Conventional extraction of Z. acanthopodium *fruit*

The conventional extraction of *Z. acanthopodium* fruit was carried out using maceration. Briefly, 100 g of *Z. acanthopodium* fruit dry powder was soaked with 1 L of 96% ethanolic in a glass container for 3 × 24 h. The mixture was filtrated to gain the filtrate and evaporated using a rotary evaporator (PRIO, RE-2000-VN). The extract (non-optimized) was collected in a dry container and kept at 2–8 °C before being used in the experiment [33].

Antioxidant activity of the optimized extract

The antioxidant activity measurement from the optimized and non-optimized extracts was established by observing the effect of extracts on inhibiting the radical DPPH. Briefly, a 0.5 mL sample was introduced into a 3.5 mL solution of DPPH° (0.2 mM DPPH° solution diluted in ethanol pro analysis) and allowed to incubate at room temperature for 30 min. The absorbance values of the sample, ethanol pro analysis, and distilled water were determined at a wavelength of 517 nm. The sample absorbance (As), solvent absorbance (Ae), and blank absorbance (Aw) [34]. The

antioxidant activity was determined by employing Eq. (1);

$$\text{Scavenging capacity (\%)} = \left(\frac{As - Ae}{Aw}\right) \times 100\% \quad (1)$$

■ RESULTS AND DISCUSSION

Total Phenolic Content of *Z. acanthopodium* Fruit Extract

The previous studies by Sibero et al. [35] and Farida et al. [36] revealed the phenolic obtained in *Z. acanthopodium* fruit extract, although the value has not been reported. This study showed a range of TPC of *Z. acanthopodium* in Table 3. TPC is expressed in mg GAE/g units. The highest TPC obtained was 152.41 mg GAE/g, extracted with 50% X_1, 450 W X_2, and 8.5 min of X_3. Meanwhile, the lowest TPC was 43.36 mg GAE/g, extracted with 100% X_1, 300 W X_2, and 14 min of X_3. This condition described the extraction factors that have a crucial impact on a level of TPC. The TPC of *Z. acanthopodium* is highest if compared to other species of *Zanthoxylum,* which of *Z. armantum* fruit was 46.12 ± 0.40 mg GAE/g [37]. However, further research must be carried out to understand the factors influencing variations in phenol levels in *Z. acanthopodium* fruit. Previous studies have mentioned that the factors that influence the phenolic content are the extraction process, the type of *Z. acanthopodium* variety used, plant growth conditions, and the method of processing and storing the fruit after harvest [38]. In addition, environmental factors such as temperature, humidity, and light intensity can also influence phenol levels in *Z. acanthopodium* fruit [39].

RSM Application in Optimizing TPC of *Z. acanthopodium* Fruit Extract

Model selection

After analysis with Design Expert®13, conclusions were obtained for each fit summary response, shown in Table 4. The recommended model is the quadratic model, for this model produces a p-value < 0.05 and a high adjusted R^2 value of 0.6858. This model is also a highest-order polynomial with significant additional terms and is not aliased.

The quadratic model includes significant additional terms that contribute to its ability to adjust responses [40]. A high adjusted R^2 figure illustrates that the amount of variation in the data that can be expressed by the quadratic model is relatively high [41]. In the lack of fit results of the quadratic model, a significant p-value was obtained (< 0.05). This shows that the actual data received is not in line with the predicted results, and unknown

Table 3. Analysis results of TPC of *Z. acanthopodium* fruit extract

Run	Space type	X_1 Solvent concentration (%)	X_2 Microwave power (W)	X_3 Extraction time (min)	Total phenolic content (mg GAE/g)
1	IBFact	100	300	3	8.55
2	IBFact	100	450	8.5	120.72
3	IBFact	50	180	8.5	126.90
4	IBFact	75	450	3	105.75
5	IBFact	50	300	14	111.40
6	Center	75	300	8.5	113.59
7	IBFact	100	300	14	43.36
8	Center	75	300	8.5	114.36
9	Center	75	300	8.5	111.56
10	IBFact	50	300	3	109.77
11	Center	75	300	8.5	111.77
12	IBFact	100	180	8.5	132.30
13	IBFact	75	180	14	130.49
14	IBFact	75	180	3	137.42
15	Center	75	300	8.5	112.05
16	IBFact	75	450	14	135.88
17	IBFact	50	450	8.5	152.41

Table 4. Fit summary for optimization model of TPC

Source	Sequential p-value	Lack of fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.4081	< 0.0001	0.0072	−0.6842	
2FI	0.6056	< 0.0001	−0.0824	−2.4785	
Quadratic	0.0081	< 0.0001	0.6858	−1.1949	Suggested
Cubic	< 0.0001		0.9973		Aliased

factors greatly influence the experiment. Thus, a model reduction is reasonably necessary [42].

Analysis of variance

The relationship between each factor and the response value is shown in Table 5. The accuracy of the model was determined by ANOVA and correlation coefficient. Based on Table 5, the p-value of the model is 0.0242, indicating that this method is accurate and reliable. The p-value for lack of fit was 0.6105 of $p > 0.05$, which means that the actual data obtained was in line with the predicted results, and unknown factors had a minor influence on the experiment [43]. A non-significant lack of fit value is a requirement for a good model because it shows the suitability of the response data to the model. This indicates that the model used in this study is highly accurate in explaining variations in the response variable [44]. A lack of significant fit value suggests that the model fits the data quite well, thus increasing confidence

Table 5. ANOVA results with various treatments (X_1, X_2, X_3) on the response of TPC

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Model	7894.40	9	877.16	4.88	0.0242	significant
Main effects						
X_1	1826.41	1	1826.41	10.16	0.0153	
X_2	19.07	1	19.07	0.11	0.7542	
X_3	23.90	1	23.90	0.13	0.7261	
Interaction effects						
X_1X_2	278.52	1	278.52	1.55	0.2532	
X_1X_3	502.21	1	502.21	2.79	0.1385	
X_2X_3	410.73	1	410.73	2.29	0.1744	
Quadratic effects						
X_1^2	387.98	1	387.98	2.16	0.1852	
X_2^2	3812.16	1	3812.16	21.21	0.0025	
X_3^2	985.22	1	985.22	5.48	0.0517	
Residual	1258.14	7	179.73			
Lack of Fit	1252.01	3	417.34	272.37	0.6105	not significant
Pure Error	6.13	4	1.53			
Cor Total	9152.54	16				
Regression analysis and response equation						
Std. Dev.	13.41					
Mean	115.08					
C.V. %	11.65					
R^2	0.8625					
Adjusted R^2	0.6858					
Predicted R^2	−1.1949					
Adequate precision	9.2913					

in its validity. However, further analysis is needed to fully understand the relationship between the independent and dependent variables [45].

The R^2 in multiple correlation analysis measures the extent to which the predicted value of a quadratic equation is related to its actual value [46]. Table 5 shows the value of $R^2 = 0.8625$, which indicates that 86.25% of the sample variables in the total phenol response are influenced by independent variables (factors X_1, X_2, and X_3), and only 13.75% of all variables cannot be explained by the model. This shows that there is a strong relationship between the factors and the response. Mean is the average percentage of the total phenolic content of *Z. acanthopodium* extract. Coefficient of variations (CV) describes the standard deviation percentage of the average result data [47]. In the Table 5, it can be seen that the standard deviation is 11.65% of the average results.

A high R^2 value indicates that the model can explain most of the variability in the data [48]. The adjusted R^2 value shows the amount of variation in the data that can be explained by the model. In contrast, the predicted R^2 shows the amount of variation in further predictions that can be explained by the model [49]. The adjusted R^2 value = 68.58 shows that 68.58% of the sample variables on the total phenol response can be predicted, and only 31.42% of all variables cannot be explained by the model. Predicted R^2, which is negative (–), indicates that the average (mean) of the data has predictions that are almost as accurate as the model. When the dataset is too small, the model may not have enough information to learn the underlying patterns, leading to negative predicted R^2 [50]. However, the adjusted R^2 and predicted R^2 values are expected to have a difference of < 0.2, wherein the results obtained the difference between the two is 1.8807. This indicates that there is a possibility of imperfection in the model or data results obtained [51]. Adequate precision measures the signal-to-noise ratio. The expected ratio is > 4. The ratio obtained in the results of 9.291 indicates an adequate signal, and the regression stated by the model is sufficient, and this model can be used to navigate optimization designs [52].

From Table 5, we get a relatively high deviation value of 13.41. A high deviation value indicates that the model is inaccurate, namely the combination of X_1, X_2, and X_3. This shows that the combination of X_1, X_2, and X_3 has a non-linear relationship to the response. Therefore, further investigation and refinement of the model may be necessary to improve its accuracy [53]. The mathematical model equation with coded variables suggested by the Design Expert®13 software is as in Eq. (2);

$$\begin{aligned} TPC = {} & 112.117 - 15.1559X_1 - 1.54375X_2 - 1.73377X_3 \\ & - 8.31879X_1X_2 - 11.205X_1X_3 + 10.1021X_2X_3 \\ & - 9.59925X_{12} + 30.5646X_{22} - 15.2967X_{32} \end{aligned} \quad (2)$$

Table 5 also describes factor X_1, factor X_2, and factor X_3 in ANOVA analysis. A large F-value and a small p-value indicate a significant influence of each factor. Based on the results obtained, the variables that had the largest to smallest influence on TPC were the quadratic influence of microwave power and the linear influence of solvent concentration with a p-value > 0.05. This indicates that X_2 plays an important role in determining the response. Further research is needed to understand the specific relationship between X_2 and response. Apart from that, the interaction between the factors did not have a significant influence with a p-value above 0.05.

Eq. (1) shows that the variables X_2X_3 and X_3^2 significantly influence increasing phenol levels in the sample. However, it is essential to note that X_2 has no direct effect on phenol content. The response surface curve is a three-dimensional spatial surface map consisting of response values in relation to each factor X_1, X_2, and X_3. The optimal parameters and interactions between parameters on the response surface curve can be seen more intuitively and visually [54].

Based on Eq. (1), the X_2 was proven to be highly influential on *Z. acanthopodium* phenolic content. This is following research conducted by Lovrić et al. [55], which states that higher microwave power and temperature of extraction improved the value of phenolic compounds. However, Alara et al. [7] stated that TPC yields declined with increasing irradiation time and microwave power. This could be associated with the influence of microwave power in degrading phenolic compounds at prolonged irradiation time. Le et al. [56]

showed increasing phenolic levels at 80–400 W, and the rise continued up to 500 W of X_2. As the X_2 reached 720 W, the TPC obtained reduced. The X_2 is directly related to the thermal effect of the extraction process; high microwave power results in high temperature, which decomposes the phenolic compounds. Thus, microwave power of 300–500 W is suggested for higher TPC results [7].

Fig. 1(a) visually represents the relationship between factors and TPC in the response surface curve. The curve in Fig. 1(a) shows that increasing or decreasing microwave power can provide a higher total phenol response, and the response shows lower results at microwave power of 300 ± 30 W. The curve also shows that decreasing the X_1 can increase the TPC obtained. These results contradict the research by Mikucka et al. [57], which explains that as the X_1 increases, a higher TPC is obtained. However, this has an optimum point. If left unchecked, the phenolic levels will decrease. These results also contradict research conducted by Liyana-Pathirana and Shahidi [58], which stated that the TPC decreased with increasing temperature. Microwaves work by emitting microwave radiation to the molecules contained in the material so that these molecules will absorb electromagnetic energy [59]. These conditions cause the TPC to increase. However, phenol is very sensitive to heat. If the temperature continues to increase, the TPC produced will decrease, and if it is shown at a power of 325–400 W, the total phenol decreases [60].

The curve in Fig. 1(b) shows that increasing or decreasing X_2 can provide a higher TPC, and the response shows lower results at X_2 of 300 ± 30 W. This curve also shows that increasing X_3 can increase the TPC obtained. These results are those reported by Liyana-Pathirana and Shahidi [58] that the longer the X_3, the

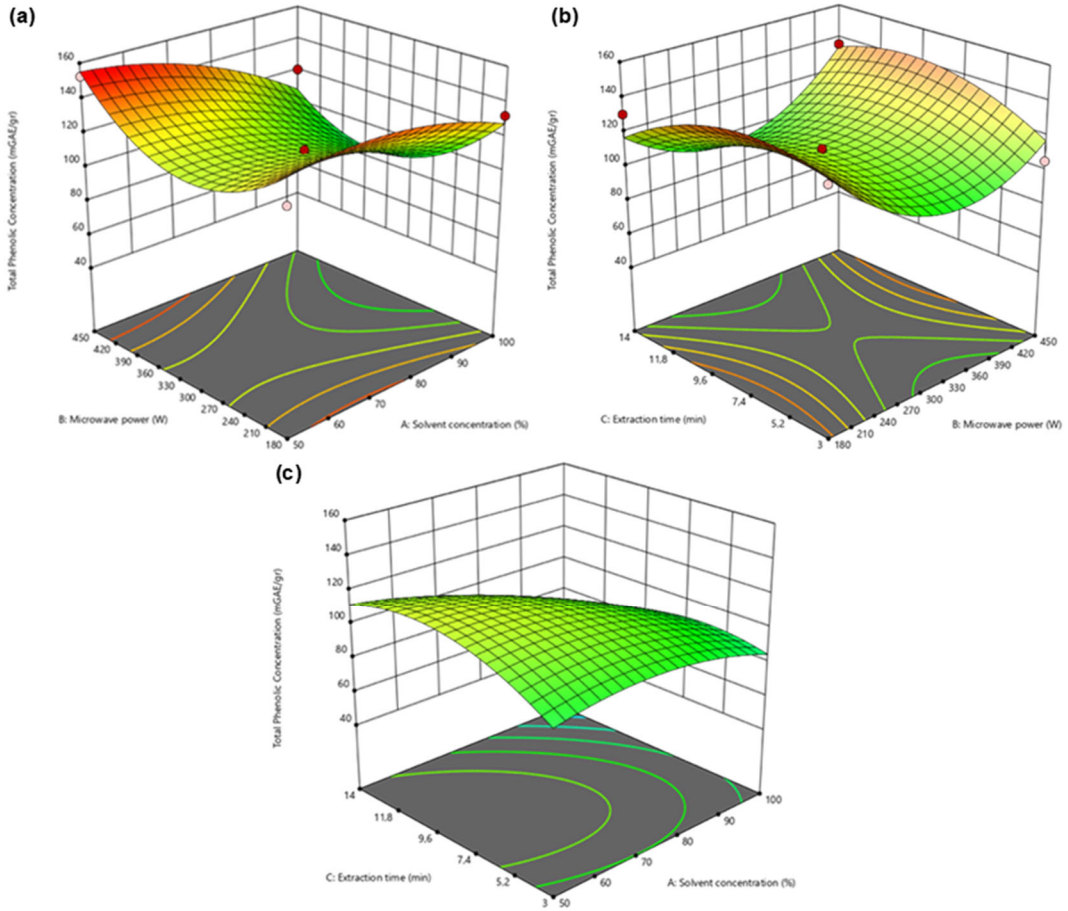

Fig 1. Representation of the relationship between entire variables and TPC in response surface curve. (a) X_1X_2, (b) X_2X_3, and (c) X_1X_3

higher the temperature, and the greater the solubility of phenolic compounds in the solvent. Meanwhile, the curve in Fig. 1(c) shows that increasing the X_1 and X_3 can cause a higher TPC. However, it should be noted that using an X_1 of 100% (absolute) and an X_3 of 14 min can produce a lower TPC than using an X_1 of 50% and an X_3 of 8.5 min.

From the three graphs above, the optimum point for each independent variable is 155.013%. The optimum point is the combination of X_1 and X_3 that produces the highest TPC in the *Z. acanthopodium*. This information is critical for determining the most effective conditions for extracting phenol from *Z. acanthopodium* fruit in subsequent experiments.

Model validation

Model validation is carried out to determine the model's validity and adequacy to predict data. The model is validated by analyzing residuals, the results of subtracting predicted data from actual data. Residuals can also be called noise contained in the data. Residuals are examined by looking at several graphic plots [48-51]. Fig. 2(a) shows the normal distribution and linear relationship between the expected response and actual values for each process. The proximity of the residual plot to a straight line and the absence of variance deviation in Fig. 2(b) indicate a normal distribution of the response variable. The current model significantly improves the relationship between process and outcome variables. Fig. 2(c) shows an excellent agreement between the calculated and measured values. Nearly all data points were found to be within the permissible range

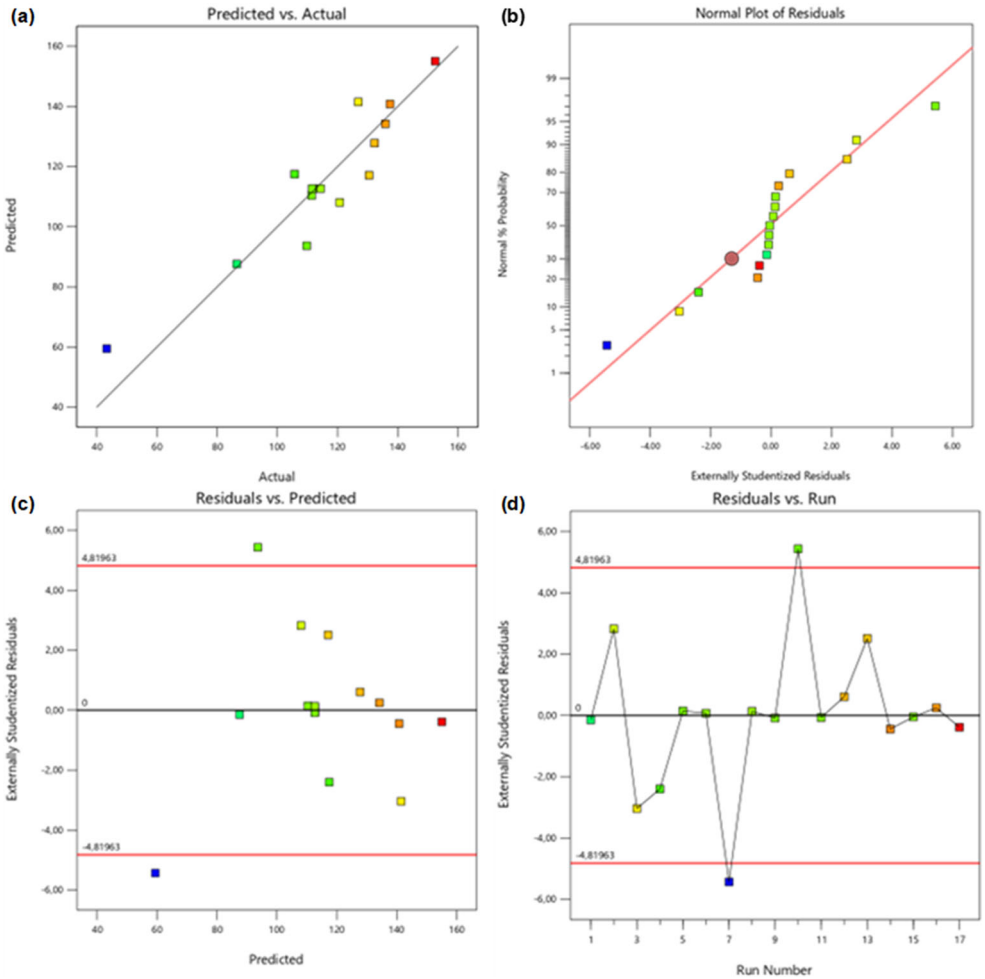

Fig 2. Plot of the residual graph, namely (a) Predicted vs. actual plot graph, (b) Normal plot of residuals, (c) Residual vs. predicted graph, (d) Residuals vs. run graph

after examining internally studentized vs. internally studentized residuals run experiments (Fig. 2(d)). However, two points are outside the permitted range. This could happen because of the significant difference between the predicted data and the actual results obtained during the experiment [43,46].

Total phenolic content optimization solutions

The optimum point was obtained based on the results, as presented in Table 6. The optimum point is the combination of X_1 and X_3 that produces the highest TPC. This information is critical for determining the most effective conditions for extracting phenol from samples in subsequent experiments. The ideal process solution was obtained based on optimization data processing using RSM with a BBD model, especially at an X_1 of 50%, X_2 of 450 W, and X_3 of 8.5 min and the value obtained for the predicted phenol content was 155.013 mg GAE/g. The best formula is selected based on the maximum desirability value (value range of 0 to 1). This number is used to assess the accuracy of the ideal solution. The desirability value of an ideal solution indicates how close the solution is to the desired outcome. A higher desirability value indicates a more accurate and optimal solution, while a lower value may indicate room for improvement in the process [61].

The solution conditions in MAE to optimize the TPC of *Z. acanthopodium* fruit extract were determined in real experiments. The results in Table 6 show TPC in real experiments highest compared to the conventional extraction of 159.637 ± 5.72 and 98.196 ± 3.84 mg GAE/g, respectively. The distinction of TPC between optimized extraction and conventional extraction in this experiment is significantly different. This experiment described the optimized conditions of TPC using MAE to gain better TPC than the conventional extraction. The previous study by Ahmad et al. [62] showed that MAE gave the best process for the TPC of *Eleutherine bulbosa* compared to conventional extraction. On the other hand, Saifullah et al. [63] revealed similar results, namely that the obtained TPC of lemon myrtle using MAE is higher than that obtained by conventional extraction. Moreover, the MAE was applied in the TPC optimization of various plants and successfully increased the TPC [64-67].

Antioxidant Activity of Optimized and Non-optimized Extract

The antioxidant activity of optimized and non-optimized extracts was revealed by inhibiting the radical DPPH. The optimized extract was prepared under optimum conditions using MAE. MAE is reported as a promising alternative for polyphenol compound extraction. Thus, MAE was compared to the traditional methodology of maceration. The inhibition effect of each extract against DPPH radical is summarized in Fig. 3.

The optimized extract and non-optimized extract revealed antioxidant activity through DPPH radical inhibition. This study performed several concentrations, beginning at 31.25 to 500.00 µg/mL. These results described the antioxidant activity of the optimized and non-optimized extract in a concentration-dependent manner. The increasing concentrations affected DPPH radical reduction. Remarkably, the antioxidant activity of the optimized extract was more effective than the non-optimized extract in the entire concentration test. At the lowest concentration, the optimized extract reduced the DPPH radical to 38.643 ± 0.787%. In contrast, the reduction of DPPH radical by non-optimized extract in a similar concentration was only 26.127 ± 1.528%, and this result showed a significant difference with $p < 0.0001$. The DPPH radical led to a decrease after the concentration test reached 500.00 µg/mL. Fig. 3 revealed the highest reduction of radical DPPH occurred at 500.00 µg/mL of optimized and non-optimized extracts of 82.457 ± 1.852% and 73.050 ± 1.911%, respectively. Although the concentrations of the optimized and non-optimized

Table 6. Optimization results for phenolic levels in samples with 3 independent variables, including solvent concentration (X_1), microwave power (X_2), and extraction time (X_3) on the response of TPC

X_1 (%)	X_2 (W)	X_3 (min)	TPC (mg GAE/g)			Desirability	
			Predicted value	Experimental value	Conventional extraction		
50	450	8.5	155.013	159.637 ± 5.72	98.196 ± 3.84	1.000	Selected

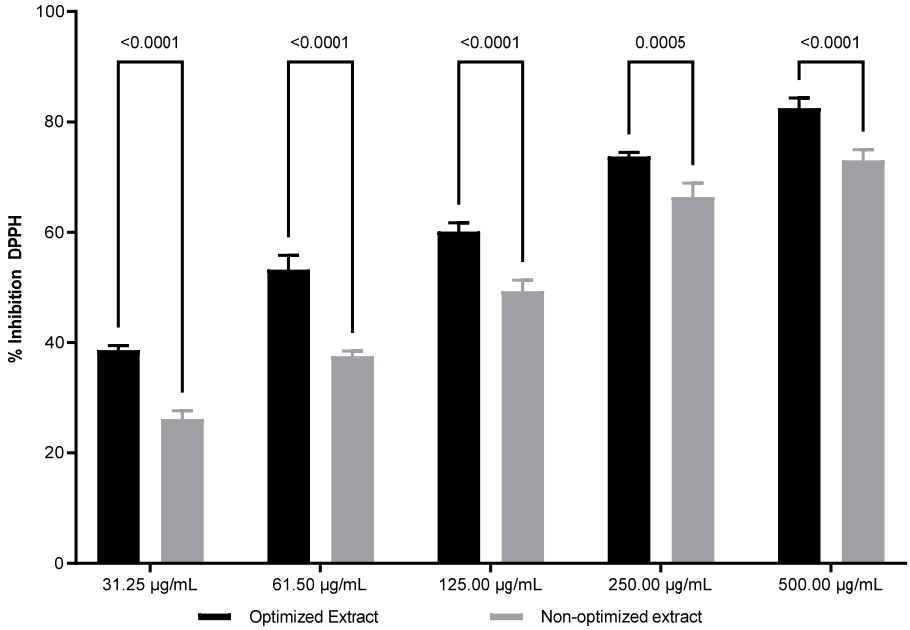

Fig 3. The inhibition DPPH effect of optimized and non-optimized extracts at a range concentration of 31.25–500.00 µg/mL

Table 7. The phenolic compounds of *Z. acanthopodium* extract

RT (min)	Compound	Molecular formula	MW (g/mol)	Compound nature
4.49	7-Hydroxycoumarine	$C_9H_6O_3$	163.03	Phenolic
5.73	Quercetin-3-galactoside	$C_{12}H_{20}O_{12}$	465.10	Phenolic
3.56	(1r,3*R*,4s,5*S*)-4-{[(2*E*)-3-(3,4-dihydroxyphenyl)prop-2-enoyl]oxy}-1,3,5-trihydroxycyclohexane-1-carboxylic acid	$C_{16}H_{18}O_9$	355.10	Phenolic
6.33	Kaempferol-3-*O*-galactoside	$C_{21}H_{20}O_{11}$	449.10	Phenolic
6.33	Quercetin	$C_{15}H_{10}O_7$	303.04	Phenolic
2.03	Procatechuic acid	$C_7H_6O_4$	155.03	Phenolic
0.82	2-Methoxy-phloroglucinol	$C_7H_8O_4$	139.03	Phenolic
6.87	Kaempferol	$C_{15}H_{10}O_6$	287.05	Phenolic
9.19	5-Pentylresorcinol	$C_{11}H_{16}O_2$	181.12	Phenolic

extract are similar, the reduction effect of the DPPH radical is significantly different with $p < 0.0001$.

This study revealed the effect of extract preparation on antioxidant activity. The optimized extract prepared using MAE showed the highest antioxidant activity compared to maceration. The MAE revealed the effective process of gaining polyphenol compounds and their antioxidant activity. The microwave irradiation caused damage to the structure of the cell plant and improved polyphenol extraction. The polyphenol compounds of the optimized extract were determined using LC-HRMS and summarized in Table 7.

Several phenolic compounds were determined in the optimized extract of *Z. acanthopodium* fruit in Table 7. This result is similar to the previous study by Dalimunthe et al. [67], which successfully identified quercetin and kaempferol. Quercetin is a phenolic group with the strongest antioxidant activity via donating electrons to unstable free radicals and stabilizing the radical [68]. Jeszka-Skowron et al. [69] revealed the antioxidant activity of quercetin-rich tea infusion is increasing compared to normal tea infusion. Meanwhile,

Zhu et al. [70] described the increase in quercetin concentration in a *Dendrobium officinale* extract causes an increase in the inhibition of DPPH. Besides quercetin, kaempferol is a phenolic group identified in many plants [71]. The mechanism of kaempferol as an antioxidant was revealed in many studies. Kaempferol was reported to have activities as an antioxidant and prevents DNA damage via scavenging reactive oxygen species, strengthening DNA, and reducing chemical damage [72]. As Altemimi et al. [73] reported, the kaempferol extract of *Ocimum basilicum* has the most potent activity compared to the conventional extract. Furthermore, this study revealed that the optimized extract of *Z. acanthopodium* fruit has the highest TPC compared to the conventional extract, which affects its antioxidant activity.

■ CONCLUSION

By applying the BBD, the extraction conditions for the TPC of *Z. acanthopodium* fruit were successfully optimized. The optimum extraction conditions involved X_1 of 50%, X_2 of 450 W, and X_3 of 8.5 min. The optimal TPC was obtained of 159.637 ± 5.72 mg GAE/g sample. Analysis of factors interactions impacts on phenolic levels shows that X_2 has the major influence on TPC in *Z. acanthopodium* extract. X_2 plays an important role in determining the TPC, and further research is needed to understand the specific relationship between X_2 and TPC. The interaction of X_1 and X_3 also significantly influences phenolic levels, which is critical for determining the most effective conditions for extracting phenol from samples in subsequent experiments. Finally, the optimized extract of *Z. acanthopodium* fruit has the strongest antioxidant activity compared to the non-optimized extract.

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■ CONFLICT OF INTEREST

We declare there are no conflicts of interest regarding the publication of this manuscript.

■ AUTHOR CONTRIBUTIONS

Conceptualization and methodology: Sumaiyah and Muhammad Fauzan Lubis; Analysis: Didi Nurhadi Illian, Muhammad Fauzan Lubis, and Keshia Tampubolon; Investigation and supervision: Sumaiyah and Retno Murwanti; Writing draft preparation: Didi Nurhadi Illian, Muhammad Fauzan Lubis, Keshia Tampubolon; Review and editing: Sumaiyah and Retno Murwanti. All authors have approved this article for publication.

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