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RESEARCH ARTICLE

Green microwave extraction of antioxidants and phenolic compounds from *Moringa oleifera* leaf: An optimization study using response surface methodology

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ABSTRACT - *Moringa oleifera* is a good source of nutrition and very beneficial for health improvement. This study aimed to optimize the process parameters for the extraction of bioactive substances from *M. oleifera* leaf using microwave-assisted extraction (MAE). The response surface methodology (RSM) based on Box-Behnken designs (BBD) was used to optimize the process parameters including solid-to-solvent ratio (1 to 3 g/100 mL), microwave power (100 to 200 W), and extraction time (15 to 25 min) on the total phenol content (TPC) and antioxidant activity (AA). The optimum conditions were 2.01 g/100 mL solid-to-solvent ratio, 152.7 W microwave power, and 20.54 min extraction time to obtained to obtain the optimum TPC and AA. The optimum TPC and DPPH were 529.801 mg GAE/g dry sample and 87.67%, respectively. The results were successfully fitted into the second-order polynomial model with R^2 values of 0.9958 and 0.9865, respectively. The predicted TPC and AA deviate from the experimental data by 1.40% and 2.69%, respectively. Thus, the model accuracy is proved for the modeling of *M. oleifera* leaves extraction by MAE

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1.0 INTRODUCTION

Medicinal plants have provided a wide range of bioactive substances that contain therapeutic properties. These substances have been widely used in their natural state or after being further processed to treat and heal various diseases. Nearly 60% of the world's population utilize medicinal plants as their primary resource for healthcare treatment, and there is a rising demand for it in some of the developing nations [1]. In addition, the food and pharmaceutical sectors are exploring the possible usage of natural sources of antioxidants, especially from plants, to avoid the adverse effects of commercial synthetic antioxidants, such as their cytotoxicity effect on the lungs and liver. *M. oleifera*, a member of the Moringaceae family, is becoming more famous as a "superfood" because of its abundant nutrients and polysaccharides content. The plant is also described as a multi-purpose herbal plant because of its medical benefits, including strong anti-inflammatory, antioxidant, and tissue-protective characteristics [2]. The leaves are widely used as a dietary ingredient and as a global alternative therapy. For example, *M. oleifera* has been added in as Orthoherb and Septilin formulations in India for the remedy of ailments [3]. The World Health Organization (WHO) has also recommended *M. oleifera* as one of the treatment alternatives for people who are suffering from malnutrition [4].

Extraction is a prevalent separation method to isolate bioactive substances such as phenolic compounds and antioxidants from other plants using a suitable solvent. Several studies have reported the utilization of various extraction techniques, either conventional or non-conventional, to meet the growing needs of bioactive substances from plants like *M. oleifera*. The conventional techniques of extracting bioactive substances from plants, such as Soxhlet and immersion, are no longer preferred. The extraction techniques of *M. oleifera* leaves still have room for improvement, particularly in optimizing the process parameters [5]. Microwave-assisted extraction (MAE), which uses microwaves to improve solvent extraction operations, has attracted significant attention compared to conventional extraction techniques. This technique allows rapid and simultaneous heat transfer to the solvent and plant material during extraction via electromagnetic radiation [6]. The electromagnetic radiation may cause dipolar rotation and ionic conduction in the solution, and this action further generates heat through friction, weakening and rupturing the sample cell wall [7]. The benefits of MAE are time saving, less solvent usage, faster heating, shorter extraction time and higher yield of extracts [8] [9-11].

MAE process has been studied for the extraction of bioactive substances such as antioxidants and phenolic compounds from plant foods and herbal materials such as passion fruit peel [7], *Careya sphaerica Roxb.* flowers [12], shiitake mushrooms [10], pomegranate [13], red sorghum grain [14], *Phaleria macrcocarpa* [8] and *Gentiana asclepiadea* L. [15]. The MAE process parameters investigated include the choice of solvent, the amount of sample to solvent volume, microwave power, sample size, time, and temperature. Researchers commonly conduct experimental works using the one-factor-at-a-time (OFAT) approach, where one parameter is changed at a time while the remaining parameters are

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constant. These limitations can be resolved using the response surface methodology (RSM). RSM is a powerful mathematical tool that enables users to design experimental works and examine multiple factors or process parameters simultaneously. In addition, it will provide a comprehensive assessment of their interactions. RSM can also facilitate the development of a mathematical model that can be used to predict the process dependent variables or responses with the change in process independent parameters [16].

The optimization studied of MAE parameters for *M. oleifera* also has been reported by several researchers. For example, Makkiyah et al. [17] optimized MAE of the *M. oleifera* MAE with ethanol as a solvent (40 to 80%) at 135 W using the RSM on the phenolic content and antioxidant activity. The other parameters investigated include solid-solvent ratio (5 to 15 mL/g) and time (1 to 3 min). Other work by Gunalan et al. [18] also optimized the *M. oleifera* using MAE parameters namely microwave power (500 to 700 W), temperature (30 to 50℃), and time (20 to 40 min) with ethanol as a solvent using the Central Composite Design method under RSM. Chen et al. [4] also optimized MAE for *M. oleifera* Lam. leaves in 90wt% of ethanol concentration on the polysaccharide yield. The time, microwave power, temperature and liquid to solid ratio were 60 to 80 min, 500 to 700 W, 60 to 80 and 25 to 35 mL/g, respectively.

Therefore, this study focused on optimizing MAE with water as a green solvent for extracting bioactive substances from *M. oleifera* leaf using RSM. The Box-Behnken designs (BBD) were applied to design the experimental works and maximize the total phenol content (TPC) and antioxidant activity (AA) in the extract. The developed design also evaluates the interaction between the MAE process parameters (independent variables) and process responses (dependent variables). The MAE process parameters investigated include solid-to-solvent ratio (1 to 3 g/100 mL), microwave power (100 to 200 W), and extraction time (15 to 25 min).

2.0 METHODS AND MATERIAL

2.1 Materials and Chemicals

M. oleifera leaf was obtained from Ethno Herbs Resources, Selangor, Malaysia. The leaves were dried in an oven and ground into powder using a Panasonic MX-8005 dry blender. The moisture content of the dried leaves was 0.8065%± 0.0384. The powder was sieved manually using a Prada Test Siever tray. Powder with a size between 125 and 520 µm was collected and kept in a zip-locked plastic container until used for the MAE process. The analytical grades chemicals and reagents required for TPC and AA analysis, namely ascorbic acid, gallic acid, Folin-Ciocaltaeu, sodium carbonate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), and methanol were obtained from Merck Sdn. Bhd., Malaysia. All the chemicals and reagents were used as received, but some were diluted. The water used in this work was prepared in-house using a distilled water system and used as the extracting solvent.

2.2 Optimization Analysis of MAE Parameters

Table 1 lists the MAE process parameters (independent variables) investigated in this study. The range was established based on the findings from the screening work performed using the OFAT method reported by Musa [19]. Table 2 tabulates the experimental design, which consists of 17 runs with three center points was developed using response surface methodology - Box-Behnken Design (RSM-BBD) (Design Expert software, Version 6.0.8 developed by Stat-EaseInc., Minneapolis, USA). The TPC expressed in mg GAE/ g dry sample and AA in % of the targeted bioactive substances were selected as the response variables. The results were statistically evaluated using analysis of variance (ANOVA). The p-value of ≤ 0.05 indicates the significance of the model. The model's suitability was also examined by assessing the Fischer test value (F value), model p-value, non-significant lack-of-fit ($p \geq 0.05$), coefficient of determination (R^2) and coefficient of variation (CV).

Table 1. Independent process parameters and their levels used for Box-Behnken designs (BBD)

Independent parameters	Levels		
	-1	0	+1
Solid-to-solvent ratio, x_1 (g/mL)	1	2	3
Microwave power, x_2 (W)	100	150	200
Extraction time, x_3 (min)	15	20	25

2.3 Microwave-Assisted Extraction (MAE) Process

A microwave-assisted extraction unit laboratory system (Milestone ATC-FO 300, North America) was used for the *M. oleifera* leaf powder extraction process. About 2 g of dried *M. oleifera* leaf powder for the first experimental run was weighed and placed in a beaker containing 100 mL of water. Then, the mixture was transferred into a closed vessel, gently whisked for one minute, and placed in the microwave extraction unit. Batch extraction was performed for 15 min, as stated in Table 2. After that, the residual solid samples in the extract solution were removed using 0.45 µm Whatman filter paper. The extract was further concentrated using a rotary evaporator (Buchi Rotavapor R-100, Switzerland). The same procedures were repeated with experimental run two until 17. The extraction temperature was fixed at 25 ℃ for all the experimental runs. The extracts were analyzed to determine the TPC and AA.

Table 2. Box-Behnken design (BBD) matrix for MAE with actual values of independent variables as well as experimentally and predicted TPC and AA as responses

Run	Solid-To-Solvent Ratio, x_1 (g/100 mL)	Microwave Power, x_2 (W)	Extraction Time, x_3 (min)	Actual TPC, (mg GAE/g dry sample)	Predicted TPC (mg GAE/g dry sample)	Actual Antioxidant Activity (%)	Predicted Antioxidant Activity (%)
1	2.00	200.00	15.00	312.477	328.11	72.8618	75.55
2	3.00	200.00	20.00	138.949	128.51	59.1860	57.56
3	3.00	100.00	20.00	118.679	115.45	52.7617	53.19
4	2.00	150.00	20.00	541.757	528.24	88.6379	87.63
5	2.00	200.00	25.00	377.117	368.69	76.9069	76.26
6	3.00	150.00	15.00	162.673	157.47	60.4245	59.36
7	2.00	150.00	20.00	532.523	528.24	86.5307	87.63
8	3.00	150.00	25.00	179.489	198.35	60.5356	62.80
9	2.00	150.00	20.00	520.811	528.24	86.6100	87.63
10	1.00	100.00	20.00	94.7748	105.21	52.5590	54.18
11	1.00	150.00	15.00	169.550	150.69	63.9939	61.73
12	2.00	100.00	25.00	368.108	352.47	75.9388	73.25
13	1.00	200.00	20.00	122.703	125.93	55.9036	55.48
14	2.00	150.00	20.00	519.234	528.24	89.1719	87.63
15	2.00	100.00	15.00	302.117	310.54	72.2488	72.89
16	2.00	150.00	20.00	526.892	528.24	87.1900	87.63
17	1.00	150.00	25.00	187.117	192.32	58.2812	59.35

2.4 Analysis Method

2.4.1 Total Phenolic Content (TPC)

The TPC was measured as described by Krim et al. [20] with minor modifications. Briefly, 1125 µL of extracted sample was added into the cuvette and mixed with 375 µL of Folin-Ciocalteu reagent. The solution was incubated at ambient temperature for 15 min to allow reaction. Then, 500 µL of sodium carbonate solution (Na_2CO_3) was added to the cuvette and incubated in the dark at room temperature for 2 hours. The sodium carbonate solution was prepared by dissolving 3.75 g of sodium carbonate in 50 mL of distilled water. The Folin-Ciocalteu reagent must be added before the alkaline solution to prevent phenol oxidation [21]. The absorbance of the solution was determined at a wavelength of 765 nm using a UV-vis spectrophotometer (U-1800, Japan). The results were quantified as milligrams of gallic acid equivalents per gram of dried weight sample (mg GAE/g d.w.). A gallic acid standard calibration curve was generated using concentrations ranging from 0.5 to 5.0 mg/mL (R^2=0.99). The analyses were conducted three times.

2.4.2 Antioxidant Activity (AA)

The AA of the extract samples was determined using the methodology described by Rodríguez-Pérez et al. [9], with minor adjustments. About 3.95 mg of DPPH powder was mixed with 50 mL of methanol solution with a concentration of 90 wt% to prepare the DPPH solution. Then, 10 mL of the extracted sample was mixed thoroughly with 10 mL of 90 wt% of methanol solution until homogenous. After that, 750 µL of the mixture was mixed thoroughly with 300 µL of DPPH solution. The solution was kept for one hour in the dark at room temperature. After that, the absorbance of each solution in a cuvette was measured using a UV-vis spectrophotometer (U-1800, Japan). The wavelength was set at 517 nm. The DPPH scavenging effect or AA was calculated using Equation (1):

$$\text{Scavenging effect (\%)} = \left[\frac{A_0 - A_1}{A_0}\right] \times 100\% \quad (1)$$

where A_0 and A_1 are the absorbance of the blank and sample, respectively [22]. The 90 wt% of methanol solution was used as the blank. The analyses were conducted three times.

3.0 RESULTS AND DISCUSSION

3.1. Model Fitting and Analysis of Variance (ANOVA) of Total Phenolic Content (TPC)

Table 2 shows the TPC obtained from the experimental and predicted using the RSM-BBD method. The TPC obtained from the experimental work ranges from 94.7748 to 541.757 mg GAE/g dry sample. The highest TPC of 541.757 mg GAE/g dry sample was obtained from the process parameters of 2 g/100 mL at 150 W for 20 min. The second quadratic

model was used to express the TPC (response) relationship as a function of MAE process parameters (independent variables). The model is shown in Equation 2.

$$\text{TPC (Actual)} = -2910.33033 + 1159.12913x_1 + 14.92568x_2 + 110.28904x_3 - 0.038288x_1x_2 - 0.037538x_1x_3 - 1.35135 \times 10^{-3}x_2x_3 - 287.35738x_1^2 - 0.048844x_2^2 - 2.64715x_3^2 \quad (2)$$

where x_1 is the solid-to-solvent ratio in g/ 100 mL, x_2 is the microwave power in W and x_3 is the extraction time in min.

The summary of ANOVA results for the TPC of *M. oleifera* leaf extract is tabulated in Table 3. The ANOVA results were used to estimate the model's coefficients, check each parameter's significance, and indicate the interaction strength of each parameter. According to the findings, the regression model is significant since the F-value is high (183.09) and the p-value is less than 0.0001. Lin et al. [23] reported that a model and process parameters with a p-value of less than 0.05 are considered significant, while the lack of fit of the model was not statistically significant. As seen in the ANOVA Table 3, the primary linear interaction is x_3, and the quadratic of x_1^2, x_2^2, and x_3^2 are significant as its p-value level is less than 0.05. Meanwhile, the linear effect of x_1 and x_2 and the interaction of x_1x_2, x_1x_3, and x_2x_3 exceed the p-value level of 0.05, thus indicating that the effects are insignificant in this model.

The coefficient of determination (R^2) and adjusted coefficient of determination (R^2 adj) of the quadratic model obtained were 0.9958 and 0.9903, respectively. The values are close to 1, thus indicating that the model showed a high correlation between the experimental values of TPC and predicted values of TPC, which was calculated using Equation 2. The data variation is also acceptable and fits the model satisfactorily. The coefficient variation (CV) is measured by expressing the standard deviation as a percentage of the mean. In this study, a CV of 5.52% was obtained. Smaller values of CV give better reproducibility and suggest that the model can be used to represent the design space [4]. Figure 1 illustrates the linear correlation plot between predicted and experimental TPC values. The figure reflects the well-fitting models since the predicted TPC values are close to the experimental values.

Table 3. ANOVA for Box-Behnken designs (BBD) quadratic model of total phenolic content (TPC)

Source of Variation*	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-Value** Probability
Model	4.64E+005	9	51640.34	183.09	< 0.0001 [a]
Linear					
x_1	82.21	1	82.21	0.29	0.6060 [b]
x_2	570.67	1	570.67	2.02	0.1979 [b]
x_3	3404.74	1	3403.74	12.07	0.0104 [a]
Interaction					
x_1x_2	14.66	1	14.66	0.052	0.8262 [b]
x_1x_3	0.14	1	0.14	4.996E-004	0.9828 [b]
x_2x_3	0.46	1	0.46	1.619E-003	0.9690 [b]
Quadratic					
x_1^2	3.477E+005	1	3.477E+005	1232.69	< 0.0001 [a]
x_2^2	62782.13	1	62782.13	222.59	< 0.0001 [a]
x_3^2	18440.49	1	18440.49	65.38	< 0.0001 [a]
Residual	1974.35	7	282.05		
Lack of Fit	1635.20	3	545.07	6.43	0.0521 [b]
Pure Error	339.16	4	84.79		
Correction Total	4.667E+005	16			
R^2	0.9958				
Adjusted R^2	0.9903				
CV (%)	5.52				

*x_1 = solid-to-solvent ratio (g/ 100 mL), x_2 = microwave power (W) and x_3 = extraction time (min).
([a] significant, [b] not significant).

3.2. Model Fitting and Analysis of Variance (ANOVA) of Antioxidant Activity (AA)

The experimental values of AA tabulated in Table 2 range from 52.559 to 89.1719%. The lowest antioxidant activity of 52.559% was obtained at the process variable of 1 g/100 mL at 100 W for 20 min. In contrast, the highest antioxidant activity of 89.1719% was obtained from the process variable of 2 g/100 mL at 150 W for 20 min. The second quadratic model in Equation (3) shows the relation of independent variables with AA response:

$$\text{AA (Actual)} = -137.58254 + 84.54928x_1 + 1.12119x_2 + 5.36393x_3 + 0.015398x_1x_2 + 0.29119x_1x_3 + 3.551 \times 10^{-4}x_2x_3 - 23.1029x_1^2 - 3.76905 \times 10^{-3}x_2^2 - 0.14866x_3^2 \quad (3)$$

where x_1 is solid-to-solvent ratio (g/ 100 mL), x_2 is microwave power (W), and x_3 is extraction time (min).

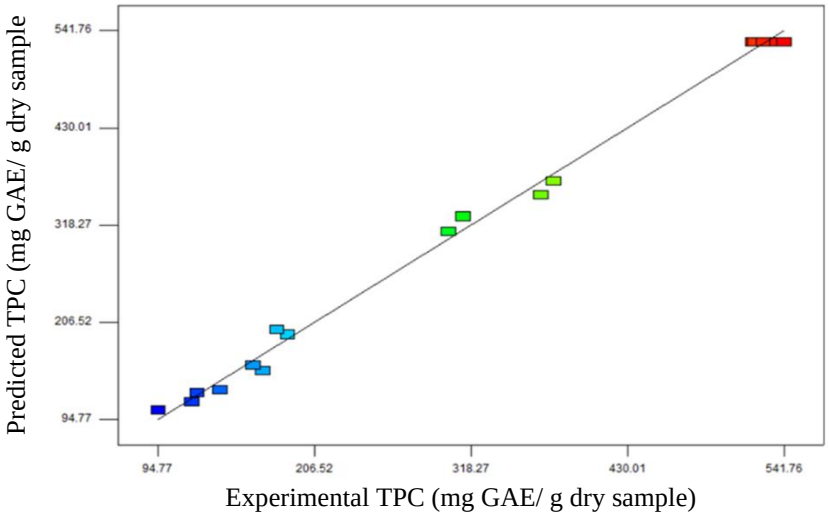

Figure 1. Correlations between predicted and experimental values of TPC from *M. oleifera* leaf extracts

The summary of ANOVA results for the *M. oleifera* leaf AA is shown in Table 4. The lack of fit with a p-value of 0.592, which was insignificant, indicates the adequacy of the developed model. In addition, the high F-value (57.01) and small p-value (<0.0001) suggested that the regression model was significant. Based on the calculated p-values in the ANOVA table, the quadratic of x_1^2, x_2^2, and x_3^2 are significant as their p-value was less than 0.05. In contrast, the linear of x_1, x_2, and x_3, the interaction of x_1x_2, x_1x_3, and x_2x_3 exceeded the p-value level of 0.05, thus indicating that the effects were insignificant in this model. The coefficient of determination (R^2) and adjusted coefficient of determination (R^2 adj) of the quadratic model obtained were 0.9865 and 0.9692, respectively, while the CV value obtained was 3.36%. Figure 2 illustrates the linear plot of the correlation between predicted and experimental values of AA. The predicted AA versus the experimental values reflected the well-fitting models, as the predicted values of antioxidant activity are close to the observed values.

Table 4. ANOVA for Box-Behnken designs (BBD) quadratic model of antioxidant activity

Source of Variation*	Sum of Squares	Degree of Freedom	Mean Square	F-value	p-Value Probability
Model	2882.56	9	320.28	57.01	< 0.0001 [a]
Linear					
x_1	0.59	1	0.59	0.10	0.7556 [b]
x_2	16.10	1	16.10	2.87	0.1343[b]
x_3	0.57	1	0.57	0.10	0.7596 [b]
Interaction					
x_1x_2	2.37	1	2.37	0.42	0.5367 [b]
x_1x_3	8.48	1	8.48	1.51	0.2590 [b]
x_2x_3	0.032	1	0.032	5.611E-003	0.9424 [b]
Quadratic					
x_1^2	2247.34	1	2247.34	399.99	< 0.0001 [a]
x_2^2	373.84	1	373.84	66.54	< 0.0001 [a]
x_3^2	58.15	1	58.15	10.35	0.0147 [a]
Residual	39.33	7	5.62		
Lack of Fit	33.49	3	11.16	7.65	0.0592 [a]
Pure Error	5.84	4	1.46		
Correction Total	2921.89	16			
R^2	0.9865				
Adjusted R^2	0.9692				
CV (%)	3.36				

*x_1 is the solid-to-solvent ratio in g/ 100 mL, x_2 is the microwave power in W and x_3 is the extraction time in min. ([a] significant, [b] not significant)

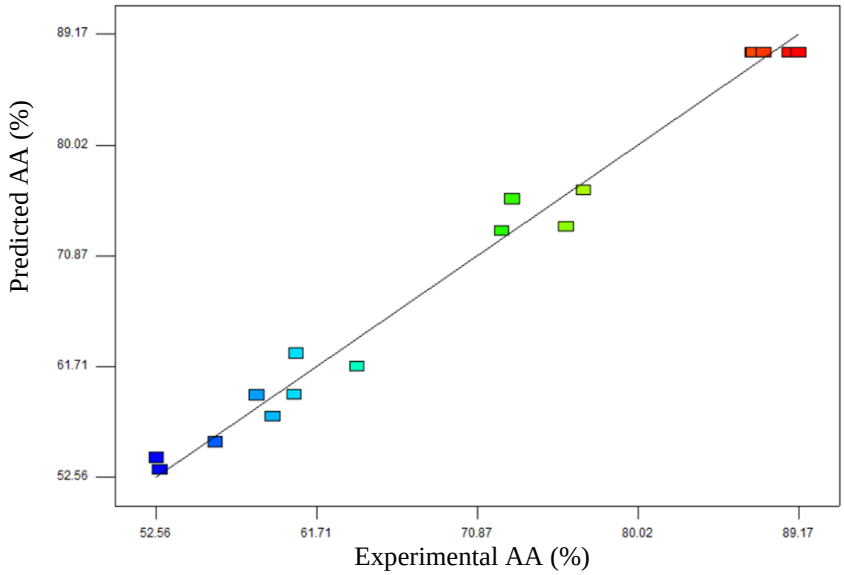

Figure 2. Correlations between predicted values with the experimental values of antioxidant activity (AA) in *M. oleifera* leaf extracts

3.3. Effect of MAE Parameters and Response Surface Analysis of Total Phenolic Content (TPC) and Antioxidant Activity (AA)

Figure 3 and Figure 4 illustrate the interaction effects of the MAE process variables in a three-dimensional (3D) response surface plot for TPC and AA, respectively. The 3D response surface plot for TPC and AA as a function of microwave power, x_2, and solid-to-solvent ratio, x_1, is shown in Figures 3 (a) and 4 (a), respectively. The findings show that the TPC and AA depend on the interaction between microwave power, x_2 and the solid-to-solvent ratio, x_1. The TPC and AA increased when the microwave power and solid-to-solvent ratio increased to a certain point. However, when the amounts of microwave power and solute-to-solvent rise, the TPC and AA decrease. The optimum value of TPC and AA was achieved at a microwave power of 150 W and a solid-to-solvent ratio of 2 g/100 mL.

Chen et al. [4] also reported that microwave power positively affected the values for TPC and AA. This is because the increase in microwave power can amplify the energy transmitted to the plant cell walls through electromagnetic waves. As the microwave power increases, more electromagnetic waves penetrate the plant materials and solvent. This phenomenon can lead to an increase in the pressure inside the plant cells, which enhances the breakdown or rupture of cell walls and promotes the release of bioactive substances [24]. These electromagnetic waves can also induce more vigorous vibration and rotation of the water molecules. Hence, it consequently enhances the diffusivity of the bioactive substances into the solvents, leading to an increase in the speed and efficiency of the extraction process [24].

However, the microwave power cannot be increased too much. This is because the increase in microwave power often leads to an increase in solution temperature [10]. Although the increase in solution temperature could enhance the separation of bioactive substances from the sample matrix due to improved solubility, it will also tend to degrade and damage the plant cell, thus ultimately reducing the yield [25] [26]. Zhang et al. [27] also discovered that the microwave power used during MAE increased the yields of TPC from *A. blazei*, but it cannot be too high since it resulted in a drop in yields. Similarly, Vinatoru et al. [24] asserted that microwave power significantly impacted the TPC values and AA of *Coriolus versicolor* mushroom extracts. This is because high microwave power might release higher energy in microwave form, increase the temperature in the solution, and expose the bioactive compound to thermal degradation.

Figures 3 (a) and 4 (b) also show that increasing the solid-to-solvent ratio up to a certain point at fixed microwave power and extraction time leads to an increase in TPC and AA. This is because fewer solid materials come in contact with the solvent at low solid-to-solvent ratios. However, the solid-to-solvent ratio cannot be too high since it will impede the diffusivity and solubility of the bioactive substances from the plant materials to the solvent. Thus, a suitable range of solid-to-solvent ratio is needed to ensure sufficient contact between plant materials and the solvent [28]. According to Mohamad et al. [29], an incomplete extraction process is expected when a smaller volume of solvent is used. In contrast, the larger solvent volume causes the extraction procedure to be wasteful and more complicated. Based on the mass transfer principle, the driving forces depend on the concentration difference between the solute and solvent. When the solid-to-solvent increases, the concentration gradient of the solution that will undergo the extraction process later will also increase. Thus, the diffusion rate will increase to allow an effective extraction process. Furthermore, when the amount of *M. oleifera* leaves increases, the possibility of its bioactive substances coming into contact with the solvent used in this study also increases, leading to a higher leaching-out rate.

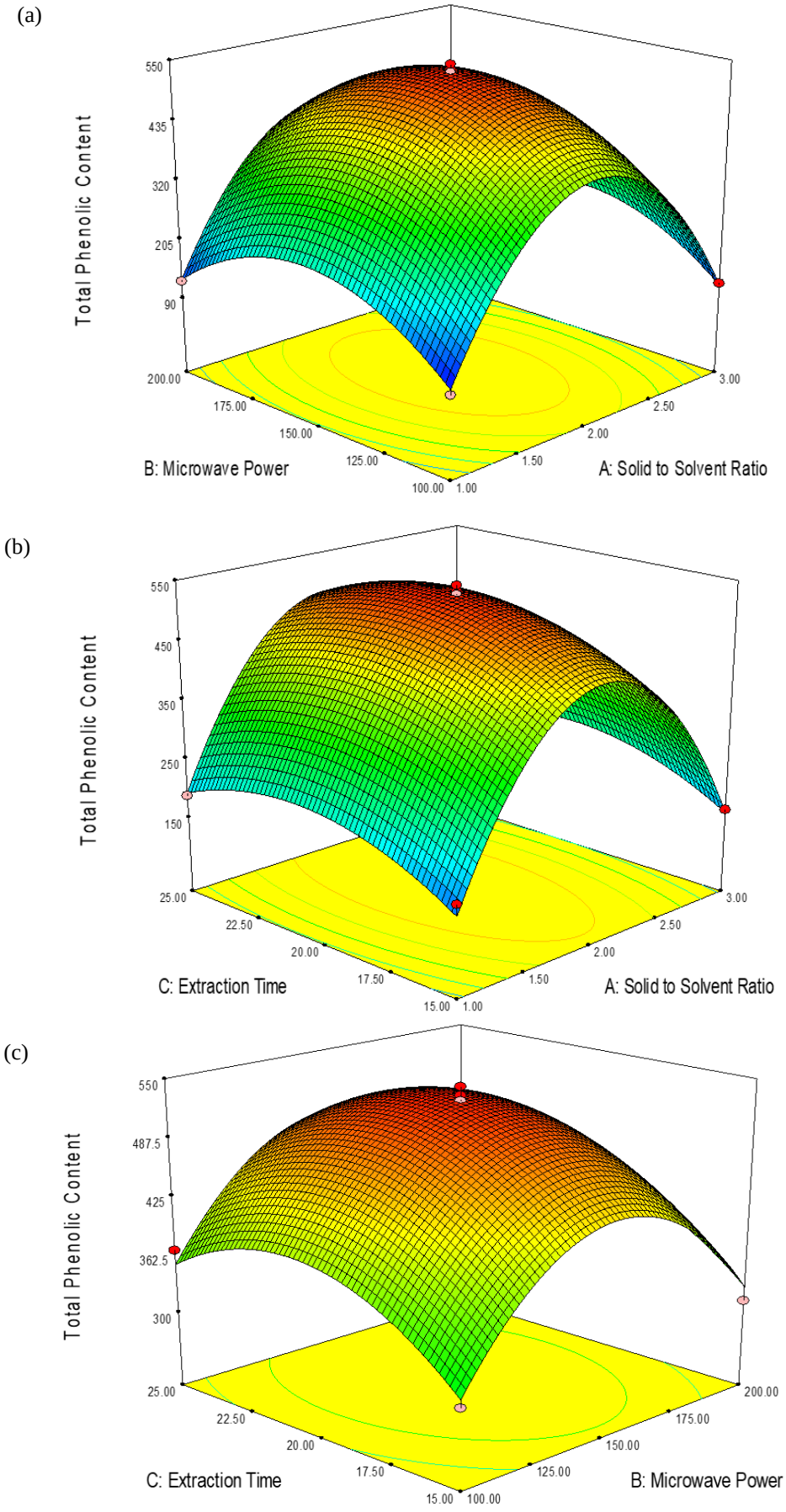

Figure 3. Response surface plot showing the effect of the interaction parameters (a) solid-to-and solvent ratio, x_1 (g/100 mL) and microwave power, x_2 (W); (b) extraction time, x_3 (min) and solid-to-solvent ratio, x_1 (g/ 100 mL); and (c) extraction time, x_3 (min) and microwave power, x_2 (W) on the total phenolic content, TPC (mg GAE/g dry sample).

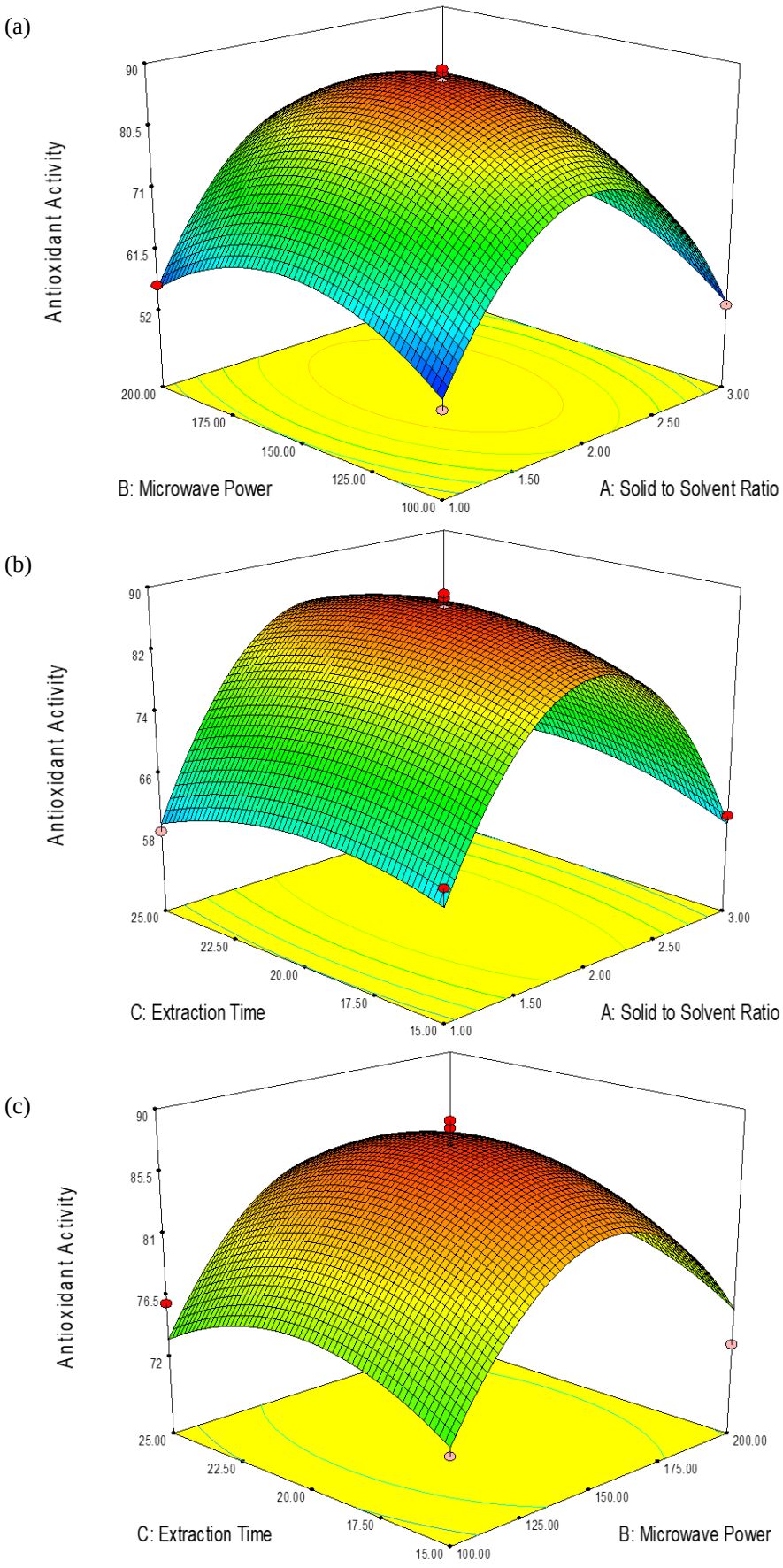

Figure 4. Response surface plot showing the effect of the interaction parameters (a) solid-to-and solvent ratio, x_1 (g/100 mL) and microwave power, x_2 (W); (b) extraction time, x_3 (min) and solid-to-solvent ratio, x_1 (g/ 100 mL); and (c) extraction time, x_3 (min) and microwave power, x_2 (W) on the antioxidant activity, AA (%).

Extraction time is important to allow sufficient contact time for the plant material sample and solvent at the desired process parameters [28]. Figures 3 (b) and 4 (b) illustrate the response surface plot of TPC and AA as a function of extraction time and solid-to-solvent ratio. Increasing the extraction time and solid-to-solvent ratio, to a certain extent, generally improves the solubility of the bioactive substances, leading to higher TPC and AA values. An extraction time of 20 min and the solid-to-solvent ratio of 2 g/ 100 mL showed the optimum value of TPC and AA of 528.24 mg GAE/ g dry sample and 87.63%, respectively. The best extraction time obtained in this work is consistent with the previous work of Rodríguez-Pérez et al. [9]. However, further increments of extraction time may result in the thermal decomposition of certain bioactive compounds since the duration of bioactive substances being exposed to a high temperature environment becomes longer [30]. Mahdi et al. [31] also reported that the TPC and AA increased with extraction time up to a certain duration.

Figures 3 (c) and 4 (c) showed that the TPC and AA of the *M. oleifera* leaf extract increased with the extraction time of up to 20 min and microwave power of up to 150 W. This result could be due to the thermolabile compounds of *M. oleifera*, which have antioxidant properties that may degrade with further increase of the microwave power and time. Thus, setting a suitable microwave power and time might control this side effect.

3.6. Model validation

The optimum MAE parameters generated from RSM-BBD that might maximize the TPC and AA are shown in Table 5. The RSM-BBD suggested only one numerical solution within the range of the studied process parameters. The predicted TPC and AA were compared with an experimental result for validation. A strong correlation between the experimental and the predicted TPC and AA was observed since it deviated with 1.4028% and 2.6917%, respectively. Thus, verifying that the response models are adequate to reflect the MAE within the parameters studied in this work. The maximum TPC (529.801 mg GAE/g dry sample) and AA (87.6657%) from *M. oleife*ra leaf extract can be obtained by using solid-to-solvent of 2.01 g/100 mL and microwave power of 152.7 W for 20.54 min extraction time. Gunalan et al. [18], who optimized the MAE extraction of *M. oleifera* leaves only obtained 63.36–76.40 mg GAE/gram of TPC when using ethanol as the extraction solvent. The study by Kheyar et al. [32] obtained 58.45 ± 0.68 mg GAE/g DW of TPC under optimal extraction conditions (48.86% ethanol, 626.53 W, 99.48 s, 29.67 mL/g solvent to solid ratio and 21.12 W/mL power density). The choice of extraction solvent and MAE parameters might cause the differences.

Table 5. Solution and validation of the model equation

Solid-To-Solvent Ratio (g/100 mL)	Microwave Power (W)	Extraction Time (min)	Total Phenolic Content, TPC (mg GAE/g dry sample)		Deviation (%)
			Predicted	Actual	1.4028
			529.802	522.478	
2.01	152.72	20.54	Antioxidant Activity, AA (%)		Deviation (%)
			Predicted	Actual	2.6917
			87.6656	85.3678	

4.0 CONCLUSION

The optimization process by response surface methodology (RSM) with Box-Behnken designs (BBD) suggested a quadratic model for modeling the *M. oleifera* leaf extraction process using microwave-assisted extraction (MAE) technique. The MAE parameters, namely solid-to-solvent ratio, microwave power, and extraction time, significantly affect the optimal TPC and AA in the extracts. This is because the TPC and AA began to decline after reaching the optimum condition. The lowest values of TPC (94.77 mg GAE/g dry sample) and AA (52.559%) were obtained at the MAE variables of 1 g/100 mL, 100 W and 20 min. Meanwhile, the highest TPC (541.757 mg GAE/g dry sample) and AA (89.1719%) were obtained at the MAE process variable of 2 g/100 mL, 150 W, and 20 min. The optimization suggested that the maximum TPC (529.802 mg GAE/ g dry sample) and AA (87.6656%) from *M. oleifera* leaf can be obtained using MAE parameters of 2.01 g/100 mL, 152.7 W and 20.54 min. The validation shows that the predicted TPC and AA deviate from the experimental data by 1.4028% and 2.6917%, respectively. These findings showed MAE as a promising green alternative for extracting a wide range of bioactive compounds with water as solvents at optimized extraction power, solid-to-solvent ratio, and time. The extracts could be further exploited in the formulation of food products or as supplements through encapsulation technology.

5.0 CONFLICT OF INTEREST

The authors declare no conflicts of interest.

6.0 AUTHORS CONTRIBUTION

T. K. Cheong (Conducting experiment, Data curation, Writing-original draft)

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