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Response Surface Optimization of Extraction of Phenolic and Flavonoid Contents of *Ipomoea batatas* Leaves

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ABSTRACT
The study aimed at optimizing the yield of extraction of flavonoids and phenolics in *Ipomoea batatas* leaves as a function of drying temperature (65-105 °C) and ethanol concentration (60-80 v/v %) at constant drying time (95 minutes) using Response Surface Methodology (RSM). From the results, quadratic term was significant for phenolic content, while 2F1 model was significant for flavonoid content. The coefficients of determination (R^2) and for phenolic and flavonoid contents were 0.9362 and 0.8102 respectively, showing a good fit model. The model terms were significant ($P<0.05$) for all the responses which provided its suitability for prediction purposes. The coefficients of variation (CV) for phenolic and flavonoid were 3.21 and 4.21, respectively, both of which were less than 10%. Using desirability function, the optimum operating conditions to obtain higher extraction of phenolic and flavonoid was found to be 65 °C and 70.33 (v/v %) ethanol concentration. The study implies that *Ipomoea batata*s leaves possess robust antioxidant abilities making them suitable for therapeutic purposes.
Keywords: Potato leaves, RSM, flavonoid, phenolic, optimisation

INTRODUCTION
Sweet potato leaves (*Ipomoea batatas*) are the green and flat structures that grow from the stem of the potato plant. They are often overlooked and disregarded as a simple byproduct of the potato plant, but they are actually a source of valuable nutrients and have been used for centuries in traditional medicine (Remya and Subha, 2014; Shahidul, 2006). Potato leaves are rich in various vitamins, minerals and protein that are essential to human health (Yoshimoto *et al.*, 2003). For example, they are a good source of vitamin C, which is important for immune function and skin health. They also contain vitamin K, which is necessary for proper blood clotting, and vitamin A, which is crucial for vision and skin health. Additionally, potato leaves are a good source of minerals such as potassium, magnesium, and iron. Potato leaves are a rich source of

bioactive compounds, including phenolic compounds, flavonoids, and tannins, which have been linked to potential health benefits (Nakachi *et al.*, 2016). Phenolic compounds are known for their antioxidant properties and flavonoids for their antioxidant, anti-inflammatory, and anti-cancer properties (Nguyen *et al.*, 2021). Tannins are known for their astringent properties and anti-inflammatory and anti-bacterial effects. The contents of these compounds can vary depending on various factors, but potato leaves contain significant amounts, making them a potential source of natural antioxidants and health benefits (Ayelesoı *et al.*, 2016). Furthermore, some studies suggest that potato leaves may have potential in the treatment of diabetes due to their ability to lower blood sugar levels. Aside from their nutritional and medicinal benefits, potato leaves also have other uses. In some cultures, they are used as a culinary ingredient, particularly in African and Caribbean cuisine. They are often cooked in stews, soups, and other dishes, and are said to have a slightly sweet and nutty flavor (Remya and Subha, 2014)

However, despite their many benefits, it is important to note that potato leaves should not be consumed in large quantities as they contain solanine, a toxic compound that can cause gastrointestinal distress and other health problems if ingested in large amounts (Chen *et al.*, 2018). Therefore, it is important to cook potato leaves properly before consumption and to only consume them in moderation. Response surface methodology can effectively optimize the antioxidant activities of plants by combining different processing methods with modeling tools. Response surface methodology (RSM) is a statistical technique used to model and optimize complex processes by exploring the relationship between multiple input variables and their output response (Bas D and Boyacı, 2007). This technique is particularly useful for identifying the optimal combination of input factors that can yield the desired output response. RSM involves the use of mathematical models and statistical analysis to analyze and optimize the response surface, which is a graphical representation of the relationship between the input variables and the response (Khuri and Mukhopadhyay, 2010). The present study aimed to explore the effect of drying temperature and ethanolic concentrations on the bioactive constituents of *Ipomoea batatas* by utilizing response surface methodology.

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MATERIALS AND METHODS

Response surface methodology was used to optimize the extraction parameters of *Ipomoea batatas* leaf. Central Composite Design (CCD) was employed to identify the relationship between the response functions and process variables. The independent variables in this study were drying temperature (X_1: 65–105 °C) and ethanol concentration (X_2: 60–80 % v/v ethanol/water). The dependent variables (responses) measured were phenolic content (PC) and flavonoid (FC). The experimental data were evaluated using the response surface methodology. The generation of response surface plots and statistical analysis were performed using Design expert software (STAT-EASE, MINNEAPOLIS, MN, USA). The regression analysis was performed on the data of response variables obtained as affected by the process variables and was fitted into a second-order regression equation as shown in the following equation;

$$Y_k = bk_0 + \sum_{i=1}^{m} bk_i x_i + \sum_{i=1}^{m} bk_{ii} X_i^2 + \sum_{i=1}^{m} bk_{ij} X_i X_j \ + € \qquad (1)$$

Where Y represents the response variables to be modeled, bk_o is the value of the fitted response at the center point of the design, and bk_i, bkii, and bk_{ij} are the linear, quadratic, and interaction regression terms, respectively. k is the number of variables and € is the random error of the model.

Collection of Plant Sample

Hand-plucked mature fresh leaves of *Ipomoea batatas* were collected from parent plants located in Babcock University, Ilishan-remo, Ogun State, Nigeria.

Preparation and Extraction of Plant Material

The freshly harvested leaves of *Ipomoea batatas* were washed thoroughly with tap water and then rinsed with distilled water. They were then dried at different temperatures in a hot air oven, as per the experimental design. Once cooled, the leaves were ground to a fine powder using a USHA MG 3473 laboratory grinder. The extraction process was carried out through maceration of the leaves in ethanol, using varying weight-to-volume ratios based on the experimental design. The mixtures were

shaken vigorously and allowed to stand for 48 hours at room temperature. The mixture was filtered through a Whatman No. 1 filter paper and the residue was macerated again in an equal volume of ethanol for 24 hours to obtain more extract. The mixtures were then combined and evaporated to dryness under reduced pressure at approximately 40°C, using an Eyela N-1001 vacuum rotary evaporator.

Phenolic Content

The method described by (Singleton *et al.*, 1999) was utilized to determine the phenolic content of the sample. The assay involves the reduction of Folin-Ciocalteu reagents, phosphomolybdate and phosphotungstate, by the phenolic compounds present in the extract. A reaction mixture was prepared by mixing 0.5 mL of ethanolic solution of the sample (containing 100 μg/mL), 2.5 mL of 10 % aqueous solution of Folin-Ciocalteu reagent, and 2.5 mL of 7.5 % $NaHCO_3$ solution. A blank was simultaneously prepared by mixing 0.5 mL ethanol, 2.5 mL of 10 % aqueous solution of Folin-Ciocalteu reagent, and 2.5 mL of $NaHCO_3$ solution. The sample was then incubated in a laboratory water bath at 45°C for 45 minutes, and the absorbance was measured at 765 nm using a spectrophotometer (JENWAY 6305, Staffordshire, UK). Standard solutions of gallic acid were treated in the same way, and their absorbance values were used to create a standard calibration curve. The phenolic content was then calculated as gallic acid equivalent (mg of GA/g) of the sample from the measured absorbance using the standard calibration curve. All samples were analyzed in triplicate.

Flavonoids

The spectrophotometric method of Dewanto *et al.* (2022) was used to determine the flavonoid content of the crude extract samples. Quercetin was used as the standard substance. In a 10 ml volumetric flask, 1 ml of the sample (containing 100 μg/ml), prepared in ethanol, was mixed with 4 ml of distilled water. Then, 0.3 mL of 5% $NaNO_2$ solution was added, followed by 0.3 mL of 10% $AlCl_3$ after 5 minutes. At the 6th minute, 2 mL of 1.0 M NaOH was added. After thoroughly shaking the mixture with 2.4 mL of distilled water, the absorbance was measured at 510 nm on a spectrophotometer (JENWAY 6305, Staffordshire, UK). A reagent blank containing 1 ml of ethanol (instead of the extract) was prepared and

treated in the same manner as the samples. A calibration curve was constructed by repeating the same procedure for standard solutions of Quercetin (2 to 10 µg/ml, R^2 = 0.986). The total flavonoid content was estimated from the measured absorbance of the samples using the Quercetin calibration curve, and the results were expressed as mg Quercetin Equivalent per gram (mgQE/g) of the sample on a dry weight basis. The analysis was done in triplicate.

Table 1: Design Matrix with Factors and Levels

Factor	Unit	Low	High
X_1- Drying temperature	°C	65	105
X_2- Ethanol Concentration	% v/v	60	80

Table 2: Design Summary for Response Surface Model for Phenolic and Flavonoid Content

Response	Name	Unit	Transformation	Model	Minimum	Maximum
Y1	Phenolic	mg/g	None	Quadratic	10.22	13.37
Y2	Flavonoid	mg/g	None	2FI	4.02	5.64

Where Y = response variable

Statistical Analysis and Optimization

By utilizing statistical parameters such as adjusted multiple correlation coefficients (adjusted R^2), multiple correlation coefficients (R^2), coefficient variation (C.V%), lack of fit, regression F-value, and regression p-value through analysis of variance (ANOVA), the most suitable model for response can be attained. This statistical method summarizes the outcomes obtained in all experimental conditions, with a confidence interval of 95% to test the significant impact of the factors and their interaction. To determine the optimal extraction conditions for obtaining the highest total phenolics and flavonoids content in *Ipomoea batatas* leaves, the desirability function approach in design expert software was used. The resulting polynomial equation was represented as three-dimensional surface plots to demonstrate the correlation between the responses and the experimental variables used.

Model Verification

The experimental and predicted values of phenolic and flavonoid contents were compared in order to determine the validity of the model.

RESULTS AND DISCUSSION

Table 3: Central Composite Design Arrangement and Responses

Run	Drying Temperature X_1 (°C)	Ethanol Concentration X_2 (v/v%)	Phenolic content (mg/g GAE)	Flavonoid content (mgQE/g)
1	105.00	80.00	10.73	4.48
2	85.00	70.00	12.99	4.58
3	85.00	84.14	13.37	4.62
4	65.00	80.00	12.85	4.92
5	65.00	60.00	11.57	5.64
6	85.00	70.00	12.99	4.58
7	85.00	70.00	12.99	4.58
8	85.00	70.00	12.99	4.58
9	113.28	70.00	10.22	4.24
10	85.00	70.00	12.99	4.58
11	56.72	70.00	13.14	4.92
12	85.00	55.86	10.98	4.32
13	105.00	60.00	10.46	4.02

The experimental design and the corresponding three response variables are presented in Table 2. The result of extracted phenolic content and flavonoid contents of potato leaves ranged from 10.46 - 13.37 mg QE/g and 4.02-5.64 mg/g GAE/g. The highest phenolic content was obtained from run 3, with a value of 13.37 mg/g, while the lowest phenolic content was obtained from run 9, with a value of 10.22 mg/g. The highest flavonoid content was obtained from run 5, with a value of 5.64 mg/g, while the lowest flavonoid content was obtained from run 13, with a value of 4.02 mg/g. Higher temperatures are associated with lower phenolic and flavonoid content, as seen in runs 1, 3, and 13, while lower temperatures are associated with higher phenolic and flavonoid content, as seen in runs 4 and 11. Ethanol concentration also have an effect on the phenolic and flavonoid content, with higher ethanol concentrations associated with higher phenolic and flavonoid content, as seen in runs 3, 6, 7, 8, 10, and 11. The higher phenolic and flavonoid content could be explained by the

natural polarity of the solvents used Tan *et al.*, 2013). The final empirical regression model of the relationship between responses and the two tested variables for phenolic and flavonoid contents in terms of actual factors could be expressed by the following equation.

Phenolic = $+12.99-0.92X_1+0.62X_2-0.79X_1^2-0.54\ X_2^2-0.25\ X_1X_2$

Flavonoid$=+4.62-0.38\ X_1+0.29\ X_1X_2$

Table 4: Analysis of Variance (ANOVA) based on quadratic model for Phenolic contents

Sources	Sum of square	Degree of freedom	Mean of square	F-value	P-value
Phenolic Content (mg/g GAE)					
Model	15.71	5	3.14	20.54	0.0005
A	*6.77*	*1*	*6.77*	*44.25*	*0.0003*
B	*3.04*	*1*	*3.04*	*19.86*	*0.0029*
A^2	*3.63*	*1*	*3.63*	*23.71*	*0.0018*
B^2	*2.02*	*1*	*2.02*	*13.20*	*0.0084*
AB	*0.26*	*1*	*0.26*	*1.67*	*0.2377*
Residual	1.07	7	0.15		
Pure Error	*0.000*	*4*	*0.000*		
Cor Total	16.78	12			

R^2 = 0.9362, Adj R^2= 0.8906, Adeq precision = 12.558, coefficient of variation (CV) = 3.21

Table 4 showed the analysis of variance (ANOVA) based on quadratic model for phenolic contents. The Sum of square represented the total variation in the dependent variable that is explained by each predictor variable or group of predictor variables. In this case, the total number of degrees of freedom is 12, which is equal to the sum of the DF for the model and the DF for the residual. The mean square represented the average amount of variation in the dependent variable that is explained by each predictor variable or group of predictor variables. It is calculated by dividing the sum of squares by the degrees of freedom. The F-value is a measure of the ratio of the mean square for each source of variation to the mean square for the residual. A high F-value indicates that the predictor variable or group of predictor variables is significant in explaining the

variation in the dependent variable. A small P-value (typically less than 0.05) indicated that the predictor variable or group of predictor variables is significant in explaining the variation in the dependent variable.

According to the results presented in the table, the model is significant, as indicated by a highly significant F-value (20.54) and a small P-value (0.0005). This means that the model, which included all the predictor variables, explained a significant amount of the variation in the dependent variable. Among the predictor variables, A, B, A2, and B2 are all significant, as indicated by highly significant F-values and small P-values. This means that these predictor variables explain a significant amount of the variation in the dependent variable, even after controlling for the other predictor variables. The interaction term AB was not significant, as indicated by a non-significant F-value (1.67) and a relatively large P-value (0.2377). This means that there was no evidence of an interaction effect between A and B on the dependent variable.

The residual and pure error sources of variation also had small mean squares, indicating that there is little unexplained variation in the dependent variable. The R-squared value (0.9362) and the adjusted R-squared value (0.8906) indicated that the model explained a high proportion of the variation in the dependent variable. The adequate precision value (12.558) and the coefficient of variation (CV) value (3.21) provided information on the accuracy and reliability of the model. The adequate precision value represented the signal-to-noise ratio, with higher values indicating a more reliable model.

The results of the analysis show that the model is statistically significant, with an F-value of 20.54 and a p-value of 0.0005. The model's goodness-of-fit was assessed using R^2, which was found to be 0.9362, indicating that 93.62% of the variability in the response variable (flavonoid contents) can be explained by the independent variables. The adjusted R-squared value of 0.8906 indicates that the model is also a good fit for the data. The analysis also shows that the sum of square has a relatively high F-value of 15.71 and a low p-value of 0.0005. The next most significant independent variable is A, with an F-value of 6.77 and a low p-value of 0.0003. B and A2 are also found to be significant with F-values

of 3.04 and 3.63, respectively, and p-values of 0.0029 and 0.0018, respectively.

The interaction term AB, however, is found to be not significant, with an F-value of 0.26 and a high p-value of 0.2377, indicating that the interaction between A and B does not have a significant effect on flavonoid contents. The results suggest that the model is a good fit for the data and provides a reliable estimation of the relationship between the independent and dependent variables.

Table 5: Analysis of Variance (ANOVA) Based on 2F1 Model for Flavonoid Contents

Sources	Sum of square	Degree of freedom	Mean of square	F-value	P-value
Flavonoid Content (mg/g GAE)					
Model	1.49	3	0.50	12.81	0.0013
A	*1.14*	*1*	*1.14*	*29.38*	*0.0004*
B	*3.373E-003*	*1*	*3.373E-003*	*0.087*	*0.7749*
AB	*0.35*	*1*	*0.35*	*8.96*	*0.0151*
Residual	0.35	9	0.039		
Lack of Fit	*0.35*	*5*	*0.070*		
Pure Error	*0.000*	*4*	*0.000*		
Cor Total	1.84	12			

R^2 = 0.8102, Adj R^2= 0.7470, Adeq precision = 12.306, Coefficient of variation (CV) = 4.27

Table 5 presents the results of an analysis of variance (ANOVA) based on a quadratic model for flavonoid contents. The sources of variation are identified as Model, A, B, and AB. The results of the ANOVA show that the model as a whole is statistically significant (P-value = 0.0013), indicating that the model is a good fit for the data. The A factor is also statistically significant (P-value = 0.0004), indicating that the level of factor A has a significant effect on the flavonoid content. However, the B factor is not statistically significant (P-value = 0.7749), indicating that the level of factor B does not have a significant effect on the flavonoid content. The interaction between A and B (AB) is marginally significant (P-value = 0.0151), indicating that the effect of factor A on the flavonoid

content may depend on the level of factor B. The R2 value of 0.8102 indicated that the model explains 81.02% of the total variation in the data, while the adjusted R2 value of 0.7470 took into account the number of predictors in the model. The adequacy precision value of 12.306 indicated that the model is adequate for making predictions, while the CV value of 4.27 indicated the precision of the model in predicting new observations.

Table 6: Diagonostic Case Statistics

Standard Order	Phenolic (Actual value)	Phenolic (Predicted value)	Flavonoid Content (Actual value)	Flavonoid (Predicted Value)
1	11.57	11.72	5.64	5.27
2	10.46	10.38	4.02	3.93
3	12.85	13.45	4.92	4.72
4	10.73	11.11	4.48	4.56
5	13.14	12.72	4.92	5.15
6	10.22	10.12	4.24	4.09
7	10.98	11.04	4.32	4.59
8	13.37	12.78	4.62	4.65
9	12.99	12.99	4.58	4.62
10	12.99	12.99	4.58	4.62
11	12.99	12.99	4.58	4.62
12	12.99	12.99	4.58	4.62
13	12.99	12.99	4.58	4.62

Table 6 Showed the differences between the actual value and predicted value for phenolic content and flavonoid content. The results are presented in terms of mean values of phenolic content and flavonoid content, where the mean actual value for phenolic content is 11.32 mg/g and mean phenolic content is 11.47 mg/g, and mean actual value for flavonoid content is 4.70 mg/g, and mean flavonoid content is 4.63 mg/g. The differences between actual values and flavonoid contents in both phenolic and flavonoid content are relatively small, with most values falling within a range of 0.1-0.5. This showed that the predictive models used in this study are generally accurate and can provide useful estimates of phenolic and flavonoid content in plant samples.

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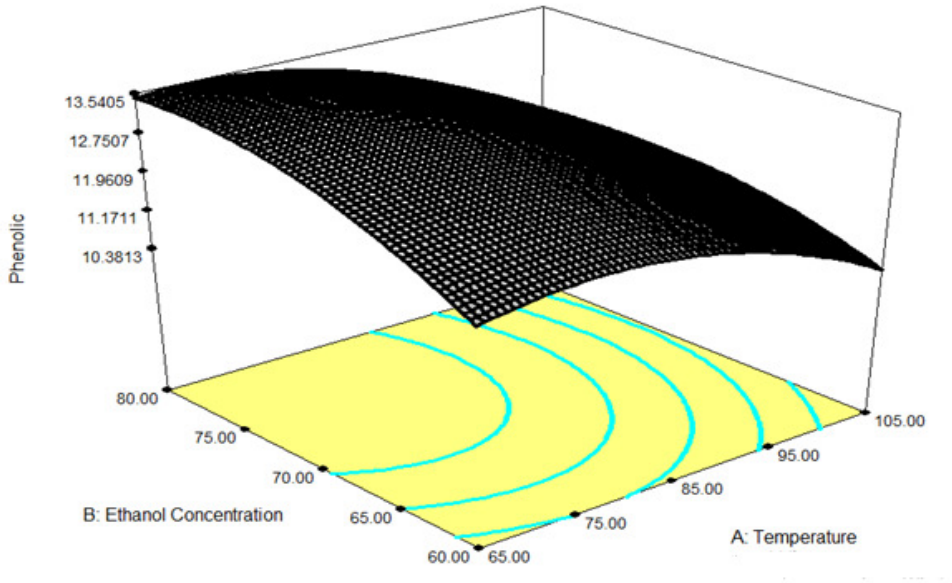

Figure 1: 3D Response surface plot depicting the effect of temperature and ethanol concentration on phenolic levels

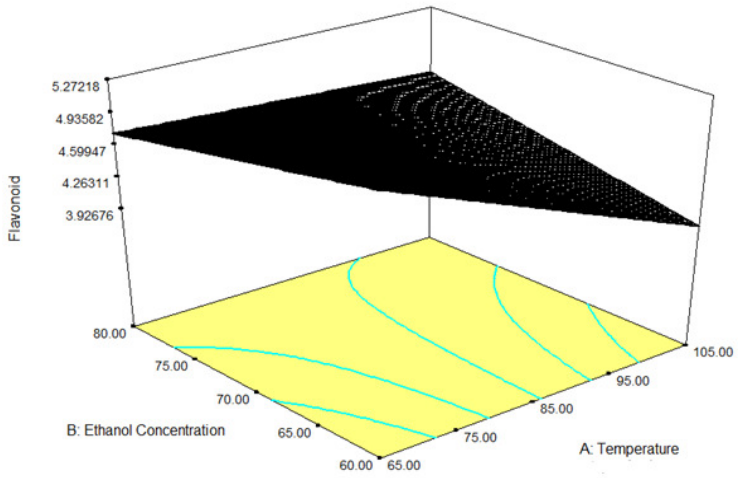

Figure 2: 3D Response surface plot depicting the effect of temperature and ethanol concentration on flavonoid levels

Figure 1 and 2 showed the response surface plot of the effect of drying temperature and ethanol concentration on flavonoids and phenolic. The independent variables were created with ethanol concentration and

drying temperature as X and Y coordinate, respectively, and the responses (FC, PC) (Anisa *et al.*, 2018). The surface plots also revealed that by increasing the extraction temperature to higher levels, the amounts of phenolic gradually dropped and this might be explained by the fact that the final equilibrium between the solvent concentrations in the plant matrix and the temperature will be achieved after a certain concentration level (Sai-Ut *et al.*, 2015). It could be observed in figure 1 and 2 that the values of phenolic and flavonoid contents increased with increased ethanol concentration and low temperature. Decrease in the phenolic and flavonoid contents was also observed with higher drying temperature and lower ethanol concentrations. According to Bazykina *et al.*, 2002, the use of ethanol as a solvent is beneficial for the effective extraction of flavonoids and their glycosides from plant materials. This is due to the diffusion of particles that leads to the rupture of plant tissue, resulting in increased solubility of the solvent. However, it is important to note that over time, the solvent may degrade, resulting in decreased effectiveness (Yoswathana, 2013).

Table 7: Numerical Optimization for Phenolic and Flavonoid Contents

Name	Goal	Lower Limit	Upper Limit	Lower Weight	Upper Weight	Importance
Drying Temperature	is in range	65	105	1	1	3
Ethanol Concentration	is in range	60	80	1	1	3
Phenolic Content	maximize	10.22	13.37	1	1	3
Flavonoid Content	maximize	4.02	5.64	1	1	3

Solution

Number	Drying Temperature	Ethanol Concentration	Phenolic Content	Flavonoid Content	Desirability
	65.00	70.33	13.1521	4.98853	0.75

Table 7 showed the result of a numerical optimization process. The optimization process aims to find the best combination of parameters that will result in the highest possible values for the phenolic and flavonoid contents while keeping the temperature and ethanol

concentration within certain ranges. This solution had a Temperature of 65.00 °C and an Ethanol concentration of 70.33 v/v %, which fell within the acceptable ranges. The Phenolic and Flavonoid contents were 13.15 mg QE/g and 4.99 mg/g GAE, respectively, which were the highest values achieved in the optimization process. The overall desirability of this solution was 0.75, which suggests that it is a highly desirable solution.

Table 8: Validation of the Model

X_1	X_2	PCAV(mg QE/g)	PCPV(mg QE/g)	FCAV(mg/g GAE)	PCPV(mg/g GAE)
65.00 (°C)	70.33 (v/v %)	13.11±0.05	13.15±0.09	4.87±0.03	4.99±0.06

PCAV- Phenolic Content Actual Value, PCPV-Phenolic Content Predicted Value, FCA–Flavonoid Content Actual Value, FCP-Flavonoid Content Predicted Value

Table 8 showed the result of the optimization. In order to verify the optimum conditions, the *Ipomoea batatas leaves* were subjected to analysis using these optimal conditions and the results were compared statistically to the predicted values given by the design expert 6.0.8 software of the RSM model. Based on the results, the predicted values of phenolic and flavonoid contents were found to be reasonably comparable with experimental values at 95% confidence level as shown in Table 8.

CONCLUSION

The current research employed Response Surface Methodology (RSM) and a design known as Central Composite Design (CCD) to identify the optimal process parameters and obtain second-order polynomial models for predicting responses. Using desirability function approach, the study determined that the most effective combination of extraction temperature and ethanol concentration was 65 °C and 70.33 ethanol-to-water ratio at constant time (95 minutes). The combination of process parameters yielded a phenolic content of 13.11 ± 0.05 mg/g GAE and a total flavonoid content of 4.87 ± 0.03 mg/g QE, signifying robust antioxidant activity in *Ipomoea batatas* leaves.

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