Antioxidant Activity of Ultrasonic Assisted Ethanol Extract of *Ainsliaea acerifolia* and Prediction of Antioxidant Activity with Machine Learning

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The antioxidant properties of *Ainsliaea acerifolia*, a wild edible plant, were examined by ultrasonic-assisted ethanol extraction methods. The primary objective was to optimize the extraction conditions and accurately predict antioxidant activities using advanced machine learning models. The extraction conditions were optimized using Response Surface Methodology (RSM). Various parameters, including temperature, extraction time, and ethanol concentration, were adjusted to maximize antioxidant activity. The optimal conditions identified were a temperature of 68 °C, an extraction time of 86 min, and an ethanol concentration of 57%. Under these conditions, the extracts exhibited the highest antioxidant activity. To enhance the predictive accuracy of antioxidant activity, an XGBoost (XGB) model was employed. The XGB model performance was evaluated and compared with the RSM model. The XGB model achieved an R^2 value of 94.71%, significantly outperforming the RSM model by 12.8%. This highlights the superiority of the XGB model in predicting antioxidant activities based on the given extraction parameters. Additionally, the study developed a graphical user interface (GUI). This GUI allows researchers and industry experts to input extraction conditions and obtain quick, accurate predictions of antioxidant activity.

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Keywords: Ainsliaea acerifolia; Antioxidant prediction; Ecofriendly; RSM; Wild edible plant; XGBoost

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INTRODUCTION

Wild edible plants have been used as medicinal resources or food since ancient times (Park *et al.* 2003; Song *et al.* 2014). Wild edible plants have greater value as medicinal resources because generally they contain a larger number of bioactive substances compared to commercially cultivated vegetables (Sánchez-Mata *et al.* 2012). Their abundant bioactive compounds, such as caffeoylquinic acid, resveratrol, and epigallocatechin, are beneficial in inhibiting metabolic diseases such as diabetes, obesity, and hypertension, as well as circulatory diseases (Martínez *et al.* 2004; Anwar *et al.* 2022). Wild edible plants have garnered attention for their functional properties, especially with the growing interest in healthy eating habits (Lee *et al.* 2011). Researchers in Korea have focused on the edible wild plant *Ainsliaea acerifolia*. The functional compounds in *A. acerifolia* are reported to protect against alcohol-induced liver damage and inhibit carbohydrate absorption, thereby aiding in the prevention of diabetes (Lee *et al.* 2015; Lee *et al.* 2020).

Methods to maximize the efficacy of extracts from wild edible plants with various beneficial effects are being considered. As interest in environmental pollution increases, there has been research on eco-friendly solvents and auxiliary methods used in the extraction process without using chemical agents (Mohan *et al.* 2022).

When ancient humans ate or stored plants, hot water treatment (blanching) was commonly used (Lee 1958). While hot water sterilized plants to facilitate storage, the heat of hot water could cause damage to phenolics or loss of antioxidants with the water (Ju *et al.* 2006; Mnich *et al.* 2020). However, hot water could also cause the degradation of plant cell walls, which facilitated the release of phenolic and flavonoid substances (Li *et al.* 2010). It was also reported that heat treatment could remove the sugar portion from the glycosides of flavonoids, resulting in free hydroxy groups, thereby increasing their radical scavenging activity (Wang *et al.* 2019).

Among eco-friendly extraction methods, ultrasound-assisted extraction generated cavitation bubbles have been shown to peel and erode the surface of wild edible plants, accelerating the exposure of new surfaces, allowing the extraction solvent to effectively penetrate the wild edible plants (Proestos and Komaitis 2006; Yusoff *et al.* 2022). Effective solvent penetration reduces extraction time and increases extraction efficiency (Tiwari 2015). Additionally, it is possible to extract using eco-friendly solvents without having to rely on hazardous chemicals (More *et al.* 2022). For rosemary, ultrasound-assisted ethanol extraction was found to be more effective than ethyl acetate extraction (Vilkhu *et al.* 2008). This indicated that ultrasound treatment could reduce dependence on solvents and provide benefits for the environment and health.

Another issue with solvent extracts is finding the appropriate conditions (dependent variables) that can maximize the target components or efficacy. Many researchers use response surface methodology (RSM) to optimize dependent variables through quantitative manipulated variables in order to optimize the efficacy of the extracts (Rodrigues *et al.* 2008; Xu *et al.* 2015; Tomšik *et al.* 2017). Meanwhile, machine learning (ML) models have recently gained traction for their potential use in biotechnology, biopharmaceuticals, and drug evaluation. ML can learn from pre-collected data, identify patterns, and predict outcomes (Mahesh 2020). In several studies, ML has demonstrated superior predictive performance compared to the quadratic equations of RSM. Particularly, ML can find trends and make predictions even with limited information about the correlations between variables. Therefore, the limitations of RSM, which requires correlations between dependent variables for accurate predictions, can be overcome by ML. Jan and Sit successfully optimized the supercritical fluid extraction parameters of *Terminalia chebula* using ML and RSM, achieving a high coefficient of determination (R^2=0.9973) in their predictions (Jha and Sit 2021). When comparing RSM and ML models for the recovery of artemisinin, a precursor of antimalarial drugs, from *Artemisia annua*, the predictive performance of the ML model was superior (Pilkington *et al.* 2014).

The XGB (eXtreme Gradient Boosting) model, which is one of the ML models, boosts (sequentially trains and assigns weights to incorrect predictions in the next model) multiple decision tree models and arranges the trained decision tree models linearly. By applying parallel learning to the existing boosting model, faster computing is possible. Regularization is applied to prevent overfitting of the model, providing higher predictive (Mitchell and Frank 2017).

Ultrasound-assisted extraction of *A. acerifolia* was adopted to enhance the antioxidant activity of the extract. There is still a lack of research on optimizing the extraction temperature, time, and solvent concentration in ultrasound-assisted extraction. To the best of the authors' knowledge, this is the first report combining RSM and XGB to

identify the optimal extraction conditions for achieving maximum antioxidant activity in *A. acerifolia* extracts and to predict the antioxidant activity. This study aimed to find the conditions that yield maximum antioxidant activity through RSM and to predict the antioxidant activity under various extraction conditions using the XGB model. Additionally, a graphical user interface (GUI) application was developed using the trained XGB model. This application allows for accurate predictions, saving time and resources compared to traditional experiments (Hamza *et al.* 2024).

EXPERIMENTAL

Material

Ainsliaea acerifolia was procured from a natural farm in Yeoju, Gyeonggi Province. The purchased *A. acerifolia* was transplanted into pots with dimensions of 43 cm (width) × 36 cm (length) × 14 cm (height) and cultivated at the academic farm of Gyeongsang National University, with watering every 12 h. Only the leaves of the cultivated *A. acerifolia* were harvested and subjected to hot water pre-treatment.

Hot Water Pretreatment

The hot water pretreatment conditions were adopted from those with the highest ABTS scavenging activity in Table A1. Pre-treatment of *A. acerifolia* leaves involved placing the leaves and distilled water in a 1:20 (w:v) ratio into a 300 mL flask. The flask containing the sample was sealed and pre-treated in an autoclave (ST-65G, JEIO Tech, Korea) at 80 °C for 90 min. The pretreated *A. acerifolia* was then filtered by gravity using Whatman filter paper No. 2. After filtration, the residue was cooled using a deep freezer and then freeze-dried at –80 °C (FDA8505, IlShin Lab Co. Ltd, Korea). The lyophilized samples were ground into powder using a commercial pulverizer and stored at –20 °C.

Ultra-Sonic Assisted Extraction

The powdered samples were subjected to various temperatures, times, and ethanol concentrations according to the Box-Behnken design (BBD) conditions. The powdered samples were placed in Falcon tubes with different extraction solvent concentrations and subjected to ultrasonic extraction using an ultrasonic bath (JAC-2010, JINWOO, Korea) operating at a frequency of 40 kHz and a maximum input power of 300 W at room temperature. After the extraction was completed, the samples were centrifuged at 4,000 rpm for 10 min. The supernatant was then separated and stored under refrigeration.

Box-Behnken Experimental Design

In this study, a BBD experimental design with three factors and three levels was adopted to optimize the ABTS antioxidant activity of *A. acerifolia* extracts *via* ultrasound-assisted extraction. The selected independent variables and their coded levels within the respective ranges are presented in Table 1. The data analysis was performed using the statistical software Design-Expert (Version 13, State-Ease Inc., USA).

Table 1. Three-factorial Box-Behnken Design

Variable	Symbol	Coding Level		
		-1	0	1
Temperature (°C)	X_1	40	60	80
Time (min)	X_2	60	90	120
EtOH concentration (%)	X_3	50	75	100

ABTS Radical Scavenging Activity

The ABTS, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) radical scavenging activity of the extracts was analyzed following the method described by Lee *et al.* (2016). The ABTS reaction solution was prepared by mixing 7 mM ABTS and 2.45 mM potassium persulfate, allowing the mixture to react in the dark at room temperature for 24 h, and then diluting it with methanol to achieve an absorbance of 0.7 ± 0.02 at 735 nm. To 190 µL of the prepared ABTS reaction solution, 10 µL of the ultrasound-assisted *A. acerifolia* extract (1 g/50 mL methanol) was added and allowed to react at room temperature for 6 min. The absorbance was measured at 517 nm using a UV-spectrophotometer (SpectraMax 190, Molecular device, USA), and 98% ethanol was used as a control. The ABTS radical scavenging activity (ABTS) was calculated using Eq. 1,

$$\text{ABTS (\%)} = 1 - \frac{Abs\ Sample}{Abs\ Control} \times 100 \quad (1)$$

where *Abs Sample* is the *A. acerifolia* extract and *Abs Control* is ethanol (98%).

Gradient Tree Boosting algorithm (XGB)

The XGB algorithm generates and trains new decision trees by utilizing the residuals of previous decision trees to address their shortcomings. During the learning process, this method linearly combines the previous decision trees to achieve higher accuracy, functioning as a supervised learning algorithm. To prevent overfitting during training, regularized model formalization was employed, enhancing overall accuracy. This approach prunes the nodes of decision trees that contribute minimally to the final prediction, thereby reducing the overall size of the decision trees. Figure 1 presents a schematic diagram of the computational process of the XGB model. The dataset was randomly split into two subsets, with 70% used for training and 30% for testing. The implementation was carried out using the XGBoost library in Python (version 3.10.14). The training of the model aimed to minimize the mean squared error (Eq. 2), calculated as follows,

$$\frac{1}{n}\sum_{i=1}^{n}(y_i^* - \tilde{y}_i^*)^2 \quad (2)$$

where MSE is mean squared error, y_i is the correct answer of the i-th training data, and $\tilde{y}_i$ is the predicted value for the i-th training data.

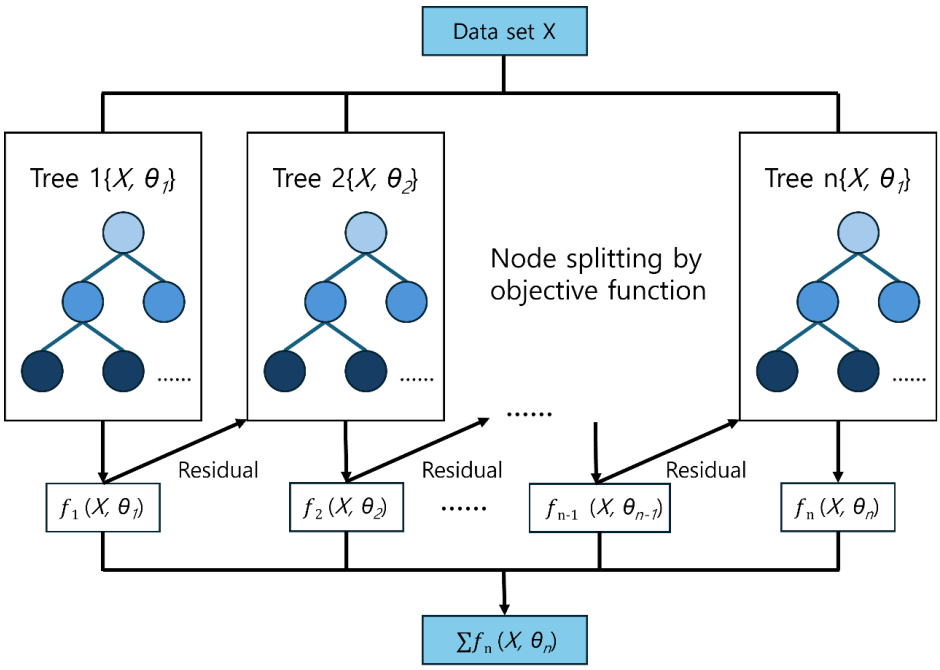

Fig. 1. Schematic diagram of the XGB model

Graphical User Interface (GUI)

A graphical user interface (GUI) was developed using the PyQt5 (version 5.15.10) library in Python (version 3.10.14), leveraging the optimal structure of the XGB model. A GUI displays the status of a computer program on a monitor, making it easy to interact with the device *via* mouse pointer and keyboard. The visual application allows users to quickly receive accurate predictions from trained models by adjusting conditions using buttons. The GUI supports researchers and developers in the field of predictive analytics by providing visualizations and insights into model performance.

RESULTS AND DISCUSSION

Antioxidant Activity of *A. acerifolia* Leaf Extracts using BBD

The *A. acerifolia* antioxidant activity was highest at 77.7% with hot water pretreatment 80 °C, 90 min, leaf and distilled water 1:20 (w:v). These conditions were adopted for ultrasonic extraction (Table A1 in the Appendix). The Box-Behnken Design (BBD) was used to set the factors (temperature, time, ethanol concentration) to achieve the desired ABTS antioxidant activity values of *A. acerifolia* leaf extracts. As presented in Table 2, 27 experimental runs were conducted with three factors and three levels. The predicted coefficient of determination (R^2) indicates that the model showed good fit, explaining 81.96% of the response variability. Equation 3 represents the quadratic polynomial model describing the BBD data,

$$\text{ABTS radical scavenging activity (\%)} = -366.64533 + 8.38361A + 1.59592B + 2.25303C - 0.0098AB - 0.013952AC + 0.006218BC - 0.044167A^2 - 0.007405B^2 - 0.010958C^2 \quad (3)$$

where A is temperature (°C), B is time (h), and C is EtOH concentration (%).

Table 2. Experimental and Predicted Values of ABTS Radical Scavenging Activity from Various Machine Learning Models

	Variables			Experimental	Prediction	
No	Temperature (°C)	Time (min)	EtOH (%)	ABTS	RSM	XGB
1	60	120	75	90.57±0.11	93.05	90.57
2	60	120	50	87.31±0.61	78.79	87.31
3	60	90	100	94.61±0.16	101.06	94.60
4	80	120	100	91.19±0.93	87.57	91.19
5	40	60	75	26.81±2.65	33.19	26.81
6	40	90	75	68.33±3.80	48.04	68.33
7	40	90	50	20.62±7.14	27.94	20.62
8	40	60	50	18.17±1.05	15.84	18.17
9	60	120	100	92.86±0.58	101.55	92.86
10	80	90	75	90.24±0.17	95.30	90.24
11	40	90	100	72.80±1.35	62.38	72.80
12	80	120	75	90.81±0.43	87.67	90.81
13	80	90	50	86.32±0.48	92.39	86.32
14	80	60	100	91.38±0.64	85.53	91.38
15	80	120	50	85.55±2.81	82.01	85.55
16	80	60	50	87.95±0.63	91.01	87.94
17	40	120	75	44.28±14.50	51.12	44.28
18	60	60	75	90.01±0.25	85.82	90.01
19	60	90	50	85.60±0.90	83.82	85.60
20	80	60	75	90.54±0.36	91.15	90.54
21	40	120	100	72.84±2.96	68.23	72.84
22	40	120	50	22.85±6.13	28.26	20.62
23	60	90	75	89.07±1.05	95.32	89.67
24	60	60	100	92.50±0.51	88.80	94.60
25	80	90	100	91.10±0.69	92.44	91.19
26	40	60	100	33.07±1.41	44.77	48.40
27	60	60	50	82.76±4.27	77.08	87.46

BBD was utilized to set the factors (temperature, time, ethanol concentration) to achieve the desired ABTS antioxidant activity values of *A. acerifolia* leaf extracts. A, B, and C represent extraction temperature, extraction time, and extraction solvent concentration, respectively. AB, AC, and BC denote the interactions between factors, with BC reflecting the interaction between extraction time and extraction solvent concentration.

Table 3. ANOVA for RSM Model

Source	Sum of Squares	df	Mean Square	F-Value	p-Value	
Model	16559.07	9	1839.9	24.06	< 0.0001	Significant
A[1)]	10048.84	1	10048.84	131.43	< 0.0001	
B[2)]	235.13	1	235.13	3.08	0.0975	
C[3)]	1338.33	1	1338.33	17.5	0.0006	
AB	343.96	1	343.96	4.5	0.0489	
AC	887.67	1	887.67	11.61	0.0034	
BC	91.41	1	91.41	1.2	0.2895	

A^2	3356.19	1	3356.19	43.9	< 0.0001	
B^2	207.77	1	207.77	2.72	0.1176	
C^2	49.77	1	49.77	0.651	0.4309	
Residual	1299.8	17	76.46	1	1	1
Corrected Total	17858.88	26				

Note: [1)] Temperature (°C); [2)] Time (h); [3)] EtOH concentration (%)

To assess the statistical significance of the proposed model, an analysis of variance (ANOVA) was performed. The model was found to be significant with a high F-value (24.06) and a low p-value (<0.0001). As shown in Table 3, extraction temperature (A) had a substantial impact ($p<0.0001$), followed by extraction solvent concentration (C) ($p<0.0006$). This suggests that extraction temperature and solvent concentration significantly influenced the nutrient availability from the plant's leaf cell walls. Increasing the temperature enhanced the solubility of polyphenols, promoting their release. However, exceeding the optimal temperature may lead to the degradation or coagulation of polyphenols along with proteins (García-Márquez *et al.* 2012). The 3D response surface graph can be used to observe the interactions between independent variables. The elliptical contour graph shown in Fig. 2 reflects the importance of the interaction of temperature, time, and solvent focus on antioxidant activity. One factor is fixed at an optimal value while the other two factors are changed within the experimental range. The main purpose of the RSM is to effectively detect the optimal extraction conditions to maximize antioxidant activity. Each contour curve represents a random combination of values within the range of the two variables.

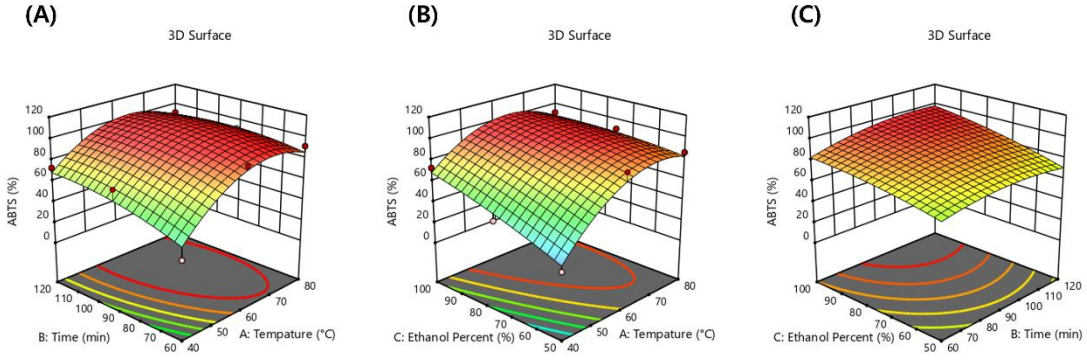

Fig. 2. 3D response surface plots for the ABTS antioxidant activity of the ethanol extract of *A. acerifolia* are shown based on the effects of (A) temperature, (B) time, and (C) solvent concentration

Figure 2(B) shows the interaction between temperature and solvent concentration when time is kept constant at 120 min. The surface plots of the interaction conditions match well with the interaction conditions in Table 3 of the analysis of variance. The maximum antioxidant activity of 96.3% was achieved at an extraction temperature of 68 °C, extraction time of 86 min, and 57% ethanol concentration. The hot water pretreatment samples showed about a 20% increase in antioxidant activity after ultrasonic extraction.

Gradient Tree Boosting algorithm modeling

The experimental data on the antioxidant activity of ultrasound extracts from pretreated *A. acerifolia* under various conditions were used to train and test the XGB model.

The XGB framework consisted of three variables (extraction temperature, extraction time, extraction solvent concentration) and one outcome (ABTS scavenging activity). A high R^2 value serves as an indicator of the model's performance. The R^2 values for the training and testing data are shown in Fig. 3. The model structure consisted of 100 decision trees. The R^2 value for the training data was 0.9999, while the R^2 value for the testing data was 0.9471, indicating a prediction accuracy of 94.71%. Additionally, the MSE value was 6.66, showing a low prediction error. The XGB model demonstrated a 12.75% increase in prediction accuracy compared to the quadratic equation-based predictions of the previous RSM model. This indicates that the machine learning (XGB) model outperformed the quadratic equation model of RSM.

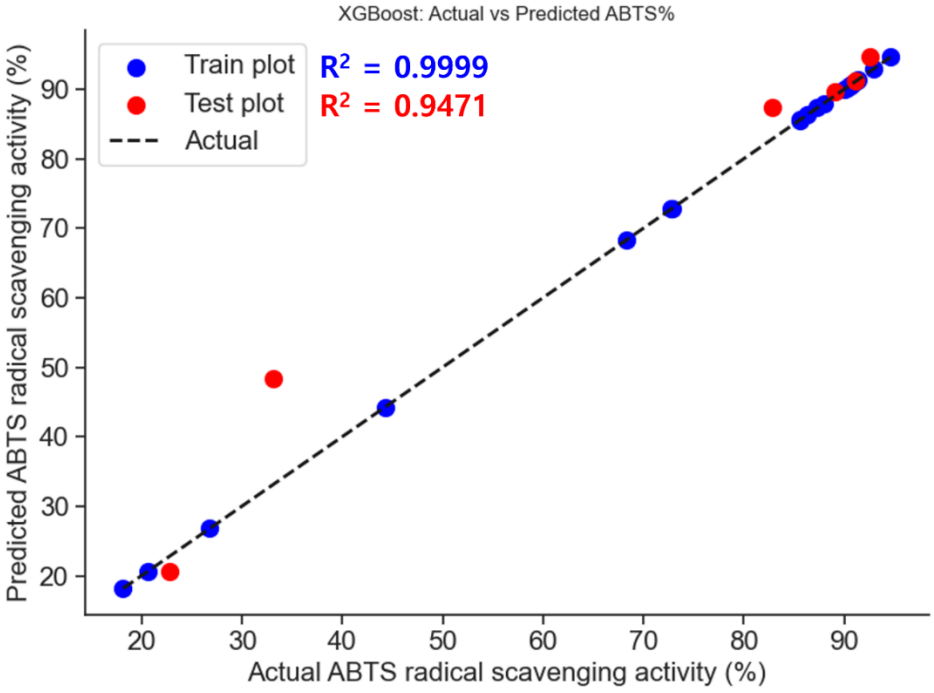

Fig. 3. Prediction plots of train data and test data for the XGB model

Comparison of RSM and XGB Results

The performance of RSM and XGB were compared based on the predicted results. The antioxidant activity data of the leaf extracts were used to optimize and predict the outputs with the XGB model. The XGB network of the developed model learns from the interactions between antioxidant activities of the extracts with changes in temperature, time, and solvent concentration. XGB outperformed RSM and had higher prediction accuracy. XGB's superior prediction performance stems from its data processing characteristics. RSM models are typically used for numerical optimization of each variable, but they can only create quadratic regression models, which limits their predictive capabilities. In contrast, the XGB model can capture and learn the nonlinear features of process variables in each tree, making it applicable across various fields. The predicted values of antioxidant activity for *A. acerifolia* leaf extracts using RSM and XGB are compared in Fig. 4. The MSE values for the RSM regression prediction and the XGB prediction are 1.78 and 0.36, respectively. Thus, XGB efficiently optimized and predicted the antioxidant activity of the extracts as a function of temperature, time, and solvent concentration. However, XGB models are limited to making predictions within the datasets of models trained under the

same conditions. To achieve higher predictive accuracy and wider application of the model, future researchers will need to unify the training conditions and increase the training data.

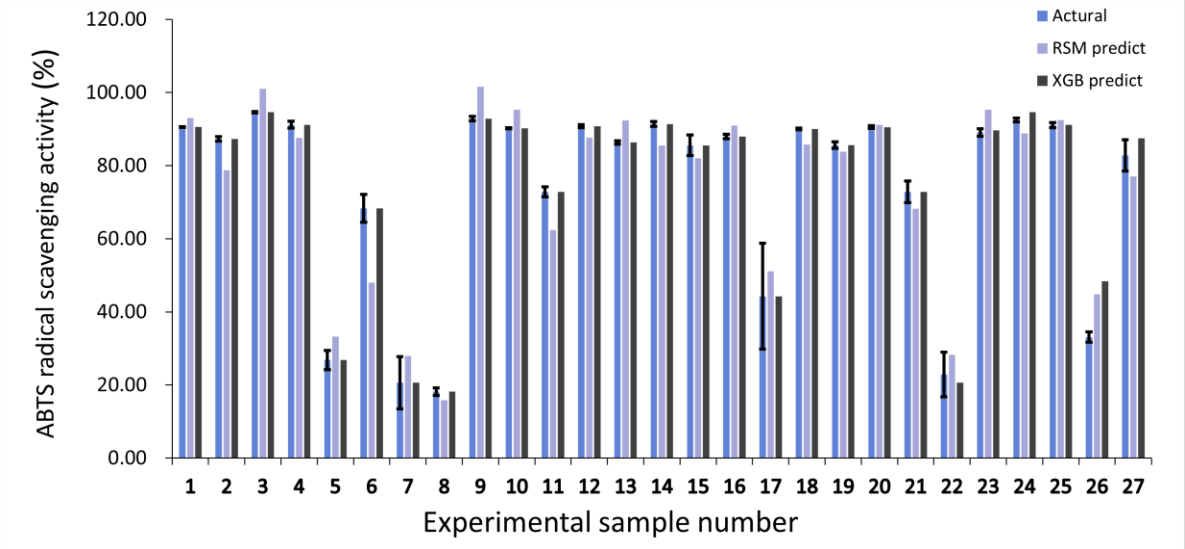

Fig. 4. Comparison of actual ABTS antioxidant activity data with predicted data from RSM and XGB

SHapley Additive exPlanations Values and Pearson Correlation Analysis

SHapley Additive exPlanations (SHAP) calculates the contribution of each feature to the prediction, thereby explaining the predicted value. SHAP makes it possible to understand the most influential features in the model. Figure 5 (A) illustrates the SHAP values based on extraction temperature, time, and solvent concentration. Temperature showed the greatest contribution to the prediction of the antioxidant activity of the extracts. As the temperature increased, the predicted value also increased, indicating a positive correlation between temperature and antioxidant activity. Time and solvent concentration also demonstrated positive correlations. The Pearson correlation coefficient for the temperature variable was observed to be the highest at 0.7 (Fig. 5 (B)). This result aligns with the earlier SHAP values, showing the relationship between changes in input feature values and their corresponding predicted values. With this understanding, researchers can thoroughly explore the features that influence the outcome of the whole experiment.

Development of a Graphical User Interface

A user-friendly Graphical User Interface (GUI) was developed to predict the antioxidant activity of extracts. Figure 6 shows a schematic of the GUI. In the GUI, users can input extraction factors such as extraction temperature, time, and solvent concentration to predict the antioxidant activity of the extract. The GUI successfully made predictions in less than 0.4 seconds. The high prediction accuracy of the XGB model allows the tool to make precise predictions. This framework can simplify the analysis and decision-making process for researchers and industry experts.

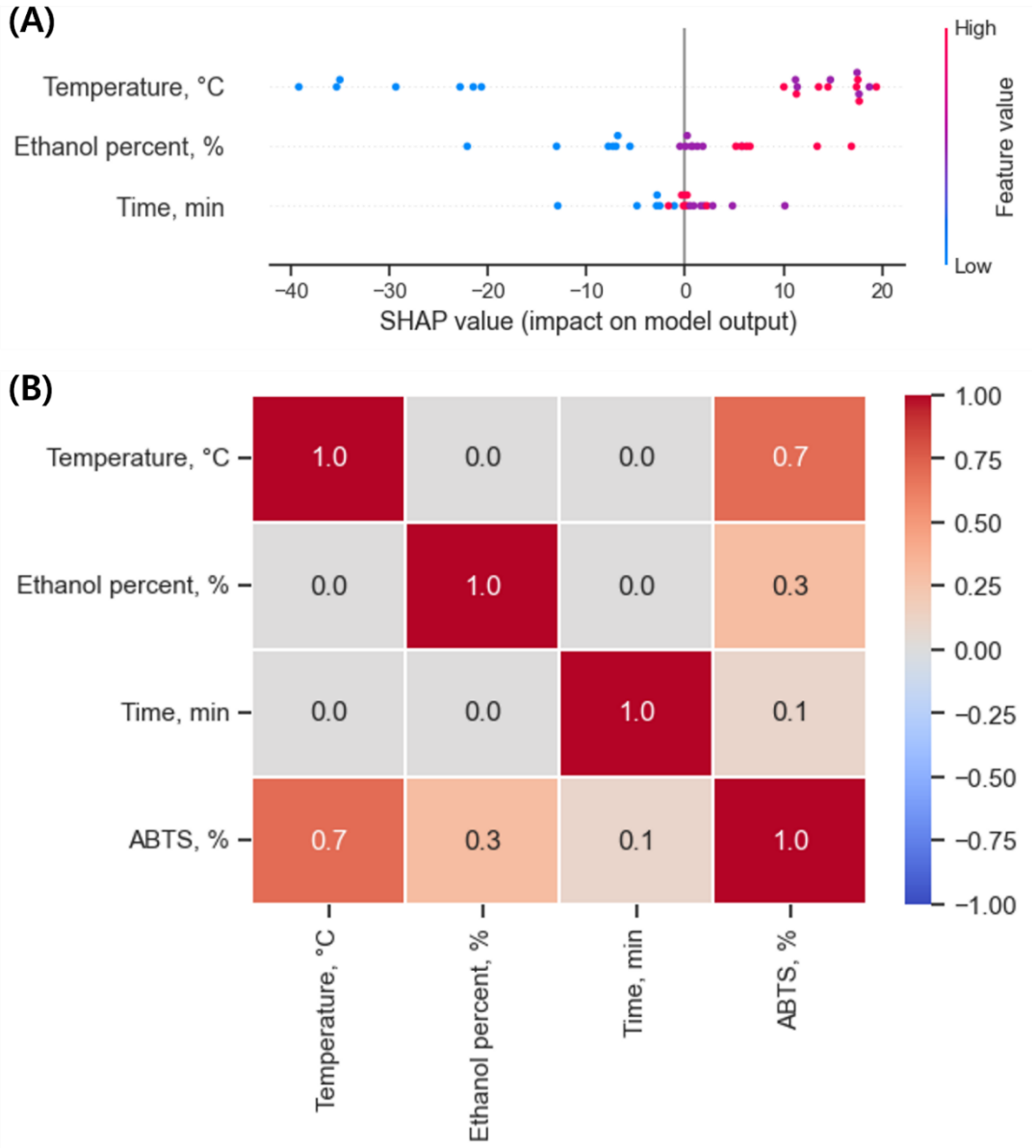

Fig. 5. Plot the SHAP values (A) of the XGB model and the Pearson correlation plot (B) of the experimental data

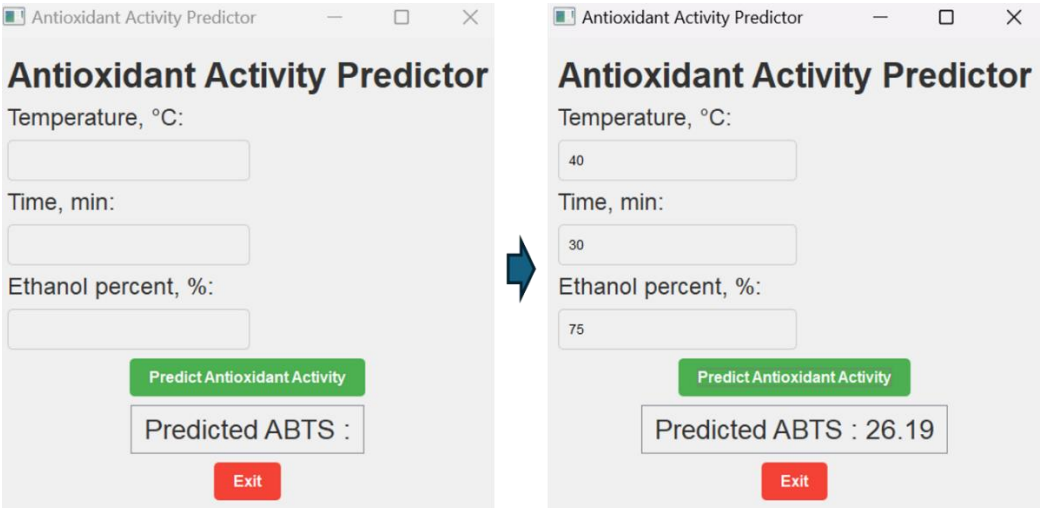

Fig. 6. Schematic illustration of GUI

CONCLUSIONS

1. The conditions for maximizing the ABTS radical scavenging activity of extracts from *A. acerifolia* were identified through Response Surface Methodology (RSM) after hot water pre-treatment and ultrasonic extraction. The antioxidant activity was then predicted using the XGB model, and the predictions were compared with those of the RSM model.
2. In the RSM, the maximum antioxidant activity of the extract was 96.3% at an extraction temperature of 68 °C, extraction time of 86 min, and 57% ethanol concentration. ANOVA analysis indicated that extraction temperature and solvent concentration were the variables with a strong influence on antioxidant activity.
3. When the XGB model was trained with the previously measured data and used to predict antioxidant activity, it achieved an R^2 value of 94.71%, which was 12.8% higher than that of the RSM model. A comparative analysis of the SHAP values of the XGB model and the Pearson correlation of the training data indicated that temperature was the most influential variable, showing consistency between the two methods.
4. The present results showed that the RSM model could identify the optimal extraction conditions to maximize antioxidant activity, and the XGB model that was trained could be used as a graphical user interface (GUI) program to make highly accurate antioxidant activity predictions. However, for researchers to utilize the model, it will need to be trained under the same conditions. The challenge remains to secure a wide range of data and validate various training conditions to improve the prediction accuracy and broaden the scope of the model.

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Data Availability

All datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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APPENDIX

Table A1. ABTS Radical Scavenging Activity of *A. acerifolia* Extract by Hot Water Pretreatment Conditions

Variables			ABTS
Temperature (°C)	Time (min)	Leaf: Distilled Water (w:v)	
Non treated			8.49±0.47
80	15	1:10	15.09±1.14
80	15	1:20	13.82±0
80	15	1:30	13.82±0
80	30	1:10	34.13±2.32
80	30	1:20	35.96±2.39
80	30	1:30	36.41±2.43
80	60	1:10	45.75±1.95
80	60	1:20	49.95±0.41
80	60	1:30	45.84±3.14
80	90	1:10	61.21±0.01
80	90	1:20	77.79±0.8
80	90	1:30	76.38±2.91
80	120	1:10	17.88±2.78
80	120	1:20	16.09±0.01
80	120	1:30	16.09±0.01
90	15	1:10	37.95±0.68
90	15	1:20	26.78±2.24
90	15	1:30	19.42±3.04
90	30	1:10	17.67±2.49
90	30	1:20	33.07±1.41
90	30	1:30	26.81±2.65
90	60	1:10	18.17±1.05
90	60	1:20	17.6±0.52
90	60	1:30	12.8±1.35
90	90	1:10	68.33±3.8
90	90	1:20	20.62±7.14
90	90	1:30	16.84±3.07
90	120	1:10	42.84±2.96
90	120	1:20	44.28±14.5
90	120	1:30	22.85±6.13
100	15	1:10	19.53±3.15

100	15	1:20	64.2±0.55
100	15	1:30	60.87±1.63
100	30	1:10	56.45±1.58
100	30	1:20	45.82±3.29
100	30	1:30	42.5±0.51
100	60	1:10	40.01±0.25
100	60	1:20	42.76±4.27
100	60	1:30	46.1±2.51
100	90	1:10	34.61±0.16
100	90	1:20	39.07±1.05
100	90	1:30	35.6±0.9
100	120	1:10	36.73±1.6
100	120	1:20	42.86±0.58
100	120	1:30	40.57±0.11
110	15	1:10	37.31±0.61
110	15	1:20	49.02±0.93
110	15	1:30	42.69±0.71
110	30	1:10	41.12±0.43
110	30	1:20	39.32±0.61
110	30	1:30	40±0.86
110	60	1:10	41.38±0.64
110	60	1:20	40.54±0.36
110	60	1:30	37.95±0.63
110	90	1:10	39.85±1.47
110	90	1:20	41.1±0.69
110	90	1:30	40.24±0.17
110	120	1:10	36.32±0.48
110	120	1:20	37.98±1.92
110	120	1:30	41.19±0.93