Beni-Suef University Journal of Basic and Applied Sciences xxx (xxxx) xxx–xxx

HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Beni-Suef University
Journal of Basic and Applied Sciences

journal homepage: www.elsevier.com/locate/bjbas

Efficient extraction of antioxidants from *Vernonia cinerea* leaves: Comparing response surface methodology and artificial neural network

Oluwaseun Ruth Alara*, Nour Hamid Abdurahman, Haruna Kolawole Afolabi, Olusegun Abayomi Olalere

Faculty of Chemical Engineering & Natural Resources, Universiti Malaysia Pahang, Lebuhraya Tun Razak, 26300 Gambang, Pahang, Malaysia

ARTICLE INFO

Keywords:
Extraction yield
Vernonia cinerea
Artificial neural network
Antioxidants
Response surface methodology

ABSTRACT

Despite response surface methodology (RSM) has been the most preferred statistical tool for optimizing extraction processes, artificial neural network (ANN) has been one of the most effective tools used for optimization and empirical modelling since the last two decades, most especially for non-linear equations. Thus, this study was carried out to compare the performance of RSM and ANN in optimizing the extraction yield and antioxidant capability of extract from *Vernonia cinerea* leaves using microwave-assisted extraction (MAE) techniques. The responses (extraction yield and antioxidant capabilities) were modelled and optimized as functions of four independent MAE parameters (irradiation time, microwave power level, ethanol concentration, and feed-to-solvent ratio) using RSM and ANN. The coefficient of determination (R^2), root mean square error (RMSE) and absolute average deviation (AAD) were employed to compare the performance of both modelling tools. ANN model has a higher predictive potential compared to RSM model with higher correlation coefficients of 0.9912, 0.9928 and 0.9944 for extraction yield, DPPH and ABTS scavenging activities, respectively. Thus, ANN model could be a better alternative in data fitting for the MAE of antioxidants from *Vernonia cinerea* leaves.

1. Introduction

Traditionally, plants are being used in treating different infectious diseases. There has been a shift from the production of synthetic drugs in treating different ailments to the use of herbal drugs because synthetic drugs have been reported having a side effect in human body. In fact, World Health Organization has suggested the use of natural-occurring products from plants in tackling most diseases (Qi, 2015). Recently, interest in the discovery of natural antioxidant has grown because most infectious diseases like cancer, diabetes and cardiovascular disorders are related to free radical cells. Likewise, the clinical and epidemical shred of evidence has suggested that frequent consumption of fruits and vegetables reduce the threat of suffering chronic diseases. In another way, consuming the phytochemicals from plants are quite safer than synthetic ones (Lahlou, 2013).

Vernonia cinerea (Asteraceae) is one the herbaceous plants mostly found in Africa, India and Asia. This plant is commonly referred to as little ironweed which is mostly grown in an open waste area, roadside and dry grassy surrounding. Traditionally, this plant has been used as medicine to treat ailments like skin diseases, cough, bronchitis, asthma, malaria, cancer, gastrointestinal disorder, diuresis, pains, and diabetes (Prasopthum et al., 2015; Youn et al., 2014). The presence of several bioactive compounds such as flavonoids and glycosides, phenolic compounds, terpenoids, sesquiterpenes, amyrin, and stigmasterols in the extracts of *Vernonia cinerea* leaf made them possess different pharmacological properties, which include anti-inflammatory, analgesic, anthelmintic, and antioxidant. The juice from *Vernonia cinerea* leaf is being given to children suffering from a urinary infection. More so, consumption of the extracts had been reported to have no adverse effect. In fact, the plant is being consumed as herbal tea for cigarette withdrawal smokers (Inpuron et al., 2013; Prasopthum et al., 2015). It has been reported that *Vernonia cinerea* leaf extract has strong anti-inflammatory and antioxidant properties. In addition, the extracts showed a protective role against DNA damage and radiation-induced oxidative stress (Prasopthum et al., 2015). Although studies had been carried out on its antioxidant activity using conventional extraction methods like Soxhlet and maceration, whereas no literature has reported the optimization of the MAE of extraction yields and the antioxidant activities (DPPH and ABTS) of *Vernonia cinerea* leaf extract comparing RSM and ANN.

Microwave-assisted extraction technique is one of the novel methods that can improve efficiency, quality yields of extract in shorter extraction time and reduced solvent consumption (Dahmoune et al., 2014). It is one of the dominant trends of green chemistry movement.

* Corresponding author.
E-mail address: ruthoalao@gmail.com (O.R. Alara).

https://doi.org/10.1016/j.bjbas.2018.03.007
Received 5 February 2018; Accepted 17 March 2018

Please cite this article as: Alara, O.R., Beni-Suef University Journal of Basic and Applied Sciences (2018), https://doi.org/10.1016/j.bjbas.2018.03.007

This method has been used to extract antioxidants from *Pistacia lentiscus* leaves in comparison with other methods (ultrasound-assisted and conventional solvent extraction), the results showed that MAE gave higher yields of antioxidant capacity (Dahmoune et al., 2014). The following conditions can affect the performance of MAE, viz, irradiation time, extraction temperature, microwave power, solvent/feed, and solvent concentration, thus, there is a need to optimize these parameters.

Response surface methodology (RSM) is a mathematical and statistical tool used in studying the effects of individual input variables on the output responses. This tool is used for building and designing experimental models, evaluating the significance of each input variable, and determining the optimal conditions for predicting response (Huang et al., 2017). Recently, artificial neural network is being used as an alternative to RSM for modelling technological process. ANN is a nonlinear computational models tool used in food analysis for solving engineering problems. Its principle is based on establishing a nonlinear mapping between dependent and independent variables that require no prior knowledge of the correlation between targeted responses. This principle has been likened to a human brain in two ways: training process is used for the network in receiving knowledge from an environment and interneuron connections are used in storing the acquired knowledge (Ameer et al., 2017). In addition, ANN has higher efficiency, accuracy and flexibility in experimental data fitting, prediction and modelling of non-linear correlation when compared with RSM (Huang et al., 2017; Sinha et al., 2012).

The main objective of this study is to model and optimize the MAE conditions, which include microwave power, irradiation time, feed/solvent ratio, and solvent concentration for the recovery yield and antioxidant activities of *Vernonia cinerea* leaf using RSM and ANN. The prediction efficiencies of both optimization tools were compared using the coefficient of determination, root mean square error and absolute average deviation.

2. Materials and methods

2.1. Plant and chemicals procurement

Vernonia cinerea leaves used in this study were procured fresh from Universiti Malaysia Pahang surrounding. The leaves were washed with tap water to remove any adhering dust and air dried until constant weight was attained. The moisture content of 0.01 ± 0.04 g water/g dry sample was recorded. The dried leaves were then finely ground to powder and stored in a polyethene bag before extraction process.

Ethanol, methanol, 2,2-diphenyl-picrylhydrazyl (DPPH), potassium persulfate, and 2,2′-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid ($ABTS^{+\cdot}$) were purchased from Sigma-Aldrich Sdn Bhd Selangor, Malaysia. Distilled water was collected from Faculty of Chemical Engineering and Natural Resources laboratory.

2.2. Microwave-assisted extraction process

The microwave-assisted extraction was carried out in an enclosed ethos reflux microwave extractor (maximum power capacity of 1000 W and frequency of 2450 MHz Milestone, Italy). A 10 g of powdered sample was extracted using ethanol as an extracting solvent based on the experimental design matrix. The effects of independent factors on the extraction yield and antioxidant capacities of *Vernonia cinerea* leaves were evaluated at different irradiation time interval (1–5 min), microwave power level (500–700 W), feed-to-solvent ratio (1:18–1:14 g/ml), ethanol concentration of 20 to 60%, and at constant temperature of 80 °C (Table 1). All the extracts were filtered and concentrated to dryness using a rotary evaporator (Buchi Rotavapor R-200 coupled with Buchi Vac V-500 pump, Switzerland). The extraction yield of *Vernonia cinerea* leaf was then determined using Eq. (1).

$$\%\ Yield\ of\ extracts = \frac{Weight\ of\ extracts\ (w)}{Weight\ of\ dried\ plant\ powder\ (w)} * 100\% \tag{1}$$

2.3. Antioxidant capacities of Vernonia cinerea leaf extract

2.3.1. DPPH scavenging capacity

The antioxidant capacity of *Vernonia cinerea* leaf extract was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to the method described by Alara et al. (2017). Briefly, 0.2 ml of the extract was added to 2 ml of 0.1 mM DPPH solution, the absorbance at 517 nm was recorded after 30 min of leaving the mixture to incubate in the dark at room temperature. The percentage of antioxidant activity was calculated using Eq. (2). The analyses were carried out in triplicate and the mean values were recorded. Methanol was used as a blank.

$$\%\ DPPH\ scavenging\ activity = \frac{A_c - A_s}{A_c} * 100\% \tag{2}$$

where A_c represents the absorbance of control (a mixture of DPPH solution and methanol) and A_s is the absorbance of the mixture of *Vernonia cinerea* leaf extract and DPPH solution.

2.3.2. $ABTS^{+\cdot}$ scavenging capacity

The scavenging activity of the *Vernonia cinerea* leaf extract on $ABTS^{+\cdot}$ was carried out according to the method previously described by Alara et al. (2017). In brief, the stock solution of 2.45 mM potassium persulfate and 7 mM $ABTS^{+\cdot}$ solutions in equal volumes were allowed to incubate for 12 h in the dark at room temperature. Prior to the analysis, fresh working solution of ABTS was diluted in methanol to obtain an absorbance value of 1.1 ± 0.02 at 734 nm. A 2850 µl of $ABTS^{+\cdot}$ solution was then added to 1.5 ml of plant extract and absorbance of the mixture was measured at 734 nm using UV–VIS Spectrophotometer (Hitachi U-1800, Japan) after 120 min of leaving it to incubate in dark at room temperature. The percentage of antioxidant activity was calculated using Eq. (3). The analyses were carried out in triplicate and the mean values were recorded. Methanol was used as a blank.

$$\%\ ABTS^{+\cdot}\ scavenging\ activity = \frac{A_c - A_s}{A_c} * 100\% \tag{3}$$

where A_c represents the absorbance of control (a mixture of ABTS solution and methanol) and A_s is the absorbance of the mixture of *Vernonia cinerea* leaf extract and $ABTS^{+\cdot}$ solution.

2.4. Experimental design

2.4.1. Screening study and response surface methodology modelling

A number of factors including irradiation time, microwave power, solvent concentration, and solute-to-solvent ratio can significantly influence the *Vernonia cinerea* leaf extraction yields and its antioxidant capacities. Thus, Response surface methodology was employed to evaluate the optimized conditions and relationship between the dependent responses (extraction yield and antioxidant capacities) and independent factors (irradiation time, microwave power, feed-to-solvent ratio, and ethanol concentration) on the extraction of *Vernonia cinerea* leaf. Face-centered central composite design (FCCCD) in RSM was applied to develop a quadratic model for describing the extraction process. The ranges and coded levels (−1, 0, +1) for the factors investigated were irradiation time (1–5 min), microwave power level (500–700 W), feed-to-solvent ratio (1:18–1:14 g/ml), and ethanol concentration (60–80%). The response factor, Y (extraction yield, % w/w and antioxidant capacities using DPPH and ABTS, %) can be expressed as a function of the independent factors using a response surface quadratic model (Eq. (4)).

Table 1
Experimental design matrix for MAE of *Vernonia cinerea* leaf using FCCCD.

Std	X_1	X_2	X_3	X_4	Observed values (experimental)			Predicted values					
								RSM			ANN		
					Yield (% w/w)	Antioxidant (%)		Yield (% w/w)	Antioxidant (%)		Yield (% w/w)	Antioxidant (%)	
						DPPH	ABTS		DPPH	ABTS		DPPH	ABTS
1	1	400	1:18	20	17.12	65.67	69.32	16.26	63.98	67.85	17.43	67.66	69.57
2	5	400	1:18	20	17.19	65.42	69.01	16.92	65.86	69.44	17.42	68.00	68.14
3	1	600	1:18	20	17.07	65.16	68.87	17.20	66.79	70.39	17.43	67.66	68.70
4	5	600	1:18	20	16.70	66.14	68.82	16.57	65.90	69.41	16.58	68.00	68.14
5	1	400	1:10	20	13.01	66.05	69.60	13.22	66.45	69.85	13.28	68.76	68.55
6	5	400	1:10	20	12.59	64.96	68.65	12.82	65.27	68.99	11.59	67.41	68.00
7	1	600	1:10	20	13.84	68.39	71.76	13.45	67.21	70.71	13.49	69.62	71.16
8	5	600	1:10	20	12.12	63.62	67.56	11.76	63.26	67.29	11.59	65.69	67.13
9	1	400	1:18	60	18.70	80.97	82.62	19.25	82.25	83.83	18.48	81.35	80.88
10	5	400	1:18	60	18.76	80.78	82.62	18.80	80.98	82.88	18.47	81.70	79.45
11	1	600	1:18	60	16.61	75.26	77.84	16.03	73.97	76.72	16.18	75.36	75.66
12	5	600	1:18	60	14.31	69.43	72.72	14.29	69.94	73.21	13.64	70.57	71.62
13	1	400	1:10	60	19.72	83.14	83.91	19.50	82.40	83.54	19.58	82.46	80.73
14	5	400	1:10	60	17.94	78.78	80.92	18.00	78.07	80.14	17.89	78.53	79.30
15	1	600	1:10	60	15.12	71.58	74.44	15.58	72.06	74.75	14.96	73.90	72.90
16	5	600	1:10	60	12.29	64.25	68.11	12.79	64.97	68.79	11.59	65.69	68.00
17	1	500	1:14	40	19.08	82.00	83.78	19.78	83.11	84.70	18.92	81.42	81.71
18	5	500	1:14	40	18.75	81.37	83.25	18.71	80.50	82.51	18.48	80.91	80.28
19	1	400	1:14	40	20.99	85.59	86.98	21.27	86.11	87.30	20.80	83.98	83.45
20	5	600	1:14	40	18.75	81.24	83.04	19.14	80.96	82.90	18.69	80.91	80.28
21	3	500	1:18	40	18.40	81.43	83.24	19.55	80.59	82.54	18.05	80.85	80.31
22	3	500	1:10	40	17.76	78.25	80.45	17.28	79.34	81.33	17.47	77.69	78.43
23	3	500	1:10	20	15.35	71.93	74.92	16.80	72.63	75.58	14.74	73.41	73.21
24	3	500	1:10	60	19.59	83.07	84.81	18.81	82.62	84.32	19.36	82.82	81.91
25	3	500	1:10	40	19.50	82.68	84.37	19.96	84.11	85.66	19.36	81.97	81.04
26	3	500	1:10	40	20.38	84.48	85.92	19.96	84.11	85.66	20.40	82.82	81.91
27	3	500	1:10	40	20.49	84.69	86.12	19.96	84.11	85.66	20.51	82.82	82.43
28	3	500	1:10	40	20.41	84.44	85.97	19.96	84.11	85.66	20.41	82.82	81.91
29	3	500	1:10	40	20.53	84.50	85.99	19.96	84.11	85.66	20.55	82.99	82.78
30	3	500	1:10	40	20.47	84.61	86.11	19.96	84.11	85.66	20.41	82.82	82.78

$$Y = \beta_0 + \sum_{i=1}^{k} \beta_1 x_1 + \sum_{\substack{i=1 \\ i<j}}^{k=1} \sum_{j=2}^{k} \beta_{ij} x_i x_j + \sum_{i=2}^{k} \beta_{ii} x_i^2 + \varepsilon \tag{4}$$

where various x_i values are independent factors affecting the dependent responses Y; β_0, β_i, β_{ii}, and β_{ij} are the regression coefficients for intercept, linear, quadratic, and interaction effects, respectively; k is the number of factors and ε is the error.

The FCCCD was generated using a Design Expert 7.0 software® (Version 7.1.6, Stat-Ease Inc., Minneapolis, USA). A total number of 30 experimental runs were carried out containing six replications at the central points (Table 1). The validity of the developed quadratic model and statistical significance of the regression coefficients were determined using analysis of variance (ANOVA). The interactions among the independent factors and their corresponding influence on the extraction yield were examined using response surface contour plots.

2.4.2. Artificial neural networks modelling

In this study, the artificial neural network (ANN) was developed for describing the *Vernonia cinerea* leaf extraction process. The same data used for RSM, viz, irradiation time, microwave power level, feed-to-solvent ratio, and ethanol concentration were used to determine the response outputs (extraction yield and antioxidant capacities) using ANN. Artificial neural network is a simplified mathematical and computational model inspired by structural and/or functional part of the biological neural network. Likewise, ANN is being used as a powerful tool in predicting the behaviour of a particular system, to evaluate the existing and design a new processes (Sinha et al., 2013). It works based on an input layer (independent factors), a number of hidden layers and output layer (response factor). Each layer consists of a number of interconnected processing units known as neurons, signals are sent when the neurons interact (Sinha et al., 2012, 2013). External sources send information to the input layer, these input data weighted singly will then pass through the hidden layer for processing and thereafter, output data will be produced based on the grand total of weighted data from input layer modified by a sigmoid transfer function (Sinha et al., 2013).

In this study, MLP neural network architectures were trained. There was an input layer of four neutrons (consisting of four extraction variables), one hidden layer of five neurons and an output of three neutrons as presented in Fig. 1. The input-output patterns used were obtained from FCCCD extraction design matrix. All the artificial neural network calculations were done using Neural Network Toolbox of MATLAB Version 7.9 (R2009b).

2.4.3. Comparison between the RSM and ANN prediction capacities for the optimization of Vernonia cinerea leaf

Different statistical analyses including coefficient of determination (R^2), root mean square error (RMSE) and absolute average deviation (AAD), are being used in comparing the efficiency of RSM and ANN (Sinha et al., 2013). These three statistical tools are calculated using Eqs. (5)–(7).

$$R^2 = 1 - \frac{\sum_{i=1}^{n} (Y_{Predicted} - Y_{Experimental})^2}{\sum_{i=1}^{n} (Y_m - Y_{Experimental})^2} \tag{5}$$

$$RMSE = \left(\frac{1}{n} \sum_{i=1}^{n} (Y_{Predicted} - Y_{Experimental})^2 \right)^{1/2} \tag{6}$$

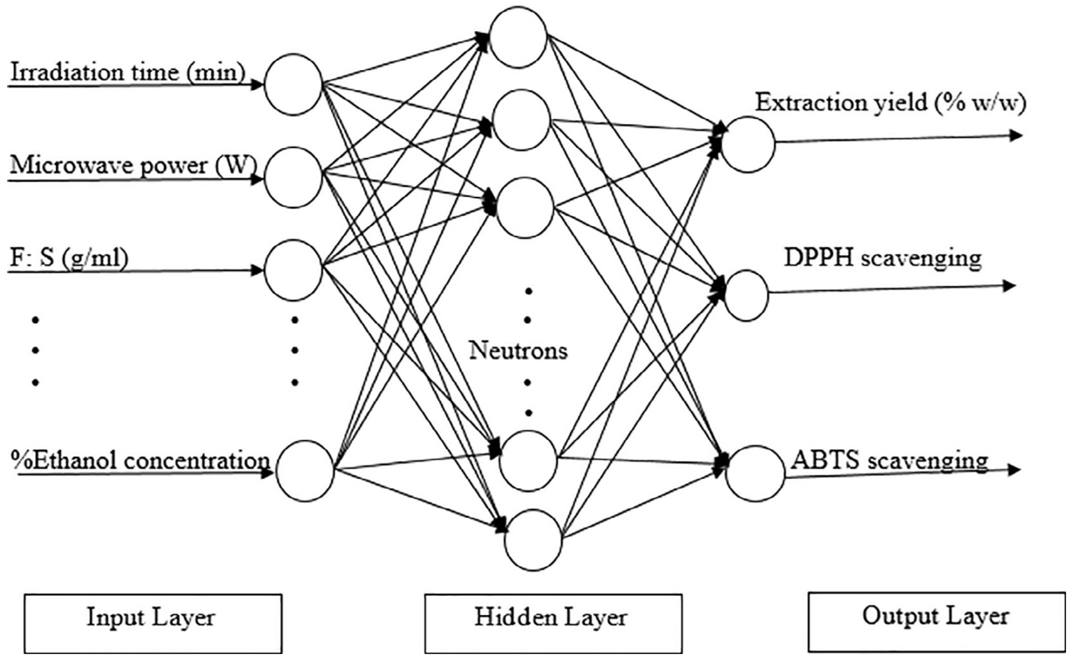


Fig. 1. Topology of ANN architecture.

$$AAD = \left(\frac{1}{n}\sum_{i=1}^{n}\left|\frac{Y_{Predicted} - Y_{Experimental}}{Y_{Experimental}}\right|\right) * 100 \tag{7}$$

where $Y_{Predicted}$ is the predicted data obtained from either RSM or ANN, $Y_{Experimental}$ is the experimental data, Y_m is the average, and n is the number of experimental runs (n = 30).

3. Results and discussion

3.1. RSM models

The experimental results obtained from the MAE of *Vernonia cinerea* leaf based on FCCCD with six centre points are shown in Table 1. The second-order polynomial models (relating independent variables with responses) in terms of coded values as predicted by Design Expert software are shown in Eqs. (8)–(10).

$$\begin{aligned} Y_{Yield}\ (\%\ w/w) = {} & 19.96 - 0.53X_1 - 1.07X_2 - 1.14X_3 + 1.00X_4 - 0.32X_1X_2 \\ & - 0.26X_1X_3 - 0.28X_1X_4 - 0.18X_2X_3 - 1.04X_2X_4 + 0.83X_3X_4 \\ & - 0.72X_1^2 + 0.24X_2^2 - 1.55X_3^2 - 2.16X_4^2 \end{aligned} \tag{8}$$

$$\begin{aligned} Y_{ABTS}\ (\%) = {} & 85.66 - 1.09X_1 - 2.20X_2 - 0.60X_3 + 4.37X_4 - 0.64X_1X_2 - 0.61X_1X_3 \\ & - 0.63X_1X_4 - 0.42X_2X_3 - 2.41X_2X_4 - 0.57X_3X_4 - 2.06X_1^2 - 0.56X_2^2 \\ & - 3.73x_3^2 - 5.71X_4^2 \end{aligned} \tag{9}$$

$$\begin{aligned} Y_{DPPH}\ (\%) = {} & 84.11 - 1.30X_1 - 2.57X_2 - 0.62X_3 + 5.00X_4 - 0.69X_1X_2 - 0.77X_1X_3 \\ & - 0.79X_1X_4 - 0.51X_2X_3 - 2.77X_2X_4 - 0.58X_3X_4 - 2.31X_1^2 - 0.58X_2^2 \\ & - 4.15x_3^2 - 6.49X_4^2 \end{aligned} \tag{10}$$

where X_1 is irradiation time (min), X_2 is microwave power (W), X_3 is feed/solvent (g/ml), and X_4 is ethanol concentration (%).

Table 2 illustrates the analysis of variance results of the second-order polynomial models. It can be clearly seen that the models (extraction yield, DPPH and ABTS scavenging activity) were highly significant, as it is evident from the F-values (25.07, 94.71 and 96.90, respectively) with a low probability value ($p < 0.0001$). The coefficients of determination (R^2) obtained for Eqs. (8)–(10) indicate that 95.90%, 98.88% and 98.91% of the variation in extraction yield, DPPH and ABTS scavenging activities, respectively can be explained by the independent variables (Irradiation time, X_1; Microwave power, X_2; Feed/solvent, X_3; and Ethanol concentration, X_4). All the independent variables have a significant effect on the responses ($p < 0.05$). Also, predicted R^2 (0.8094, 0.9286 and 0.9295, respectively) values show good agreement with adjusted R^2 values (0.9208, 0.9784 and 0.9789, respectively). Low values of coefficient of variance (CV = 4.39, 1.55, 1.31%) indicated that the models were reliable and precise (Alara et al., 2017, 2018). The lack of fits for the models are insignificant, indicating good fittings. More so, the quadratic terms of independent variables were significant for only feed/solvent and ethanol concentration.

For extraction yield, the interactions between the variables showed that microwave power and ethanol concentration, and feed/solvent and ethanol concentration significantly contributed to the recovery yields from *Vernonia cinerea* leaf. In the case of DPPH and ABTS scavenging capacities, interaction between microwave power and feed/solvent, and feed/solvent and ethanol concentration were not significant ($p > 0.05$) on the antioxidant recoveries. Thus, the models can effectively predict the extraction recovery yield and antioxidants from *Vernonia cinerea* leaf.

Contour plots were drawn to evaluate the effects of interaction between the independent variables on the responses (Fig. 2A–C) and to determine the optimal MAE conditions for maximum extraction yields and antioxidant scavenging activities. A contour plot presents a graphical technique used in representing three dimensional surface by plotting two independent variables while maintaining all others at constant level. Fig. 2A1 showed the combined effect of microwave power and irradiation time on extraction yield from *Vernonia cinerea* leaf when feed/solvent and ethanol concentration were kept at 0.07 g/ml and 46.58%, respectively. Increasing irradiation time from 1 to 2 min and microwave power from 400 to 443 W resulted in optimum extraction yield of 21.0138%w/w. Further increase in the irradiation time and microwave power tends to reduce the yield. This may be due to the fact that, the microwave heating may be too strong to breakdown the bioactive compounds in the plant matrix. Likewise, long extraction time beyond 2 min may degrade the phytochemicals in the plant sample leading to lower recovery yield. In the same vein, the interaction effect of feed/solvent and irradiation time is shown in Fig. 2A2, the optimum extraction yield was attained when irradiation time and feed/solvent were increased to 2 min and 0.07 g/ml, respectively. Increasing feed/

Table 2
Analysis of variance for the response surface quadratic models of the MAE of *Vernonia cinerea* leaves.

Extraction yield (% w/w)						DPPH inhibition (%)						ABTS inhibition (%)					
Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob > F	Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob > F	Source	Sum of Squares	df	Mean Square	F-Value	p-value Prob > F
Model	206.09	14	14.72	25.07	< 0.0001	Model	1847.13	14	131.94	94.71	< 0.0001	Model	1435.94	14	102.57	96.90	< 0.0001
X_1-Irradiation time	5.14	1	5.14	8.76	0.0098	X_1-Irradiation time	30.60	1	30.60	21.97	0.0003	X_1-Irradiation time	21.49	1	21.49	20.31	0.0004
X_2-Microwave power	20.50	1	20.50	34.91	< 0.0001	X_2-Microwave power	119.04	1	119.04	85.45	< 0.0001	X_2-Microwave power	87.38	1	87.38	82.55	< 0.0001
X_3-Feed/solvent	23.28	1	23.28	39.64	< 0.0001	X_3-Feed/solvent	7.02	1	7.02	5.04	0.0403	X_3-Feed/solvent	6.54	1	6.54	6.18	0.0252
X_4-Ethanol concentration	18.10	1	18.10	30.82	< 0.0001	X_4-Ethanol concentration	449.20	1	449.20	322.45	< 0.0001	X_4-Ethanol concentration	343.83	1	343.83	324.83	< 0.0001
X_1X_2	1.66	1	1.66	2.82	0.1136	X_1X_2	7.65	1	7.65	5.49	0.0334	X_1X_2	6.58	1	6.58	6.22	0.0248
X_1X_3	1.11	1	1.11	1.89	0.1898	X_1X_3	9.39	1	9.39	6.74	0.0202	X_1X_3	6.00	1	6.00	5.67	0.0309
X_1X_4	1.22	1	1.22	2.07	0.1708	X_1X_4	9.89	1	9.89	7.10	0.0177	X_1X_4	6.40	1	6.40	6.05	0.0266
X_2X_3	0.49	1	0.49	0.84	0.3738	X_2X_3	4.24	1	4.24	3.05	0.1014	X_2X_3	2.81	1	2.81	2.65	0.1243
X_2X_4	17.24	1	17.24	29.36	< 0.0001	X_2X_4	122.99	1	122.99	88.29	< 0.0001	X_2X_4	93.03	1	93.03	87.89	< 0.0001
X_3X_4	10.91	1	10.91	18.57	0.0006	X_3X_4	5.43	1	5.43	3.90	0.0671	X_3X_4	5.24	1	5.24	4.95	0.0518
X_1^2	1.33	1	1.33	2.26	0.1536	X_1^2	13.79	1	13.79	9.90	0.0067	X_1^2	10.95	1	10.95	10.34	0.0058
X_2^2	0.15	1	0.15	0.25	0.6223	X_2^2	0.86	1	0.86	0.62	0.4437	X_2^2	0.81	1	0.81	0.77	0.3943
X_3^2	6.23	1	6.23	10.61	< 0.0053	X_3^2	44.66	1	44.66	32.06	< 0.0001	X_3^2	35.96	1	35.96	33.97	< 0.0001
X_4^2	12.09	1	12.09	20.59	< 0.0004	X_4^2	109.19	1	109.19	78.38	< 0.0001	X_4^2	84.34	1	84.34	79.68	< 0.0001
Residual	8.81	15	1.38			Residual	20.90	15	1.42			Residual	15.88	15	1.06		
Lack of Fit	8.03	10	1.79	5.17	0.0418	Lack of Fit	17.96	10	1.83	3.06	0.1146	Lack of Fit	13.57	10	1.36	2.94	0.1226
Pure Error	0.78	5	0.54			Pure Error	2.94	5	0.60			Pure Error	2.31	5	0.46		
Cor Total	214.90	29				Cor Total	1868.02	29				Cor Total	1451.82	29			
C.V.%	4.39					C.V.%	1.55					C.V.%	1.31				
PRESS	40.96					PRESS	133.45					PRESS	102.33				
Adeq precision	17.547					Adeq precision	27.378					Adeq precision	27.513				
R^2	0.9590					R^2	0.9888					R^2	0.9891				
Adj R^2	0.9208					Adj R^2	0.9784					Adj R^2	0.9789				
Pred R^2	0.8094					Pred R^2	0.9286					Pred R^2	0.9295				

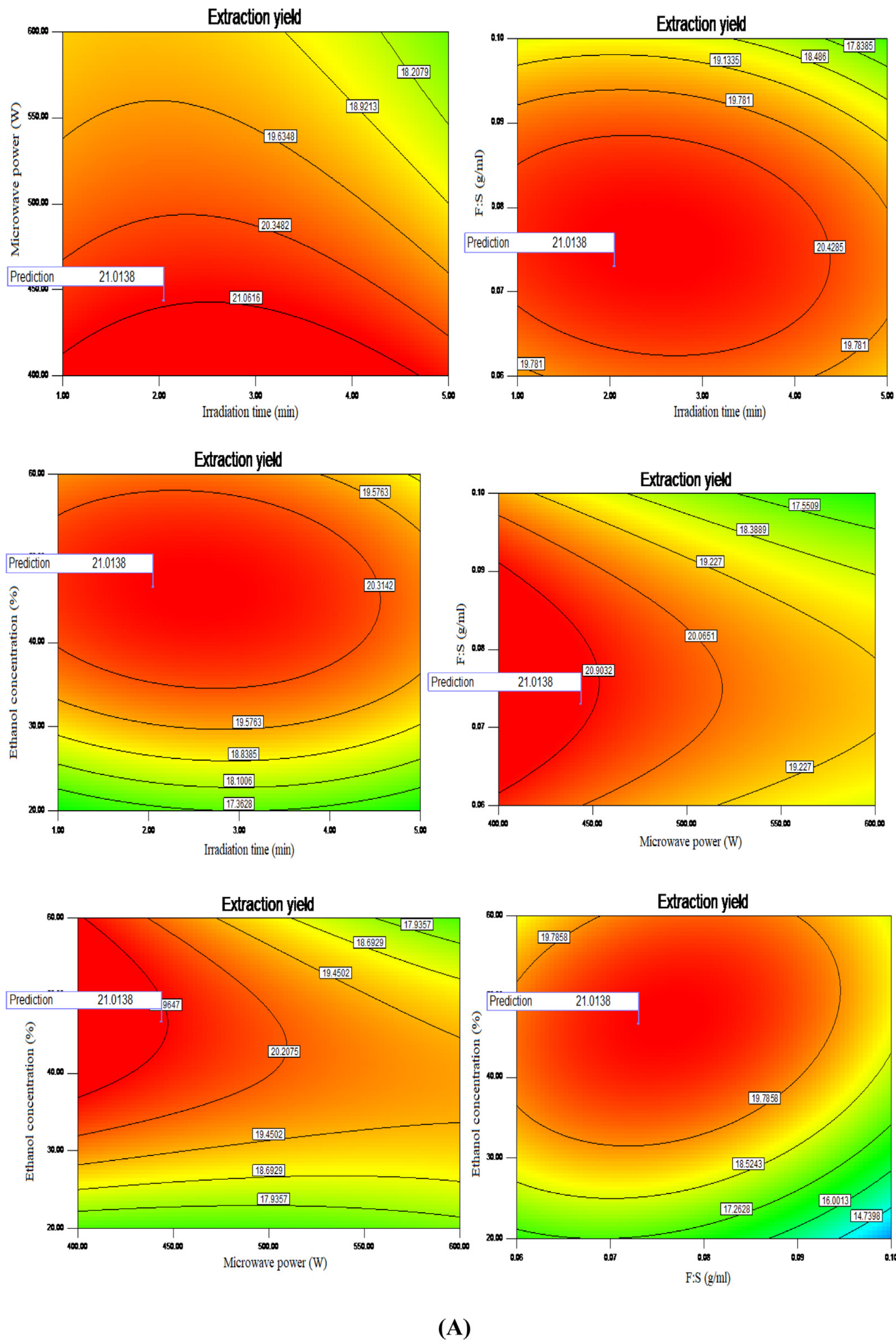
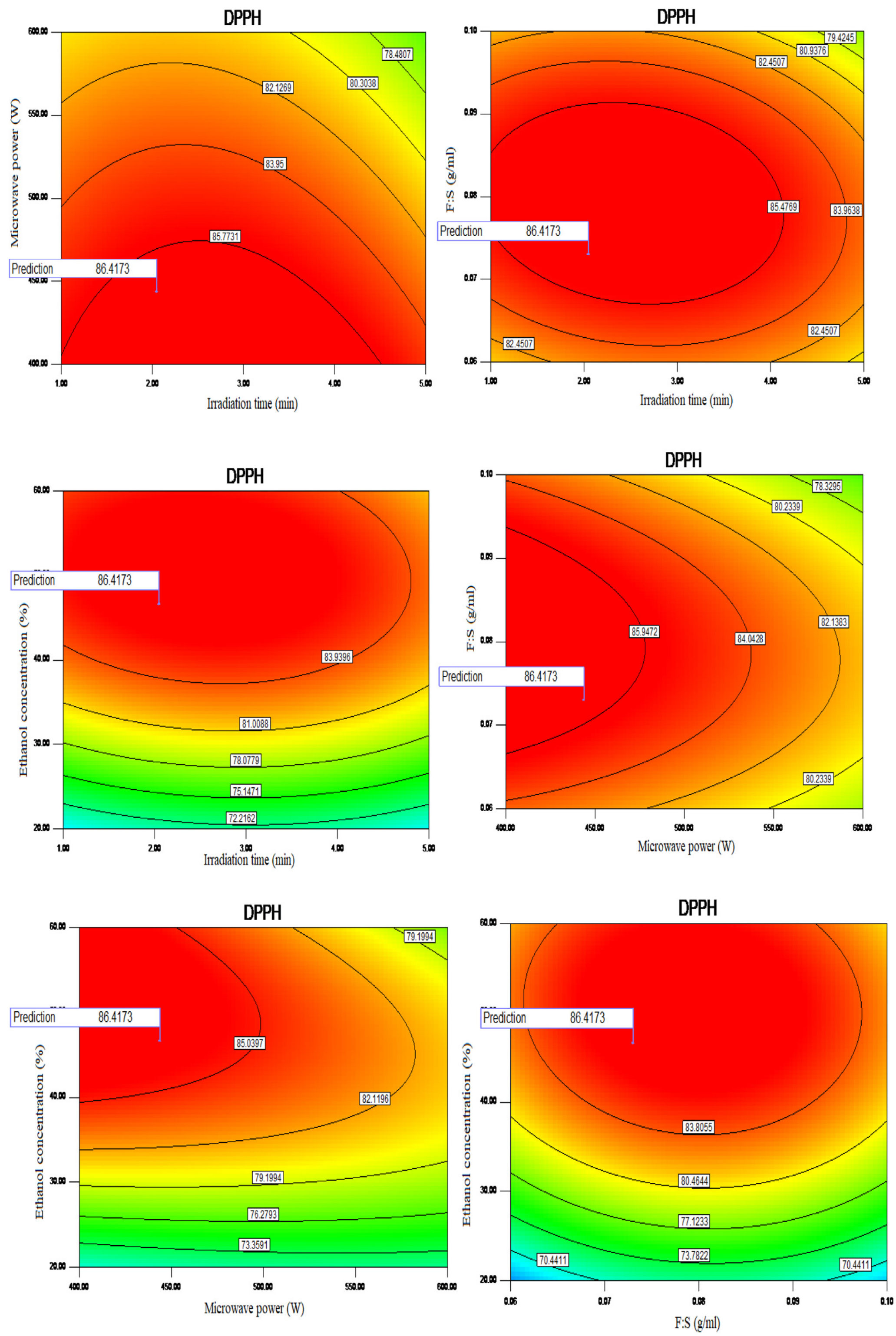


Fig. 2. RSM contour plots for the responses.

solvent beyond limit may decline the mass transfer which can cause lower heating efficiency, that result in reduced recovery yields from the plant matrix. In the same vein, extraction yield as a function of irradiation time and ethanol concentration indicated marked increase when microwave power and feed/solvent were kept constant at 443 W and 0.07 g/ml, respectively (Fig. 1A3). There was a gradual significant increase in the yield as ethanol concentration increased from 20 to 46%. Similarly, maximum extraction yield was obtained as the microwave power and feed/solvent increased to 443 W and 0.07 g/ml, respectively (Fig. 1A4). Similar results were obtained for the interaction between ethanol concentration and microwave power, ethanol concentration and feed/solvent (Fig. 1A5-6). This result is in agreement with the findings reported by Dahmoune et al. (Dahmoune et al., 2015), higher total phenolic content yield had been recovered using a binary



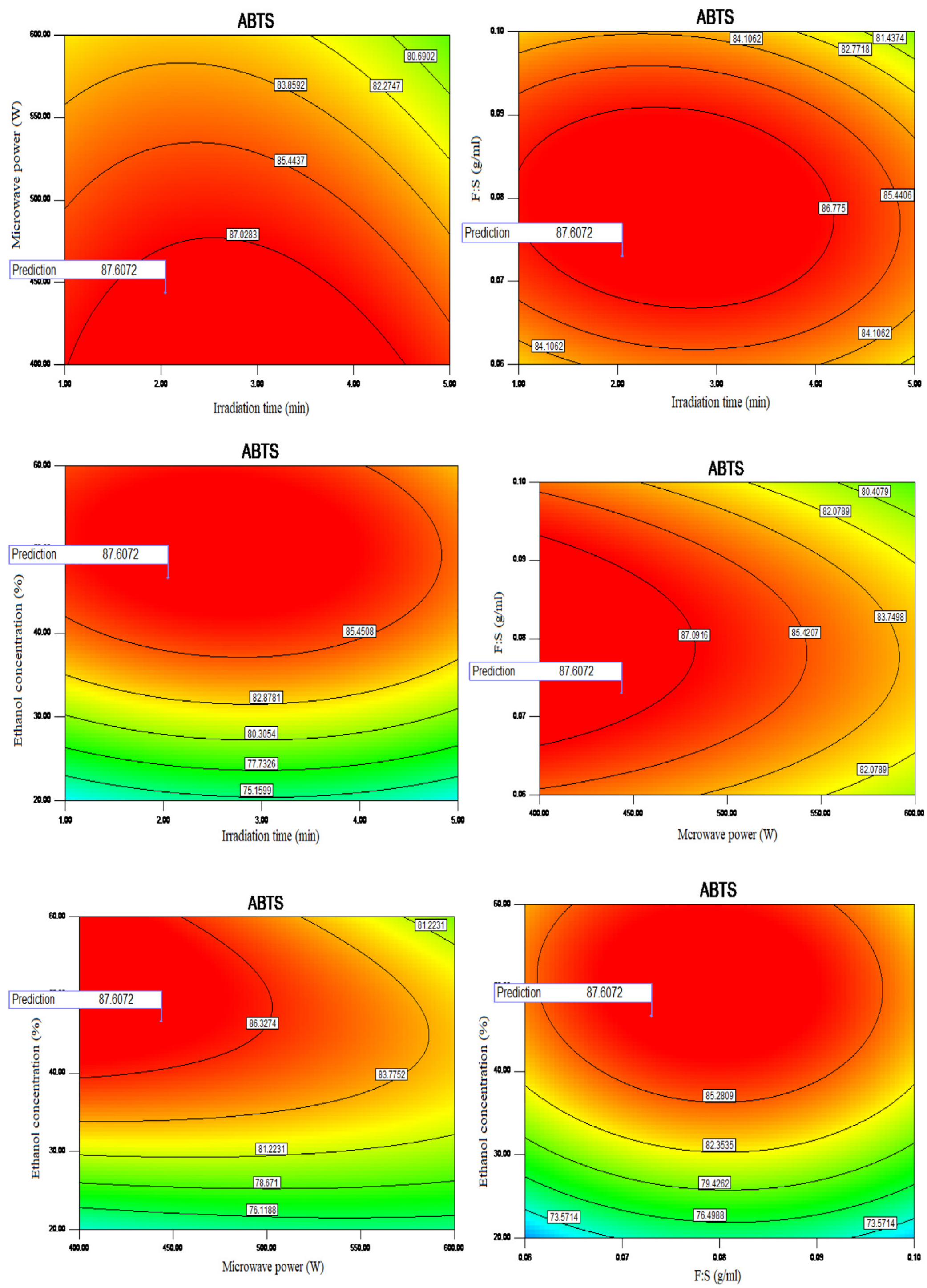
(B)

Fig. 2. (*continued*)

solution of ethanol and water (42:58, v/v) at an irradiation time of 62 s. Therefore, the predicted MAE optimum conditions for obtaining maximum extraction yields of 21.0138% w/w from *Vernonia cinerea* leaf were: irradiation time, 2.05 min; microwave power level, 443.60 W; feed-to-solvent ratio, 1:14 g/ml; and ethanol concentration, 46.58%.

3.2. ANN models

ANN has been a powerful tool for optimizing and simulating extraction processes. Thus, it was used to develop models for describing the microwave-assisted extraction of yields and antioxidant capabilities of *Vernonia cinerea* leaves. As shown in Fig. 1, a three layer (input,



(C)

Fig. 2. (*continued*)

Table 3
Comparison between RSM and ANN modelling.

Parameters	RSM			ANN		
	Yield	DPPH	ABTS	Yield	DPPH	ABTS
R^2	0.9590	0.9888	0.9891	0.9912	0.9928	0.9944
RMSE	0.54	1.54	2.40	0.36	0.83	0.74
ADD (%)	2.56	1.82	0.02	1.80	0.97	0.01

hidden and output) was employed in this study. The experimental results used for RSM was as well employed to predict the optimal architecture of ANN (Table 3). It was limited to the selection of suitable numbers of neurons in the input, hidden and output layers as defined in the experimental design. The number of neurons in the hidden layer was chosen after a minimum error of the predictive models was achieved. Firstly, the neural network was optimized to obtain an ANN model with minimal dimension and errors in training and testing. In this study, the inputs were irradiation time, microwave power, feed/

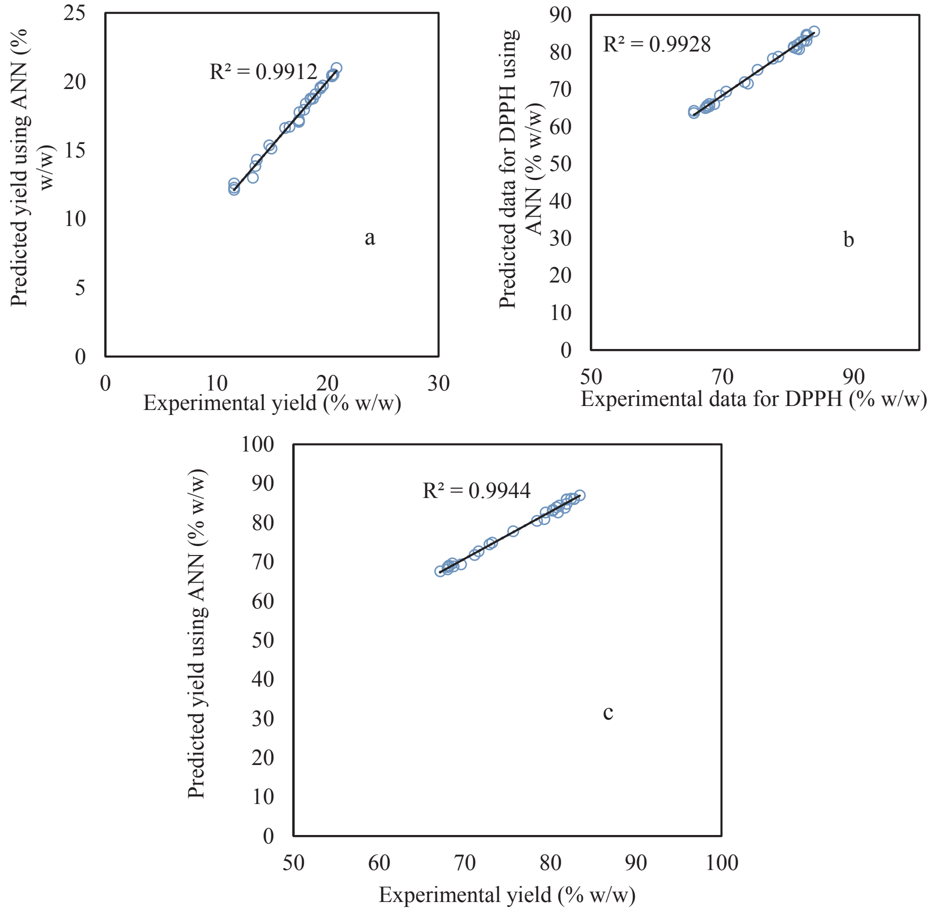


Fig. 3. Comparison between experimental data and predicted extraction yield (a); DPPH scavenging capacity (b) and ABTS scavenging capacity using ANN model.

solvent, and ethanol concentration; while the outputs were extraction yields and antioxidant capacities of the extracts using DPPH and ABTS. The data portioning (training, testing and cross-validation) were carried out to avoid excessive training and over-parameterization. The goodness of fit between the observed and predicted response data form ANN models are shown in Fig. 3a–c with correlation coefficients of 0.9912, 0.9928 and 0.9944 for extraction yield, DPPH and ABTS scavenging activities, respectively. Higher correlation coefficients reflect the reliability of the predictive models using ANN.

The performance of RSM and ANN modelling of extraction yield and antioxidant capacitity of *Vernonia cinerea* leaves were compared using the coefficient of determination (R^2), root mean square error (RMSE) and absolute average deviation (AAD) as shown in Table 3. Although, both RSM and ANN models resulted in the good quality of predictions, however, higher values of R^2 with lower values of RMSE and AAD showed that ANN models can effectively predict the responses with higher estimation capabilities compared to RSM. This improved accuracy of the ANN can be due to its universal ability to approximate the non-linearity of the system, but RSM is only based on a quadratic polynomial. In addition, ANN possesses the ability to evaluate multi-response in a single process, whereas in RSM it has to be run severally (number of parameters to be predicted). Predictions similar to the obtained results had been reported in several studies (Ameer et al., 2017; Pilkington et al., 2014; Sinha et al., 2013). Thus, it will be more reliable and adequate to interpret microwave-assisted extraction of antioxidants from *Vernonia cinerea* leaves using ANN architecture.

4. Conclusion

In this study, the MAE conditions of antioxidants from *Vernonia cinerea* leaves have been optimized comparing RSM and ANN modelling. The coefficient of determination (R^2), root mean square error (RMSE) and absolute average deviation (AAD) were employed to compare the performance of both modelling tools. ANN model has a higher predictive potential compared to RSM model. Thus, ANN model could be a better alternative in data fitting for the MAE of antioxidants from *Vernonia cinerea* leaves.

References

Alara, O.R., Abdul Mudalip, S.K., Olalere, O.A., 2017a. Optimization of mangiferin extracted from *Phaleria macrocarpa* fruits using response surface methodology. J. Appl. Res. Med. Aromat. Plants 5, 82–87.

Alara, O.R., Abdurahman, N.H., Abdul Mudalip, S.K., Olalere, O.A., 2017b. Effect of drying methods on the free radicals scavenging activity of *Vernonia amygdalina* growing in Malaysia. J. King Saud Univ. Sci. http://dx.doi.org/10.1016/j.jksus.2017.05.018.

Alara, O.R., Abdurahman, N.H., Olalere, O.A., 2018. Optimization of microwave-assisted extraction of flavonoids and antioxidants from *Vernonia amygdalina* leaf using response surface methodology. Food Bioprod. Process. 107, 36–48.

Ameer, K., Bae, S.-W., Jo, Y., Lee, H.-G., Ameer, A., Kwon, J.-H., 2017. Optimization of microwave-assisted extraction of total extract, stevioside and rebaudioside-A from Stevia rebaudiana (Bertoni) leaves, using response surface methodology (RSM) and artificial neural network (ANN) modelling. Food Chem. 229, 198–207.

Dahmoune, F., Spigno, G., Moussi, K., Remini, H., Cherbal, A., Madani, K., 2014. *Pistacia lentiscus* leaves as a source of phenolic compounds: microwave-assisted extraction optimized and compared with ultrasound-assisted and conventional solvent extraction. Ind. Crops Prod. 61, 31–40.

Dahmoune, F., Nayak, B., Moussi, K., Remini, H., Madani, K., 2015. Optimization of microwave-assisted extraction of polyphenols from *Myrtus communis* L. leaves. Food Chem. 166, 585–595.

Huang, S.M., Kuo, C.H., Chen, C.A., Liu, Y.C., Shieh, C.J., 2017. RSM and ANN modeling-based optimization approach for the development of ultrasound-assisted liposome encapsulation of piceid. Ultrason. Sonochem. 36, 112–122.

Inpuron, S., Musikachat, L., Boonmark, W., Jarmkom, K., Suchaitanavanit, S., Eakwaropas, P., 2013. Antioxidant activities of Vernonia cinerea and Murraya siamensis. 1st Academic Science and Technology Conference ASTC2013.

Lahlou, M., 2013. The success of natural products in drug discovery. Pharmacol. Pharm. 4, 17–31.

Pilkington, J.L., Preston, C., Gomes, R.L., 2014. Comparison of response surface methodology (RSM) and artificial neural networks (ANN) towards efficient extraction of artemisinin from Artemisia annua. Ind. Crops Prod. 58, 15–24.

Prasopthum, A., Pouyfung, P., Sarapusit, S., Srisook, E., Rongnoparut, P., 2015. Inhibition effects of *Vernonia cinerea* active compounds against cytochrome P450 2A6 and human monoamine oxidases, possible targets for reduction of tobacco dependence. Drug Metab. Pharmacokinet. 30, 174–181.

Qi, Z., 2015. WHO Traditional Medicine Strategy 2014-2023: Background and progress in the last decade. Glob. Heal. Hist. Semin. Tradit. Med. Ayurveda 1–28.

Sinha, K., Das, P., Datta, S., 2012. Response surface optimization and artificial neural network modeling of microwave assisted natural dye extraction from pomegranate rind. Ind. Crop. Prod. 37, 408–414.

Sinha, K., Chowdhury, S., Das, P., Datta, S., 2013. Modeling of microwave-assisted extraction of natural dye from seeds of *Bixa orellana* (Annatto) using response surface methodology (RSM) and artificial neural network (ANN). Ind. Crop. Prod. 41, 165–171.

Youn, U.J., Miklossy, G., Chai, X., Wongwiwatthananukit, S., Toyama, O., Songsak, T., Turkson, J., Chang, L.C., 2014. Bioactive sesquiterpene lactones and other compounds isolated from *Vernonia cinerea*. Fitoterapia 93, 194–200.