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Application of orthogonal optimization and feedforward backpropagation model in the microwave extraction of natural antioxidants from tropical white pepper

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Abstract

The tropical white peppercorns are common commodity crops which have been traditionally used for the treatment of many free radical-related diseases. These medicinal properties are due to the presence of natural antioxidants. This study investigated the combination of microwave extraction parameters for the recovery of natural antioxidants from the white pepper matrix. Microwave-assisted technique was used for the extraction of bioactive oleoresin from white pepper. Taguchi experimental design was employed to investigate the combination of independent extraction parameters for optimal recovery of natural antioxidants. The feed backpropagation artificial neural network model was thereafter applied to optimally predict the result for the different combination of operating parameters. This was achieved by evaluating different algorithms, transfer functions, and neurons. The result obtained from the orthogonal parametric study gave an optimal antioxidant activity of 91.02% at irradiation time of 120 min, microwave power level of 350 W, particle size of 0.300 mm, and liquid-to-solid ratio of 6 mL/g. The gradient descent (GD) algorithm, tansigmoid transfer function, and 4-x-3 topology were used to model the experimental data. A better prediction was then obtained with an overall coefficient (*R*) and mean square error (MSE) of 0.9595 and 1.4381, respectively. In this study, the feedforward backpropagation neural network was successfully applied to optimally evaluate the complex relationship between the input extraction parameters and the response.

Keywords: Antioxidants, Artificial neural network (ANN), Free radicals, Microwave extraction, Taguchi design, White pepper

Introduction

The free radical scavenging activities of some tropical commodity crops have therefore secured pivotal benefits in the production of replacement drugs for the treatment of many degenerative diseases (Meghwal and Tk 2012). The antioxidants act as principal agents that terminate the formation of free radical and inhibit other oxidative reactions in the human body (Rajkovic et al. 2015). However, the production of natural antioxidants is usually not enough to scavenge these free radicals and prevent body degeneration (Cao et al. 2009). There is, therefore, a need to outsource antioxidant from plant origin (Abd El Mageed et al. 2011). The extraction of plant-based antioxidant is, therefore, a safe alternative for the prevention and treatment of many free radical-related diseases when compared with the synthetic ones. These antioxidants are known to be present in functional dietary intakes such as fruits, vegetables, and seeds (Sovilj, 2010). The white pepper seed is an example of such commodity crops with both nutritional and therapeutic benefits (Nuurul et al. 2016). The nutritional and medicinal properties are largely due to the presence of many antioxidant components which makes them functions for the treatment of free radical- and

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oxidative stress-related diseases such as cancer and cardiovascular diseases (Singh et al. 2013). The bioactive components in white pepper extracts have proven to be an effective antioxidant with the potential of repairing and scavenging the damage cells in the human body (Mustapa et al. 2015). A need then arises to find an optimum technique in order to explore its antioxidant potentials (Olalere et al. 2017a).

In recent times, many researchers had employed the conventional method for the recovery of antioxidant constituents, but it has proven to consume time, solvents, and energy (Olalere et al. 2017b). The introduction of a classical microwave reflux combines the conventional and electromagnetic radiation for the extraction of bioactive oleoresins from natural products of plant origin (Abdurahman and Olalere 2016a). The advantage of this method is that it is a rapid and economically feasible technique coupled with a high degree of selectivity. Although many researchers investigated and evaluated the antioxidants in white pepper, none succinctly elucidated the optimum extraction conditions using Taguchi and artificial neural network (ANN). Take, for instance, Rmili et al. (2014), who extracted essential oils from black pepper using hydrodistillation and microwave-assisted hydrodistillation. The demerit from their work was the exclusion of experimental design in the determination of extraction yield. There was no clarity whatsoever on the basis with which the yield was calculated and the assurance that other parameter combination could provide better results. Zhang and Xu (2015) did a comparative study on the antioxidant activity of black and white pepper using hydrodistillation extraction method, but no optimization study was conducted to be sure the antioxidant properties were obtained at optimum condition.

In this study, the L_9-Taguchi parametric design was employed to determine a combination of extraction parameters that jointly optimize the inhibitory capacity of medicinal extracts. Artificial neural network (ANN) model was thereafter employed to further validate the experimental data. ANN model is a data-driven modeling technique whose efficiency largely depends on having more data (Adedeji et al. 2014). The model studies the trend of the input data and develops a black box dynamic model through the adjustments of weights and biases along each neuron for a set of data (Toboc and Lavric 2012).

Material and methods

This study was conducted using a standard-grade white pepper purchased from the Malaysia Pepper Board (MPB) located in Sarawak. An analytical-grade ethanol (95%) and distilled water were obtained from the Analytical Laboratory, Universiti Malaysia Pahang, Gambang, Malaysia. Sigma-Aldrich Chemical Co. was our major supplier for the DPPH (1,1-diphenyl-2-picrylhydrazyl).

Material and reagent preparation

The white peppercorn was grounded into the powdery form using an Eppendorf grinder (200-model, Germany). The sample was later sieved into different particle sizes (0.105, 0.154, 0.30, 0.45, and 0.9 mm). The DPPH solution was prepared by mixing 100 ml of 95% ethanol and an accurately 0.0238-g crystalline solid of DPPH to make up a 0.6-mM stock solution (Badwaik et al. 2015).

Experimental design

The three-level L_9-Taguchi robust experimental array was designed to study the effects of four independent extraction variables on the percentage inhibition. This was employed to investigate the effect of irradiation time (x_1), microwave power level (x_2), feed particle size (x_3), and liquid-to-solid ratio (x_4) on the radical scavenging activity of the extracts. The Taguchi parametric design presented a step-by-step optimization of various extraction variables to improve performance, quality, and cost. The advantage of this design over other robust designs is that it involves smaller experimental runs, thereby reducing time and cost of experimentation (Mandal et al. 2008). The extraction factors and levels for the L_9-orthogonal matrix were designed and analyzed using Minitab 17® software with nine experimental runs.

Microwave reflux extraction

The extraction process was conducted using an automated Milestone microwave system (Ethos-ATC/FO-300, North America). Briefly, 5 g of dried white pepper powder was loaded into the reactor containing a suitable amount of distilled water in accordance with the experimental design. Three levels of microwave heating were applied, and these include pre-heating for 10 min at 100 °C, irradiation at 80 °C, and 10 min of cooling at 30 °C. The application of intermittent heating was to prevent the degradation of antioxidant properties of the spice extracts. The extract was unloaded from the microwave reactor and centrifuged at 5000 rpm for 10 min using the 5810R Eppendorf model refrigerated centrifuge. The supernatant solution was then collected and filtered using the 0.45-μm PTFE micro-filter for subsequent DPPH free radical scavenging assay.

DPPH free radical scavenging assay

DPPH free radical scavenging activity is a biological assay commonly used in the evaluation and estimation of total antioxidant activity of extracts from a medicinal plant. The DPPH reagent is a purple crystalline solid containing free oxidizing radical. The antioxidants inside the spice oleoresin oxidize the free radicals of DPPH molecules, and this is noticeable through the disappearance of the purple color of the DPPH solution into a pale yellow solution. The crystalline DPPH reagent was due to its high sensitivity in detecting minimal free radical scavenging activities in test samples (Thakker et al. 2016). Analytical-grade ethanol was mixed with the prepared DPPH solution at a ratio of 1:5 (0.5 ml ethanol plus 2.5 ml DPPH solution) to make up the negative (A_0) control. Absorbance was taken at 517 nm after 30 min of incubation at room temperature. The absorbance of the 0.5 ml of spice extracts against 2.5 ml DPPH solution was also recorded as A_1. To eliminate the effect of extract color, an absorbance of 0.5 ml spice extract and 2.5 ml DPPH solution was taken as A_2. All absorbance was measured using the 2800-modeled HITACHI UV–vis spectrophotometer. The percentage inhibition (I%) of the DPPH free radicals by the spice extract was then estimated by using Eq. 1 below:

$$\text{Scavenging activity } (\%) = \left(1 - \left[\frac{A_1 - A_2}{A_0}\right]\right) * 100\% \quad (1)$$

Artificial neural network architecture

The feedforward backpropagation ANN model was developed using MATLAB R2014a® software with a single hidden (perceptron), input, and the output layer configuration. The results of the robust experimental design and accompanied response data were used in the development of a neural network model. Data normalization was carried out with an appropriate transfer function (tansigmoid) selected for the network, and this was trained over the hidden layers. The division into training and testing data was performed according to an 80 to 20% division, respectively. Four operating parameters (irradiation time (x_1), microwave power level (x_2), feed particle size (x_3), and liquid-to-solid ratio (x_4)) were considered as the hidden layer. The percentage inhibition (I%) was regarded as the output layer of the ANN model. Levenberg-Marquardt and gradient descent backpropagation training algorithms were then employed. Optimum neurons were retrieved to obtain minimum mean square error (MSE) and the highest regression coefficient (R). The algorithm which gave the best MSE and R value was chosen for further similar problem solving.

Results and discussion

Optimization of the microwave reflux extraction

Taguchi optimization makes use of the signal-to-noise ratio (SNR) to measure the deviation of quality characteristics from the optimal response settings (Abdurahman and Olalere 2016a). The SNR analysis was used to determine the optimal conditions in the extraction of spice oleoresin from white pepper. The test runs with the largest SNR ratio, therefore, gave the better performance characteristic and hence is adjudged as the optimal response point (Abdurahman and Olalere 2016b). From the design matrix (Table 1), the optimum extraction conditions were achieved at 120 min of irradiation time (x_1), 350 W of power level (x_2), 0.300 mm of feed particle size (x_3), and 6 mL/g of the liquid-to-solid ratio (x_4). The inhibition percent, DPPH antioxidant activity, and SNR ratio of the oleoresin extract under the optimum conditions were 91.02%, 218.05 μg/mL, and 39.1827, respectively.

The optimum extraction condition was selected based on the signal-to-noise ratio (SNR). According to Mandal et al. (2008), the optimal level of the extraction parameter is the point at which the SNR gives the largest value. The signal-to-noise ratio (SNR) and response values (inhibition percent and antioxidant activity) intercepted at run 8 (Fig. 1). The descending order of significance of the overall main effects was given as $x_1 > x_4 > x_2 > x_3$ with respect to the generated extremum difference (delta ranking) (Olalere et al. 2017b). This suggested that a nominal change in the irradiation time has a greater influence upon the extraction process. Triplicate parallel tests were conducted under the optimal response setting from the orthogonal parametric design. From the predicted (91.02%) and triplicate actual optimal yields (90.56%, 91.05%, and 91.06%); the χ-goodness-of-fit test was estimated to be 0.0546. This indicated that there is no significant difference between the predicted and actual optimum response settings. The χ^2 values were, therefore, smaller when compared with the 7.81 cutoff value for three degrees of freedom at 95%

Table 1 Experimental design using L9 (3^4)-Taguchi orthogonal array

Run	Uncoded control factors				I (%)	A (μg/mL)	Estimated
	x_1	x_2	x_3	x_4			S/N ratio
1	60	250	0.105	6	54.86 ± 0.06	109.46 ± 0.12	34.7851
2	60	300	0.154	8	54.21 ± 0.30	107.51 ± 0.03	34.6816
3	60	350	0.300	10	57.57 ± 0.62	117.60 ± 0.03	35.2039
4	90	250	0.154	10	71.32 ± 0.38	158.89 ± 0.01	37.0642
5	90	300	0.300	6	76.82 ± 0.23	175.41 ± 0.04	37.7095
6	90	350	0.105	8	74.58 ± 0.02	168.68 ± 0.11	37.4524
7	120	250	0.300	8	75.14 ± 0.11	170.36 ± 0.02	37.5174
8*	120	300	0.105	10	91.02 ± 0.15*	218.05 ± 0.03*	39.1827
9	120	350	0.154	6	89.07 ± 0.04	212.19 ± 0.43	38.9946

Mean ± SD (n = 3)

x_1 irradiation time (min), x_2 microwave power level (W), x_3 feed particle size (mm), x_4 liquid-to-solvent ratio (mL/g), I inhibition (%), A antioxidant activity (μg/mL)

*Optimum trial

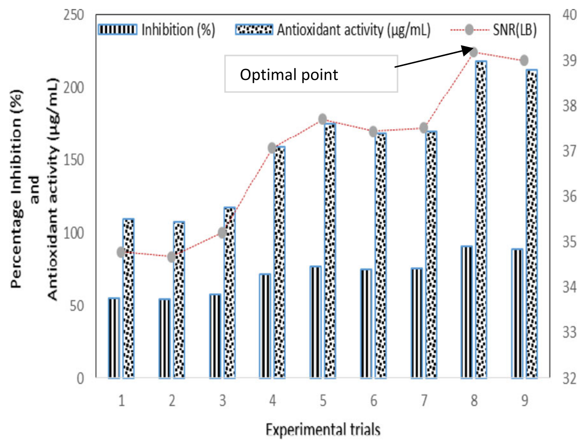

Fig. 1 The graphical illustration of inhibition percent, antioxidant activity, and SNR for each experimental runs

confidence level (Alara et al. 2017). The result indicated a higher percentage inhibition and close resemblance with the result obtained from other studies (Ilhami 2005; Cao et al. 2009; Singh et al. 2013). The difference in the inhibition percentage could be attributed to the climatic condition, geographical location, processing, and storage methods as highlighted by Abou-Gharbia et al. (1997).

Artificial neural network (ANN)

Two training algorithms (Levenberg-Marquardt and gradient descent) were selected as the hidden layer with tansigmoid transfer function. The purpose was to test the effectiveness of the ANN model in predicting familiar data. The results indicated that under the same conditions of perceptron and number of hidden layer neurons, the gradient descent achieved an optimal mean square error (MSE) and R value compared to the Levenberg-Marquardt algorithm. The training evaluation and testing of both algorithms are shown in Table 2 with an overall regression coefficient (R) and mean square error (MSE) of 0.9595 and 1.4381, respectively, with optimal gradient descent configuration. This result showed a good agreement with the optimality of parametric experimental design data. Hence, the feed-forward back-propagation ANN model can be used to optimally predict the result for the different combination of operating variables in the extraction of bioactive spice oleoresin from white pepper.

Figure 2a shows the post-regression plot of the predicted result and the expected trained network response. The primary aim of training and retraining the network is to provide a better function approximation which can enable it to predict a similar problem. For this to be achievable, the regression value of the training process must be closer to unity (≈ 1). The plot (Fig. 2a) shows a regression plot between the network outputs when used to predict new data. A regression value was recorded (R = 0.95953), and this indicated a good success in the supervised learning process for the neural network. Moreover, Fig. 2b showed the performance plot for the network using gradient descent algorithm configuration. The mean square error (MSE) for the training process experienced a reduction with increasing number of iterations which validated the literature and confirms a good training. One of the performance metrics in neural network training is that the mean square error must decrease as the network training progresses through a series of iterations known as epochs. Figure 2c shows a decrease in the mean square error as the number of iterations (epochs) increases. The best validation performance of the network was therefore achieved at the seventh iteration and a mean square error (MSE) of 1.0856. Furthermore, Fig. 2d shows a regression plot between the network outputs when used to predict new data with regression value $R = 0.93054$ recorded. This is an indication of a good network which can be used to predict other data with similar structure.

Statistical analysis

In order to validate the developed neural network model, it was further subjected to new data from the experimental data set. An analysis of variance (ANOVA) was conducted between the predicted values of the response variables and the expected responses. The result (Table 3) revealed that Fisher's values of observed data are less than the critical F statistic ($F = 0.202158 < 4.964603$). This indicated that there is no statistical significance between the values of the predicted and that of expected responses at a confidence level of 95%. The neural network model can, therefore, be used to predict similar data as supported by Thakker et al. (2016).

Conclusion

This study carefully detailed the experimental investigation of microwave parameters associated with the inhibitory and antioxidant activities of spice oleoresin extracts from white pepper. A tolerance-based Taguchi design was constructed to estimate the effects of extraction

Table 2 Network training configuration and results

Training algorithm	Number of neurons	Iterations	Transfer function	MSE	R
Levenberg-Marquardt	25	7	tansig	1.0856	0.9305
Gradient descent	25	1000	tansig	1.4381	0.9595

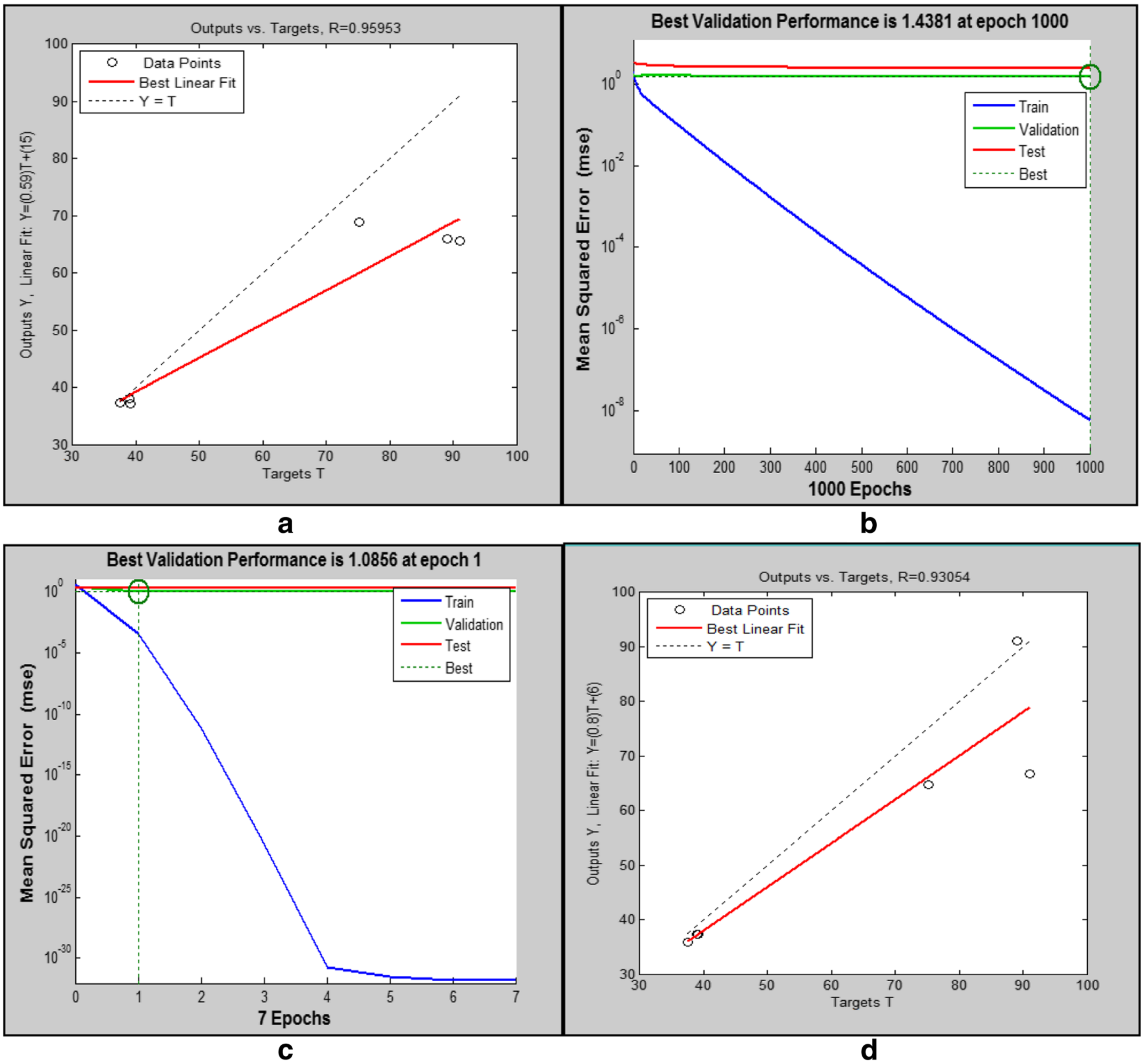

Fig. 2 **a** Post-regression plot (GD). **b** Network performance plot (GD). **c** Network evaluation regression plot (LM). **d** Network simulation with new inputs (LM)

Table 3 ANOVA between predicted and expected responses

Groups	Count	Sum	Average	Variance		
Predicted	6	333.0431	55.50718	503.7455		
Expected	6	370.9247	61.82078	679.3375		
Source of variation	SS	df	MS	*F*	*P* value	*F* crit
Between groups	119.5848	1	119.5848	0.202158	0.662573	4.964603
Within groups	5915.415	10	591.5415			
Total	6035	11				

parameters on the mean and variation of the response/ signal factor. An optimal inhibitory percent of 91.02% was achieved at 120 min of irradiation time (x_1), 350 W of power level (x_2), 0.300 mm of feed particle size (x_3), and 6 mL/g of liquid-to-solid ratio (x_4). To further validate the optimal response settings, an artificial neural network was employed to predict the corresponding inhibitory percent with known input, hidden, and output layers. The gradient descent (GD) provided a better prediction when compared with the Levenberg-Marquardt (LM) configuration giving an overall regression coefficient of 0.9595 and mean square error of 1.4381. The result obtained is, therefore, a potential blueprint of scale-up parameters for industrial diversification of the extracts in pharmaceutical industries.

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Availability of data and materials
Research data have been provided in the manuscript.

Authors' contributions
OAO designed the experiments, performed the data analysis, reviewed the literature, and drafted the manuscript. NHA supervised the experiment. ZH provided useful insight into the work. AOR provided guidance in designing, writing, and revising the manuscript. HP assisted with the data analysis. All authors read and approved the final manuscript.

Ethics approval and consent to participate
Not applicable

Consent for publication
Not applicable

Competing interests
The authors declare that they have no competing interests.

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