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Optimization of Oleuropein Extraction from Olive Leaves using Artificial Neural Network

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Abstract

In this work, the artificial neural networks (ANN) technology was applied to the simulation of oleuropein extraction process. For this technology, a 3-layer network structure is applied, and the operation factors such as amount of flow intensity ratio, temperature, residence time, and pH are used as input variables of the network, whereas the extraction yield is considered as response value. Performance indicators $RMSE$, SSE, R^2_{adj}, R^2 have been used to determine the number of optimal midway neurons.Neural network trained with an error back-propagation algorithm, was used to evaluate the effects of parameters on extraction yield.The obtained optimal architecture of artificial neural network model involved a feed-forward neural network with 4 input neurons, 1 hidden layer with 6 neurons and one output layer including single neuron.The trained network gave the minimum value in the RMSE of 1.6423 and the maximum value in the R^2= 0.9641, which implied a good agreement between the predicted value and the actual value, and confirmed a good generalization of the network.Functional structure of modeling, related to education, validation and test were obtained 0.99229,0.95591and 0.99224 respectively. The overall agreement between the experimental data and ANN predictions was satisfactory showing a determination coefficient of 0.9838.The neural network tools implemented in MATLAB software were used to predict the change process.

Keywords: *Artificial Neural Networks, Extraction, Microfluidic,Oleuropein*.

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Introduction

In the recent century, major advances have been made in the use of medicinal herbs, and well-equipped laboratories are working on the effects of these drugs around the world. It may not be conceivable to many people that the existence of a variety of chemical drugs in colorful packaging is the result of research on the effective components of medicinal plants[1,2].This has led to extensive research by pharmaceutical companies on the therapeutic properties of herbs in different parts of the world. A group of compounds known as polyphenols is the primary category of secondary metabolites in plants. The presence of polyphenols is vital for plants and have different functions including color production, pollination and improved resistance to insects and invasive animals[3]. Bio phenol activity of olive is known against pathogenic attack and scars from insects and animals.

Studies on polyphenols show that the extracts of vegetable polyphenols, including those found in fruits and vegetables, show beneficial medicinal properties that include: anti malaria, antispasmodic, anticancer activity and preventing Alzheimer's disease and also was added to cosmetics.In the study of olive, it was discovered that the main characteristic of olive isoleuropein[4].Oleuropein is one of the largest, most important and abundant phenolic compounds in fruit and olive leaves [5].It is a bitter glycoside. This substance, which is known as the most abundant polyphenol in the olive leaf, prevents lipid membrane oxidation in heart disease[6,7]. Studies have been conducted on hairless rats that exposed to ultraviolet radiation shown that the extract of olives and oleuropeinsignificantly impedes the increase in skin thickness, decreased elasticity, and cancer of the skin and tumor growth[8]. Other properties include lipid metabolism, which reduces obesity-related problems, antiviral properties, prevent diabetic neuropathy, preventing cell death in cancer patients, antimicrobial and antifungalproperties [9].

Common methods of extraction of medicinal plants are: maceration [10], percolation [11], soxhlet[12], extraction with supercritical fluid [13] extraction with ultrasound[14] and extraction using microwave waves [15]. These methods are very time consuming, not very repeatable, much solvent is consumed. Some of these methods require high temperatures or high pressures and are costly. But extracting the effective substance of medicinal plants using microfluidic systems [16] as a novel method can greatly overcome these problems. In the micro channel method, the effect of gravity is negligible due to the increase of the surface-to-volume ratio and the dominance of surface forces. Therefore, the penetration length is shortened and the penetration time decreases. So mass transfer is done more and the amount of solvent consumed decreases. For this reason, the amount of environmental pollution and the cost of the solvent is reduced and extraction percentage increases. Optimized conditions and comparative assessment for extractionOleuropein from Olive leaf were investigated between conventional methods that different extraction methods have been reported. For example, ultrasound-assisted extraction [17, 18], microwave-assisted extraction [19], supercritical fluid extraction [20], maceration and soxhlet by deionized water [21], hot blanching technique assisted with UAE [22]. The results of investigations revealed that the extraction yield using microchannel extractor device was more than that of conventional processes. The improvement of extraction performance in this method can be more than 30% compared to conventional extraction methods.In current study, it is tried to apply ANN for characterization the extraction efficiency through optimizing the conditions of extraction for achieving the maximum efficiency. Following, an artificial neural network ANN was used to predict the efficiency of extraction.

Microfluidic method

Microfluidics system is the science of controlling fluids and particles at micron and submicron dimensions.Mixing of samples and fluids in microfluidic devices is very important, especially in chemical and biological applications with different reactants composition. It is obvious that macro-scale mixing is easily achieved by creating turbulence in the current butthe flow type in microchannel is laminar, it will be difficult to create turbulence.Microfluidic mixers are designed to reduce mixing time.Micromixers are classified as active and passive mixers. The inactive model is generally cheaper than the active model [23].Micromixers, which have a considerable impact on the efficiency are one of the most important components of micro channels. Mixing in the micro-scale fluidic ones is often achieved in the microchannels with external turbulences or special microstructures at micro-level dimensions.Various techniques exist for creating turbulencein these mixers: electro hydrodynamics, magnetic field, di-electrophoretic, sound and use a circulating pump. These mixers are designed to reduce the penetration distance, increase the contact surface between mixed fluids and/or both. Two commonly used designs are the use of Y and T shapes as a mixer. Since distance is reduced in micro systems, and given the fact that in the lower distances, molecular penetration increases, therefore, the mass transfer rate is also increased.Microfluidic techniques has multiple advantages including decreased fabrication costs, increased separation efficiency, reduce response times and solvent volumes.Thus, micro channels have their numerous applications in various fields [24].

Artificial Neural Network

The basis of artificial neural networks is such as neural networks in animals and humans. As you know, in the brain, neurons have the task of processing and transmitting information in the form of electrical signals. Artificial neural networks are simplistic examples of the brain and usually, a limited number of artificial neurons are connected, depending on the network application, from tens to hundreds, each of which makes a simple calculation. The structure of these networks is such that they have the ability to learn. Each neuron (or cell) of the network has multiple input.For an artificial neuron, the weight function is a number, and represents the synapse. All inputs are summed up and modified by the weights and referred as a linear combination. Finally, an activation function controls the output[25].

After building the network, the learning process is done. These processing units are made up of input and output units. The input units receive various information and the neural network attempts to presented one output. Artificial neural networks also use a set of training rules called backpropagation, to perfect their output results.The learning of this algorithm is based on the error correction law which can be called generalization of the algorithm, is customary to the least squares mean. Learning through back propagation method returns two stages: advance step and return stage. In advance step, inputs go through layer-by-layer in the network and at the end, some output is obtained as the real response of network. In return stage, the connection weights are governed by the error correction law. The difference between the actual network response and the expected response that is called error, is released in the opposite direction to the network connections and the weights change in such a way that the actual response of the network becomes closer to the desired response[26, 27].

Experimental

Samples Preparation

Olive Tree leaves of Sevillanaspecies were collected from an olive grove in Ilam province. The leaves were washed and dried, then milled uniformly, and kept at 4 ° C.

Preparation of standard solution

A standard stoke solution at 500 mg/L was prepared by dissolving the standard oleuropeinpowder in methanol. To obtain the calibration curve, standard solutions with different concentrations,$50, 100, 200, 300\ , 400\ \frac{mg}{L}$ were prepared byadding distilled water to stock solution to reach the appropriate volume. The amount of oleuropein concentration in the aqueous and organic phase was obtained using a calibration curve.

Chemicals

All chemicals used in current study such as Methanol, Ethanol, Ethyl acetate, Acetonitrile and

Oleuropein (purity ≥ 98% by HPLC), were purchased from Merck Company.

High Performance Liquid Chromatography

High Performance Liquid Chromatography (model KNAUER) made in Germany that is equipped with a vacuum degasser, ultraviolet detector, two pumps. A reversed-phase C18 analytical column (250 mm ×4.6 mm I.D., 3µm particle size) was used. The aqueous solution was filtered through a filter paper and injected to the column using a 1µl injection loop. Flow rate was 1 ml / min and the column temperature was kept in ambient temperature. The analysis was performed at 254 nm with a UV detector.

Different solvents such as deionized water, acetonitrile, and methanol and phosphate buffer were used as mobile phase.The next step, to improve the appearance of the peak the composition of percentages and the current intensity of mobile phase were investigated.Experimental results indicated that high extract concentrations of oleuropein were obtained with a mixed solvent, of 30% acetonitrile and 70% phosphate buffer.Concentration of Phosphate buffer was 0.05 M with pH equal 3.

Liquid-liquid extraction with microchannel

In extraction from the solid phase, 10 gr olive leaves powder in 100 ml of solvent for 24 hours at ambient temperature was soaked .then solution was separated by Whatman filter paper and used as feed in micro channel.In this extraction method, glass microchannel T-shape was used to because of resist chemical solvents.In order to increase the time remaining and the mixing at the microchannel, copper coil with an inner diameter of 600µm and outer diameter of 2mm was used.In order to create flow, two syringe pumps were used in inlet streams. These pumps provided the necessary driving force to move organic and aqueous phases in the glass syringes. One of the glass syringes contains the suitable aqueous phase and the other syringe contains the extract of the sample obtained from the previous step.These two phases, through glass syringes, first enter the microchannel and then pass through the copper coil from the system. Flow rate control of two-phase was carried out using the valves on each path. In this way, the flow velocity is determined in the input paths.In order to ensure the correct operation of the valves, after adjusting the input currents, the output flows rate was measured individually and again after mixing and in the total output current. The microchannel was placed inside the water bath, in order to keep its temperature in during the test.The microchannel output was collected in the Falcon tube.In order to reduce the error, after each time setting apparatus, 2ml of output stream is throwaway.

After collecting the output stream, organic and aqueous phases were separated by syringe and the aqueous phase was injected to the HPLC for determine its oleuropein. Concentration oleuropein in the output stream can be calculated from its peak area in the chromatogram. A calibration curve was provided for oleuropein and the regression equation of the calibration curve was used to determine the outcomes.

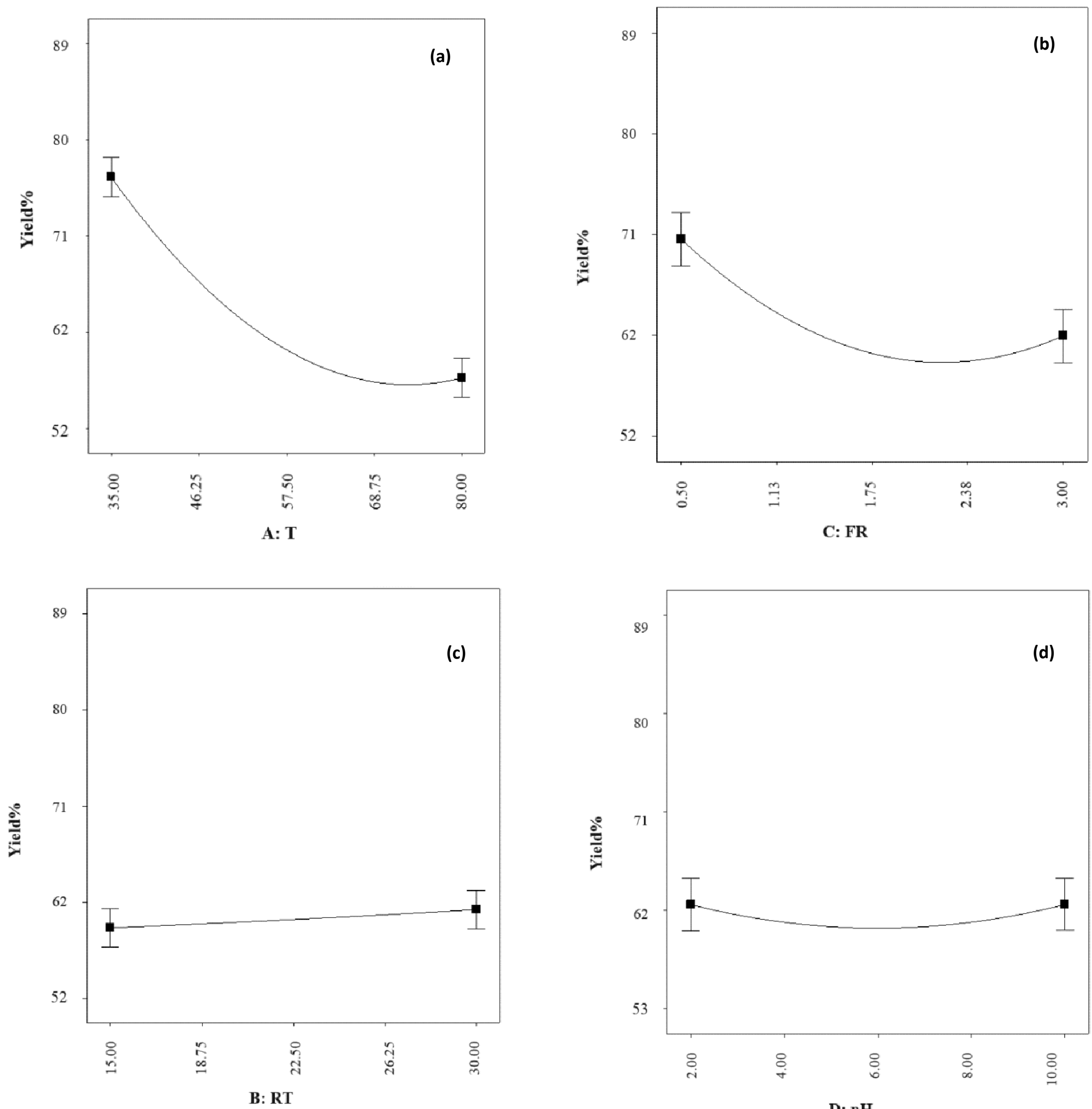

Figure1. Single-parameter effect on extraction efficiency.Chart of yieldin terms of:**(a)**Temperaturechanges under constant conditions:RT=22.5, FR=1.75, pH=6; **(b)**Flow intensity Ratio changes under constant conditions:RT=22.5, T=57.50, pH=6; **(c)**Residence Time changes under constant conditions:FR=1.75, T=57.50, pH=6; **(d)**pH changes under constant conditions:FR=1.75, T=57.50, RT=22.5

Results and discussion

Single-parameter effect

In a single-parameter study, the effect of a variable is checked when the three other parameters are assumed to be constant. As shown in the figure 1-a, temperature (T) changes have a significant and negative effect on extraction efficiency. Increasing the temperature decreases the extraction efficiency. Figure 1-bshows that flow intensityratio(FR) has a negative and decreasing effect on extraction efficiency. Residence time(RT) has a slight increase in extraction efficiency (As shown in figure 1-c). As shown in the figure 1-d,the pH graph with efficiency is an incremental graph, but it has not much slope and is gentle.

Interaction effect of parameters

Results shows, the effect of residence time on the efficiency is higher at lower temperatures.The interaction between the two parameters temperature and flow intensity shows which the simultaneous increase of both parameters reduces the efficiency. Low pH at low temperatures has a positive effect and when the temperature rises, high pH values have a better effect on the extraction efficiency. RT and the low values of the ratio of the flow rate of the organic phase to the aqueous phase increases the efficiency of the oleuropein separation. According to results, the interaction between the ratio of flow intensity and pH is increases - decreases. High pH values have a better effect at lower flow intensity while, when the ratio of the flow intensity increases, low-pH values have a better effect on efficiency. Interaction between pH and RT is linear and increases-decreases. That high pH values have a better effect on efficiency at low RT values while low pH values have a better effect on efficiency when RT values rises. According of results, addition to linear effects, interactions and their squares also effect on the efficiency. Where the graphs are linear only, their square effects are meaningless.

Artificial neural network model

An ANN method is one of the computational methods that, with the help of learning and using processors called the neuron, attempts to establish a relationship between the input space and the output layer by recognizing the inherent relationships between the data. The hidden layers process the information received from the input layer and place it on the output layer. The proposed neural network model has 4 input layers, 6 hidden layers and one output layer. Parameters of input layers are the flow intensity ratio, residence time, PH and temperature, and the output layer is the extraction efficiencyas shown in Figure2.

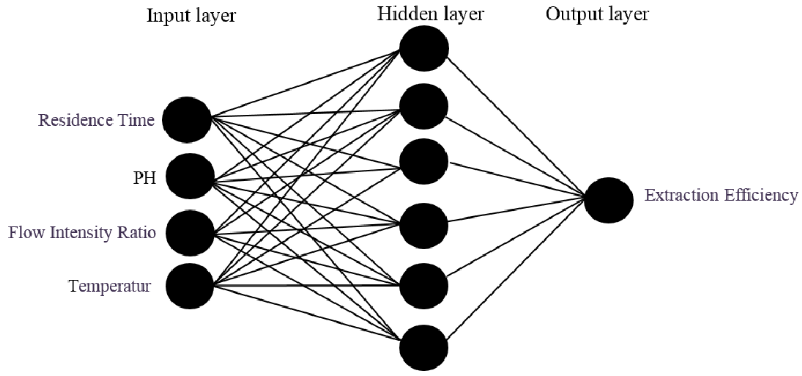

Figure 2. Neural interpretation diagram for proposed artificial neural networks modelinclude input layers, hidden layers and output layer.

The multiple linear programming (MLP) network used has three layers. The transfer function in the hidden layer is tansigmiod and in output layer is the linear. Then, the methods of try and error and the necessary optimization are used to achieve the minimum error. After entering the input and output data, the neural network divides the data set into three categories of training, validation and test. The network randomly divides 70% of the data as training data, assigning 15% to the validation category and the remaining 15% to the test category.In this model, four input variables are pH, temperature, residence time and flow intensity ratio between the two organic and aqueous phases, and the extraction efficiency is considered as the output variable. In the network training section, at first, a number of algorithms are compared according to table 1. In this stage, seven different algorithms were used for network training and the best algorithm for network training was Algorithm Levenberg-marquardt by comparing the errors. Equations 1 to 3 were used to obtain errors.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}\left(y_{i,predic} - y_{i,exp}\right)^2}{n}} \quad (1)$$

$$SSE = \sum_{i=1}^{n}\left(y_{i,predic} - y_{i,exp}\right)^2 \quad (2)$$

$$R^2 = 1 - \frac{\sum_{i=1}^{n}\left(y_{i,predic} - y_{i,exp}\right)^2}{\sum_{i=1}^{n}\left(y_{i,exp} - y_m\right)^2} \quad (3)$$

Table1.Seven different proposed ANN for network training and the best training algorithm.

Training	R^2	R^2_{adj}	RMSE	SSE
TrainBR	0.9588	0.9576	1.6628	78.3836
TrainCGB	0.8942	0.8904	2.7871	217.4961
TrainCGF	0.7180	0.7079	4.4692	559.2657
TrainCGP	0.8756	0.8712	3.1724	281.7915
TrainLM	0.9641	0.9628	1.6423	75.5160
TrainR	0.8214	0.8150	3.5841	359.6753
TrainRP	0.8709	0.8663	3.0725	264.3282

After comparing different algorithms and reaching the best algorithm, neurons from 5 to 15 were compared to obtain the best output data (predicted) by the neural network. According to Table 2, the best result was achieved in neuron 6. After training and obtaining optimal neurons of network, the indices $R^2, R^2_{adj}, RMSE, SSE$ were compared together. In this model, performance function (R) related to training, validation and testing, and all data were obtained 0.99229 ،0.95591 ،0.99224, and 0.99185respectively as shown in Figure3.

Table2. Compare neurons for finding the best output data by the neural network.

N	R^2	R^2_{adj}	RMSE	SSE
5	0.9169	0.9140	2.4101	162.6414
6	0.9838	0.9832	1.1878	39.5049
7	0.9544	0.9525	1.9202	103.2390
8	0.9649	0.9636	1.7495	85.7035
9	0.9398	0.9376	2.2828	145.9133
10	0.9641	0.9628	1.6423	75.5160
11	0.9676	0.9665	1.7356	84.3418
12	0.9340	0.9317	2.7214	207.3695
13	0.9389	0.9367	2.3909	160.0594
14	0.9736	0.9726	1.5001	63.0100
15	0.9399	0.9377	2.1747	132.4180

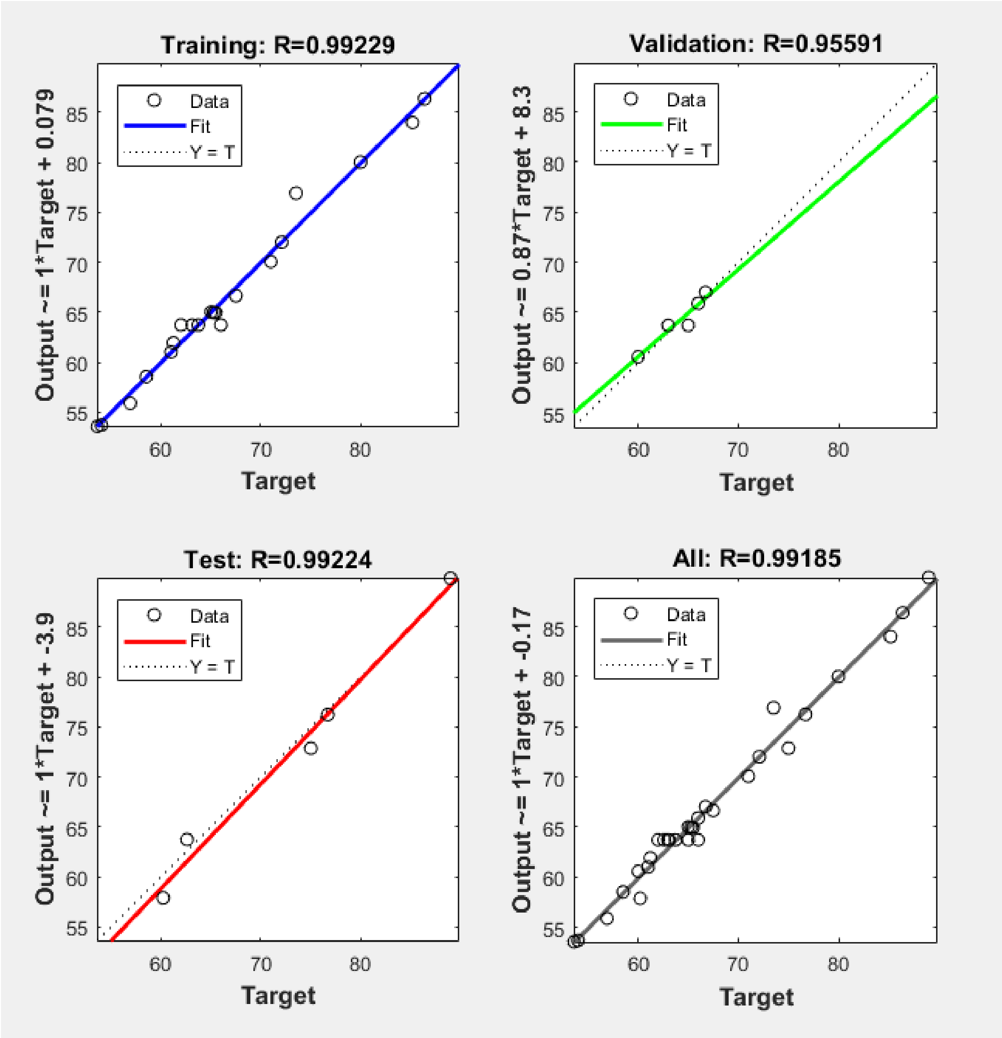

Figure 3. Performance R related to training, validation and testing, and all data in the proposed model by artificial neural network.

In Figure4, experimental data and predicted data using neural network response methods are plotted. Table 3 displays the weights and biases of optimal neuron that interconnect the layers.

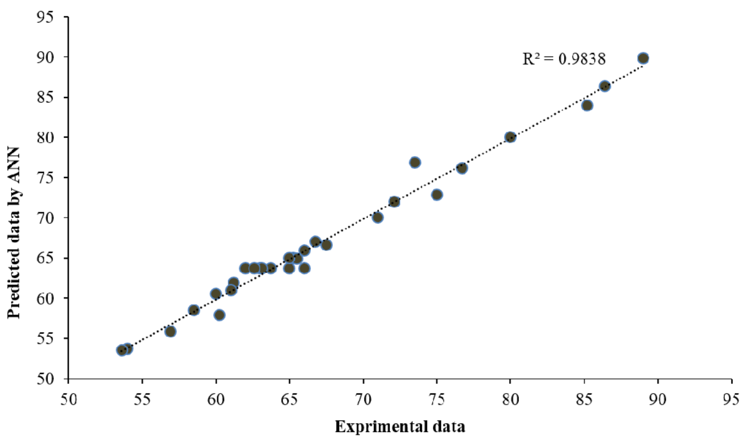

Figure 4. A comparison between the experimental and ANN predicted values of extraction yield.

Table 3. Weights and biases of optimal neuron that interconnect the layers.

	w1					B_2=0.4980
Nu. Of neurons	**Temperature**	**Resident Time**	**flow intensity**	**pH**	**w_2**	**B_1**
1	1.1055	-2.3766	-0.73878	-1.5196	-0.3655	-2.1282
2	-0.6214	-2.0729	-1.9471	-1.8713	0.21302	1.7614
3	1.5037	-1.9462	-0.64507	0.68728	0.19707	-1.3064
4	-1.0594	0.5807	-1.4297	1.2298	0.33317	-0.86005
5	2.5457	-0.60527	-0.29924	0.69729	-0.63742	0.9637
6	-1.2128	0.20709	1.9471	-0.44663	0.61834	-2.2421

The operation of the neural networks using LM is as follows: this function adds a neuron to the network sequentially. This will be done as long as the mean-square deviation (error) is less than the target value or that we have reached the maximum amount of neuron determined. The results of this study show that the network consists of three layers: input and hidden layers and output which the hidden layer shows the best performance with 6 nodes.The stopping criterion of the training process is based on an error generated by ANN. This criterion is used for the mean squared error.

In this section simulated data is compared with real data and in this way, the adequacy of the network to predict the efficiency of extraction confirmed or violated. It should be noted that this method is also presented in several algorithms and forms. One of the best and most effective methods that are available in the software package is the Leungberg-Marquette method which is briefly known as the LM in the MATLAB package and greatly increases the speed of convergence and speeds up conclusions. The correlation factor and regression relation between network results and real values, as well as prediction of experimental data extraction represent the optimal performance of the network.

Conclusion

Given the high accuracy of the neural model, it is possible to trust the results of these models prediction with high confidence.These models are used to optimize and control the process, which saves energy and time and it will create a more favorable product. By having the activation function in the neural networks, that in this study, the hypogenous sigmoid tangent function is and also having weight and bias values, it is possible to extract relations created by the neural model.Various activation functions were used to determine the best type of function in estimating the characteristics of oleuropein extraction from olive. The results showed that the best performance of the modeling is when the hyperbolic tangent activation function for the parameters studied with the topology 1-6-4 is considered. In general, the comparison of the functions showed that the activation function of the hyperbolic tangent has the highest accuracy in the predicting of extraction parameters due to the least relative error and the highest determination coefficient compared to the other functions.

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