Determination of the ultrasound power effects on flavonoid compounds from *Psidium guajava* L. using ANFIS

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Abstract: The Adaptive Neural Fuzzy Inference System (ANFIS) model was used to predict and optimize the content of flavonoid compounds in guava leaves (*Psidium guajava* L.). The extraction process was carried out by using ultrasound assisted extraction (UAE) with the variable parameters: temperature ranging from 25°C to 35°C, ultrasonic frequency (30 - 40 kHz) and extraction time (20 - 40 minutes). ANFIS learning procedure began by providing the input variable data set (temperature, frequency and time) and the output of the flavonoid compounds from the experiments that had been done. Subtractive clustering methods was used in the manufacture of FIS (fuzzy inference system) structures by varying the range of influence parameters to generate the ANFIS system. The ANFIS trainings conducted were aimed at a minimum error value. The results showed that the best ANFIS models used a subtractive clustering method, in which the ranges of influence 0.1 were 0.70 x 10⁻⁴ for training RMSE, 8.11 for testing RMSE, 2.7 % MAPE, and 7.72 MAE. The optimum condition was obtained at a temperature of 35°C and frequency of 40 kHz, for 30 minutes. This result proves that the ANFIS model can be used to predict the content of flavonoid compounds in guava leaves.

Keywords: ultrasound power, flavonoid, ANFIS, modelling

1. Introduction
Medicinal plants grown in Indonesia such as guava leaves (*Psidium guajava* L.) contain compounds that can be utilized as an alternative medicine. One of the most commonly used compounds is flavonoids, which is used to prevent infections that cause diarrhea, to be applied as antioxidant and antibacterial [1,2]. Flavonoid compounds can be obtained from the extraction process. The conventional methods have been proposed in recent decades but required relatively amounts of solvents and prolonged extraction times [3,4]. To overcome the drawbacks, alternative methods have been proposed such as ultrasound assisted extraction (UAE) [5], supercritical fluid extraction (SFE) [6], and microwave assisted extraction (MAE) [7]. In the literature review, the use of ultrasonic assisted extraction methods is more preferred than the conventional methods [8,9], since it uses less...
solvent volume, shortens extraction time and it is energy saving [10].

Modeling the extraction process with variations of parameters such as temperature, ultrasonic frequency and sonication time is necessary because it helps researchers to predict and to obtain optimum value of extraction results, so as to reduce time and operational costs. Some models of artificial intelligence are response surface model (RSM) [11], fuzzy inference system (FIS) [12], artificial neural network (ANN) [13], genetic programming (GP) [14] and regression trees [15], which increase in popularity among researchers. Most of these intelligence systems cannot connect all parameter operations to target variables with high accuracy. Thus, in order to overcome this problem, the development of existing artificial intelligence models to better control the ultrasound extraction process is required.

Some researchers have developed an ANFIS model, a combination of two systems of fuzzy logic models and artificial neural networks. The ANFIS model is based on fuzzy inference systems trained using learning algorithms derived from artificial neural network systems. Thus, the ANFIS system has all the advantages possessed by fuzzy inference systems and artificial neural network systems. Therefore, the ANFIS has higher training speed grades, more effective learning algorithms, and better predictability of artificial neural networks [16]. ANFIS model has been used to estimate delamination effect on medium density fiber board (MDF) [17] and to modelling the effects of ultrasound power and reactor dimension on the biodiesel production yield [18]. Based on our literature study, no study reported on the use of the ANFIS model for predicting of flavonoid compounds from guava leaves.

2. Materials and methods
2.1 Experimental design
The extracted object used in this study was fresh guava leaves (Psidium guajava L.) which are obtained from Temanggung, Central Java, Indonesia. Ultrasonic waves are generated by ultrasonic transducers that serve to convert electrical energy into ultrasonic waves, and vice versa.

![Figure 1. Experimental set up of extraction system using UAE](image)

For each experiment 0.5 g guava leaves were blended in 15 ml solvent. The blended mixture was put in the extraction tube for sonication. During the extraction process, variations of temperature (25-
35°C), sonication time (20-40 minute), and ultrasonic frequency (30-40 kHz) were applied. Temperature monitoring was performed to obtain optimal results. The data generated from the LM35 temperature sensor was processed by the ATSAM3X8E microcontroller, sent to the computer, and then displayed in the Delphi software application as an interface media. Furthermore, the extraction results were analyzed to find the flavonoid content of the sample using spectrophotometry UV-VIS.

2.2 Adaptive Neural Fuzzy Inference System (ANFIS)
In this research, the optimization modeling of extraction parameters using Adaptive Neural Fuzzy Inference System (ANFIS) was applied, which is a combination of fuzzy logic mechanism described in artificial neural network. The development of ANFIS model using MATLAB R2014b was done by entering the input variables, such as temperature, ultrasonic frequency and time of sonication. The output variable was flavonoid compound obtained from the extraction. The ANFIS architecture which consists of 12 rules with AND logic as a link for all rules is shown in Fig. 2.

Figure 2. Architecture of ANFIS model

The ANFIS training was conducted using 12 data sets obtained from the experimental results. Hybrid algorithms and subtractive clustering methods were used in the creation of FIS structures by varying the range of influence parameters to generate ANFIS systems, the best ANFIS architecture selected by comparing the mean root mean square error (RMSE), mean absolute percent error (MAPE) and mean absolute error (MAE) from the smallest number of training and data testing. Data testing was performed to evaluate the performance of the ANFIS system using 3 untried data. Testing of input and output sets resulting in minimal error values is the predicted result of optimal flavonoid compounds. The steps used to develop an ANFIS model are shown in Fig. 3.
2.3 Statistical analysis
To validate and examine the performance of the ANFIS prediction, 3 untried data sets with hybrid and subtractive clustering methods were performed. The criteria used to measure the performance of the best ANFIS architecture model was root mean squares error (RMSE), mean absolute percentage error (MAPE), and mean absolute error (MAE) as shown in equation (1), (2), and (3) consecutively.

\[
RMSE = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (Y_{i, \text{exp}} - Y_{i, \text{pred}})^2}
\]

\[
MAPE = \left( \frac{1}{N} \sum_{i=1}^{N} \left( \frac{Y_{i, \text{pred}} - Y_{i, \text{exp}}}{Y_{i, \text{exp}}} \right) \right) \times 100
\]

\[
MAE = \frac{1}{N} \sum_{i=1}^{N} |Y_{i, \text{exp}} - Y_{i, \text{pred}}|
\]

3. Results and Discussion
The extraction process of guava leaves conducted with variation of temperature, frequency and time of sonication using UAE has been performed. Experimental results were predicted using the ANFIS model to obtain optimal results. The ANFIS procedure began by inserting a variable set of input and output data for data training. Hybrid algorithms and subtractive clustering methods were used in the manufacture of FIS structures and range of influence parameters varied from 0.1 to 0.7 with an increase of 0.2 to generate ANFIS systems. The value of RMSE for training data is shown in Fig. 4.
Based on Figure 4, the smallest value of RMSE for training at the range of influence 0.1 with the value is $0.70 \times 10^{-4}$. It can be seen that a better learning with the smallest training error was achieved by using hybrid algorithm. This is the same result reported by Mostafaei et al. [18]. To evaluate the performance of the ANFIS system, the data were examined using 3 untried data. The data was then compared by RMSE, MAPE and MAE values obtained from the examine data (Figure 5).

From Figure 5, it can be seen that the best ANFIS architecture was obtained at the smallest value of RMSE, MAPE and MAE. Namely, at range of influence 0.1, the values of RMSE, MAPE, MAE from the examine data are 8.11, 2.7 % and 7.72. The number of system parameters of the best developed ANFIS model is shown in Table 1.
Table 1 System parameters of ANFIS model

| Parameter                        | Value |
|----------------------------------|-------|
| Number of nodes                  | 102   |
| Number of linear parameters      | 48    |
| Number of nonlinear parameters   | 72    |
| Total number of parameters       | 120   |
| Number of fuzzy rules            | 12    |

The best number of members for each input is 12, with the type of membership using the Gaussian type and the output part of each rule using a linear defuzzifier formula. Comparison of experimental flavonoid values with the ANFIS model variations in frequency, temperature and extraction time is shown in Table 2.

Table 2 Comparison of experimental flavonoid values with the ANFIS model

| Frequency (kHz) | Temperature (°C) | Extraction Time (minute) | Yields of Flavonoid (ppm) |
|-----------------|------------------|--------------------------|---------------------------|
| 30              | 30               | 20                       | 308.71                    | 300.00                    |
| 30              | 30               | 40                       | 299.23                    | 299.00                    |
| 30              | 25               | 30                       | 304.13                    | 300.00                    |
| 30              | 30               | 30                       | 302.98                    | 300.00                    |
| 30              | 25               | 20                       | 296.52                    | 297.00                    |
| 35              | 25               | 20                       | 217.05                    | 217.00                    |
| 35              | 25               | 40                       | 269.85                    | 270.00                    |
| 35              | 35               | 20                       | 262.67                    | 273.00                    |
| 35              | 35               | 40                       | 261.42                    | 261.00                    |
| 35              | 30               | 30                       | 157.56                    | 158.00                    |
| 40              | 30               | 20                       | 165.38                    | 165.00                    |
| 40              | 30               | 40                       | 204.13                    | 204.00                    |
| 40              | 25               | 30                       | 164.44                    | 164.00                    |
| 40              | 35               | 30                       | 589.02                    | 589.00                    |
| 40              | 35               | 40                       | 542.77                    | 543.00                    |

The optimum conditions of yields of flavonoid is obtained from the smallest error value of each data between the experimental results and the ANFIS prediction. The error value of each data is presented in Fig. 6. The smallest error value is shown in the 14th data with the value is 0.003.
Optimum yields of flavonoid were obtained at a temperature of 35°C and frequency of 40 kHz, for 30 minutes. These results indicated that the ANFIS model can connect all input parameters with the result of extraction at high accuracy [16]. Comparison of yields of flavonoid obtained experimentally and predicted by the ANFIS model is presented in Fig. 7.

The result shows that the deviation of yields of flavonoid predicted by the ANFIS model compared to experimental values is very small, which means that the ANFIS model has good ability for prediction of flavonoid yield. The smaller the error training value obtained, the better the predicted results of flavonoid compounds. Research done by Dahmoune [13] that is to model the effect of ultrasonic power for prediction ability of phenolic compounds recovery using ANN model. The value of training RMSE obtained 0.372 whereas in this study the value of RMSE training using ANFIS is...
0.70 x 10⁻⁴. These results prove that the ANFIS has higher training speed grades, more effective learning algorithms, and better predictability of artificial neural networks [16].

4. Conclusion

The ANFIS model can be used to predict the content of flavonoid compounds in guava leaves using UAE with the variable parameters: temperature, frequency and time of extraction. Hybrid algorithms and subtractive clustering methods with a range of influence of 0.1 are selected to build the best ANFIS architecture to obtain optimal prediction results because they have the smallest error value. ANFIS prediction capability was evaluated using statistical criteria RMSE, MAPE and MAE where the RMSE for training, RMSE, MAPE and MAE from testing values obtained are 0.70 x 10⁻⁴; 8.11, 2.7 % and 7.72. Optimum yields of flavonoid were obtained at a temperature of 35°C and frequency of 40 kHz, for 30 minutes.

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