Optimization Extraction of *Indigofera tinctoria* L. using Microwave-assisted Extraction

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**Abstract.** Dyes are many used in textiles, food, drink, and cosmetics. In the textile industry, the demand for dye is increasing, especially in natural dye. One of the natural dye that is often used in those products is *Indigofera tinctoria* L. due to its various potential. However, the time extraction needed is very long in the conventional extraction process. Because of that, some researchers have proved that microwave-assisted extraction takes a short time and high yield extraction obtained because microwave radiation is transmitted to the plant matrix that increasing solubility of solvent, and decreasing surface tension. Correlated with that, *Indigofera tinctoria* L. dye extraction using microwave-assisted extraction has been analyzed by modeling with response surface methodology with Box-Behnken Design (BBD) with parameters of ratio feed to solvent (0.02-0.1 g/ml), temperature (50-60°C) and extraction time (50-90 minutes). The obtained model could represent the extraction process and showed there an interaction among parameters on the yield, but ratio F/S had the most significant effect. Besides, the difference of optimization model obtained at optimum condition process extraction at ratio feed to solvent 0.02, temperature 60°C, and 50 minutes. The actual yield is 4.976% is closed to the predicted yield which the value is 4.85%. The compounds and similarity of the sample were analyzed using FT-IR and the similarity compound of indigo is 39.11%.

**Keywords:** *Indigofera tinctoria* L., microwave-assisted extraction, Box-Behnken Design

1. **Introduction**

Dyes are used in the textile, food, drink, and cosmetic industry. The use of dyes in the industry is very important, especially in the textile industry. The dyes which usually used are synthetic and natural. Synthetic dyes are more commonly used than natural dyes because it is easier to get it, and practically use it. Besides, synthetic dyes are more stable, more resistant to various environmental conditions, stronger color strength and have a wider color range and are not easily wear off, and have a variety of reinforcement [1].

The use of synthetic dyes in the textile or clothing industry produces waste that has an impact on the environment. This dye waste is generally a non-biodegradable organic compound that can cause environmental pollution, especially in the aquatic environment if the synthetic dye is not managed properly. Because of the pollution from the waste of synthetic dye, the alternative use is a natural dye in which the source is from natural ingredients [2].

*Indigofera tinctoria* L. is one of the plants that can be taken as a natural coloring dye. *Indigofera* plants including small shrubs with upright or scattered branches covered indumentum in the form of
two-pronged feathers. The leaves are alternating, odd-finned sometimes with three or single leaflets [3], [4]. Recently, the extraction of *Indigofera tinctoria* L. leaves is still relatively with the conventional method. During this process, it takes a long extraction time and a lot of solvents. Yields extraction of natural dyes from the conventional method is very low. From previous studies, the extraction of natural dyes from *Ricinus Communis* L. leaves using the Microwave-assisted Extraction method need less time extraction results than conventional extraction methods [5]. The extraction of anthocyanin in grape with the Microwave-assisted Extraction method can extract 60 times faster than the conventional method [6]. This shows that extraction using the Microwave-assisted Extraction method can save energy, time, and money.

2. Materials and Methods

2.1. Materials

The *Indigofera tinctoria* leaves obtained from gardens around the campus of the department of chemical engineering, Sepuluh Nopember Institute of Technology, Surabaya, Indonesia.

2.2. *Indigofera tinctoria* L. extraction

The study was taken in the Technology Process laboratory of the Chemical Engineering Department ITS. The extraction process was done using a microwave oven used for this research is the KRISBOW ELECTROLUX EMM2308X, which has a height x width x depth dimension of 292,5 mm x 485 mm x 370 mm with maximum power 800W and frequency of 2.45GHz. Four grams *Indigofera tinctoria* L. leaves and 200mL of aquadest were put in a flask 500 mL, then placed in a microwave oven and the system was turned on. The process was done at 50 minutes, then the extract added by Ca(OH)\(_2\) solution. There were two phases in solution, sediment, and liquid. The liquid was discarded and heated at 70°C until it formed solid dye extract. Next, the dye extract was analyzed by calculation of yield extraction: the weight of dye divided by the weight of *Indigofera tinctoria* L. (g/g) and identification similarity of component: Fourier Transform Infra-Red in Assessment Service Unit of Material Metalurgi ITS.

2.3. Response surface methodology

Response surface methodology (RSM) is one of the experiment design that allows researchers to study the effect of independent variables interaction on response variables. This modeling has been using widely due to can reduce the number of matrix variables design so that in practice, research can be relatively shorter cost and time [7]. Box-Behnken design, three-level factor response surface methodology, was applied for three parameters in the Design-Expert version 11 trial with 17 run numbers, including center points, and for approaching the variable response, the quadratic model was used as shown in Equation 1.

| Table 1. Design parameters of response surface methodology Box-Behnken. |
|-----------------------------|-----------------------------|
| Level | A: Temperature (°C) | B: Feed/solvent ratio (g/mL) | C: Extraction time (min) |
| -1   | 50             | 0.02           | 50             |
| 0    | 55             | 0.06           | 70             |
| +1   | 60             | 0.1            | 90             |

\[
Y = \beta_0 + \sum_{i=1}^k \beta_i x_i + \sum_{i=1}^k \beta_{ii} x_i^2 + \sum_{1 \leq i < j}^k \beta_{ij} x_i x_j + e
\] (1)

Where Y is the yield of Indigofera tinctoria L. dye as a variable response, \(\beta_0\), \(\beta_i\), \(\beta_{ii}\), \(\beta_{ij}\) are intercept, linear, square, and 2-way interaction term respectively, while \(x_i\) and \(x_j\) represent the independent variables, \(k\) is variables number, and \(e\) is the residual associated to the experiment. Furthermore, the obtained model was analyzed statistically using analysis of variance (ANOVA).
3. Results and discussion

3.1. Box-Behnken design of Indigofera tinctoria L. dye extraction

Box-Behnken design of Indigofera tinctoria L. dye extraction using microwave-assisted extraction obtained response model equation in coded form as shown in Equation 4.

\[
Y = 230000 + 0.100000A - 1.43125B - 0.018750C - 0.001250A^2 + 0.75125B^2 - 0.088750C^2 - 0.175000AB - 0.040000AC + 0.287500BC
\]

According to the analysis of variance as shown in Table 2, the p-value of the model equal to 0.0028 which the value less than 0.05. The model terms are significant if the p-value is less than 0.05. In this case, B and B^2 are significant model terms. Values greater than 0.1 indicate the model terms are not significant. The model F-value of 10.29 implies the model is significant. There is only a 0.28% chance that an F-value this large could occur due to noise.

### Table 2. Analysis of variance

| Source            | DF | Adj SS | Adj MS | F-Value | P-Value |
|-------------------|----|--------|--------|---------|---------|
| Model             | 9  | 19.32  | 2.15   | 10.29   | 0.0028  |
| Linear            | 3  | 16.47  | 5.49   | 16.55   | 0.0001  |
| A                 | 1  | 0.0800 | 0.0800 | 0.3833  | 0.5554  |
| B                 | 1  | 16.39  | 16.39  | 78.52   | <0.0001 |
| C                 | 1  | 0.0028 | 0.0028 | 0.0135  | 0.9108  |
| Square            | 3  | 2.39   | 0.7973 | 3.82    | 0.0656  |
| A^2               | 1  | 0.00007| 0.00007| 0.0000  | 0.9957  |
| B^2               | 1  | 2.38   | 2.38   | 11.39   | 0.0119  |
| C^2               | 1  | 0.0332 | 0.0332 | 0.1589  | 0.7020  |
| 2-Way Interaction | 3  | 0.4595 | 0.1532 | 0.3976  | 0.7577  |
| AB                | 1  | 0.1225 | 0.1225 | 0.5870  | 0.4686  |
| AC                | 1  | 0.0064 | 0.0064 | 0.0307  | 0.8659  |
| BC                | 1  | 0.3306 | 0.3306 | 1.58    | 0.2485  |
| Error             | 7  | 1.46   | 0.2087 |         |         |
| Lack-of-Fit       | 3  | 1.02   | 0.3410 | 3.12    | 0.1505  |
| Pure Error        | 4  | 0.4378 | 0.1095 |         |         |
| Total             | 16 | 20.78  |        |         |         |

R^2 = 0.9297; R^2adj = 0.8393; R^2pre = 0.1794

Meanwhile, the lack of fit F-value of 3.12 which implies the lack of fit is not significant relative to the pure error. There is a 15.05% chance that lack of fit F-value this large could occur due to noise. A non-significant lack of fit is good, and we want the model to fit. The adequacy of the Box-Behnken model for Indigofera tinctoria L. dye extraction using microwave-assisted extraction was also validated by regression value which approach to one as performing coefficient of determination (R^2) = 0.9297; adjusted coefficient of determination (R^2adj) = 0.8393. The predicted R of 0.1794 is not as close to the adjusted R^2 of 0.8393 as one might normally expect, the difference is more than 0.2. This may indicate that there is a large block effect or possible problem with the model or data. And adequate precision measures the signal to noise ratio which value is 10.078, a ratio greater than 4 is desirable. The ratio of 10.078 indicates an adequate signal, so this model can navigate the design space. However, the normal probability chart (Figure 1) displays the normal distribution of the data following the diagonal line. Therefore, the model can represent the Indigofera tinctoria L. dye extraction process with consideration of model reduction.

3.2. Analysis contour plot of yield versus parameters extraction

Based on the analysis of variance that showing the significant effect of interaction among the parameters on the response, then the study of Indigofera tinctoria L. dye extraction using microwave-assisted extraction will be more effective when investigated based on the effect of these parameters changing simultaneously on extraction yield compared to the effect of one factor. The contour plot is
one of the analysis terms provided by RSM. This plot is generated from the model equation, where the yield changing is analyzed when two parameters changing at the constant value of the other.

![Normal probability plot](image)

**Figure 1.** Normal probability plot

Based on Figure 3a at extraction time of 70 min, the higher ratio of feed to solvent value at the high level of temperature, the high yield obtained but using the lower temperature not recommended especially at high feed/solvent ratio because it can reduce the yield of *Indigofera tinctoria* L. dye, then the dye can not be extracted maximally. Temperature is an important parameter for extraction since it contributes to increasing yield. With increasing temperature, the solvent has a higher capacity to solubilize the target compounds, while surface tension and solvent viscosity decrease, which improves sample wetting and matrix penetration. In MAE, the temperature depends on the solvent’s ability to absorb microwaves. The temperature is so quickly homogenous in the medium, and increasing of temperature can cause opening cell-matrix, then indigo availability for extraction increases [8], [9]. Other research also states that extraction with higher temperature conditions will result in more extracted results, but can also reduce the quality of the extracted substances and can change the composition of its composition [10].

![Contour plot](image)

**Figure 2.** Contour plot

The next contour plot, shown in Figure 3b at hold value of 0.06 g/mL feed/solvent ratio, which represents the increase of temperature, and extraction time. In this contour plot, there is no significant
increase or decrease in yield caused by temperature and extraction time. Meanwhile, in the several experiments that have been carried out can be seen that there is an increase in yield along with the increase in extraction time. One of the advantages of using microwave-assisted extraction is the short time taken compared to that conventional method. At 50 min to 70, there is increasing yield although insignificant, then fall not significant too. At decreasing yield, it shows that there is degradation which causes by long extraction time [11], [12].

Meanwhile, in Figure 3c can be observed the yield changing due to the change in extraction time and ratio feed to solvent at hold value of temperature 55°C. The contour shows the relationship between the F / S ratio (g / mL) and the yield of natural dyes. Based on Figure 3a, the greater the ratio of raw materials to solvents, the smaller the yield obtained. A decrease in yield is obtained, when the greater the ratio between the material to the solvent (the ratio of Indigofera tinctoria L. leaves to aquadest). This is due to the amount of water in the flask during the extraction process of Indigofera tinctoria L.leaves using the microwave-assisted extraction method. The amount of ratio between the material with this solvent influences the yield of the dye. The amount of solvent present in the flask has the same amount in each extraction process of Indigofera tinctoria L.leaves which is 200 ml. The lower ratio of feed to solvent may give lower recoveries due to the adequate stirring of the aquadest by microwave. Moreover, the excessive aquadest can cause a dissolution of the other undesirable compounds which make lowering the selectivity of target compounds[13], [14].

Therefore based on the three contour plot, and overall high in yield of extraction will be obtained at the condition temperature extraction range of 60°C; feed/solvent ratio of 0.02 g/mL, and extraction time of 50 minutes.

3.3. Response Surface Optimization

The high yields in the range of extraction parameters aforementioned in contour analysis can be optimized to get the optimum condition of the extraction process by finding the critical point of the model equation, the point where the differential of the model equals zero [15]. In this study, that condition was obtained using optimization in Design Expert version-11 trial by set the goal of response as a maximum value in the range of parameter design temperature extraction of 50°C-60°C, feed to solvent ratio of 0.02 g/mL-0.1 g/mL, and extraction time of 50 min-90 min. The output solution was at the temperature extraction of 60°C, feed to solvent ratio of 0.02g/mL, and extraction time of 50 min with the predicted yield of 4.976%. The experiment with the same value of the optimum conditions was run and obtained the actual yield of 4.85%. This actual yield close to the predicted yield with the residual value of 0.126. Therefore, the model fits the experiment and can represent the dye extraction process of Indigofera tinctoria L. dye using microwave-assisted extraction.
3.4. Analysis component of Indigofera tinctoria L. dye
In determining the quality of the blue *Indigofera tinctoria* leaf dyes obtained using the microwave-assisted extraction methods, it is necessary to conduct an FT-IR test to determine the characteristics of the functional groups contained in the *Indigofera tinctoria* L leaf dye extract. In Table 3 showed that Indigofera tinctoria leaf extract contains a similarity of Penicilin G potassium 47.97%, Calcium carbonate 40.52%, and indigo 39.11%. It showed that the extract contains an indigo component.

| Similarity | Name of compound                                      |
|------------|-------------------------------------------------------|
| 47.97      | PENICILIN G POTASSIUM IN KBR                           |
| 40.52      | Calcium carbonate 99%                                 |
| 39.11      | Indigo, synthetic                                     |
| 36.11      | Methylenecclobutane, tech., 96%                        |
| 35.10      | DEXBROMPHENIRAMINE MALEATE IN KBR                     |
| 33.30      | Diphenylglyoxime, 97%                                 |
| 32.53      | 1,2-Cyclohexanedione dioxime, 97%                     |
| 31.32      | 5-Methylthiazole, 97%                                 |
| 30.39      | Methylenecclopentane, 97%                             |

4. Conclusion
The obtained model of Box-Behnken design can represent *Indigofera tinctoria* L dye extraction using microwave-assisted extraction with the value of $R^2 = 92.97\%$, and $R^2_{\text{adj}} = 83.93\%$. Analysis of effect parameters showed there an interaction among parameters on the yield, but ratio F/S had the most significant effect. Based on the optimization of the model, the optimum condition of process extraction was at a temperature extraction of 60°C, feed/solvent ratio of 0.02 g/mL, and extraction time of 50 min. These conditions have been validated and got the actual yield closed to the predicted yield with the residual value of 0.126. Next, the FT-IR analysis of the sample shown the similarity compounds of *Indigofera tinctoria* L dye extract was Pencilin G. Potassium (47.97%), Calcium Carbonate (40.52%), and Indigo Synthetic (39.11%).

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