Response Surface Methodology (RSM) Analysis of Antipyretic Extracted from *Aquilaria Subintegra* Leaves

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***Abstract*— In this study, the optimization condition for extraction of antipyretic properties known as acetaminophen inside the *Aquilaria Subintegra* leaves was analyze using response surface methodology (RSM). The objective of this study was to determine the optimum condition for extraction of *Aquilaria Subintegra* leaves by using RSM and to investigate the effect of drying temperature on extraction of *Aquilaria Subintegra* leaves. RSM use a series of data to obtain an optimal response with minimal effort. Effect of drying temperature on the for the concentration of extraction on *Aquilaria Subintegra* leaves was studied and the data obtained will be inserted to second-order polynomial equation using multiple regression analysis and analyzed by analysis of variance (ANOVA) to determine the importance of the model. The extraction of the compound on different drying temperature and type of leaves was based on the previous study. Based on the result, the model F-value of 2.75 and Prob>F of 0.1102 indicate that the model is suitable to use. The high value of R^2 which is 0.6623 and smaller value of adjusted R^2 which is 0.4211 was compared to show the model was sufficient. The predicted optimum of drying temperature was 30ºC. The predicted value of concentration of the compound on Aquilaria leaves was 2.697 µg/mL and the error calculated was based on the previous study by taking the average value of error on both of the studies which is 2.04%. There is no actual value in this study since there are no validation experiment was run to prove the optimum value. In conclusion, the end results show that the response surface methodology is succeeded in determining the optimum drying temperature of Aquilaria Subintegra.**

Keywords*— *ANOVA, Aquilaria Subintegra, Optimization, RSM

I. INTRODUCTION

Response surface methodology (RSM) is a compilation of statistical and mathematical techniques useful for developing, enhancing and optimizing [1]. The main principle of RSM is to use a series of data to obtain an optimal response. There are three types of RSM, the first-order, the second-order, and three-level fractional factorial. RSM also can be used for food analysis to obtain optimum condition for example, modeling for extrusion cooking process [2]. By utilizing RSM, information can be obtain with less cost and short time also obtain rapid and efficient development of new products and processes [3].

The implementation of RSM in the design optimization is focused at minimizing the difficulties of other analysis methods such as finite element method or computational fluid dynamics (CFD) analysis. RSM is functional in three different methods or technique which is first, a statistical experimental design (two level factorial or fractional factorial design) [1]. Second, a regression modeling technique and lastly, optimization methods. The relationship between a response that we set which is y and a number of related control variable which represented by $x_1, x_2, \ldots, x_n$ can be show as Eq. 1

$$y = f(x_1, x_2) + e \qquad \text{(Eq. 1)}$$

The response y depends on the variables x_1 and x_2 also variable x_1 and x_2 are the independent variables. Variable y is a function of x_1 and x_2, and term e represent the experimental error. The true response function f is uncertain and term e are not consider in f. Normally term e is consider as a statistical error and assume to have a normal distribution with mean zero and variance s^2. Since the true response function f is unknown, a low-order polynomial is use to develop an approximation for f in several small regions. If there is a linear function of independent variable responses involve, a first-order model function can be use. The first-order model is suitable when there is little curvature in f for independent variable space. The first-model with the independent variable can be denoted as Eq. 2

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + e \qquad \text{(Eq. 2)}$$

The first-order equation above is also known as main effects model, it is because the model only involves the main effects of the two variable x_1 and x_2. Then, if there is an interaction between the variables, the addition term is needed to be inserted in the new response function Eq. 3

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{12} x_1 x_2 \qquad \text{(Eq. 3)}$$

Sometimes, the first-order model is sufficient because of the curvature in the true response even with the interaction term is strong. In this case, the second-order model is used. The second-model with two variables can be denoted as Eq. 4

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_{11} x^2_{11} + \beta_{22} x^2_{22} + \beta_{12} x_1 x_2 + e \qquad \text{(Eq. 4)}$$

The second-order model is popular in response surface methodology because first, it is very flexible and be able to take a number of functional forms so it can approximate the true response surface [4]. Second, the parameters, β is easy to estimate in the second-order model by using the method of least squares. Lastly, solving problem by using second-order models is more towards practical experience. Generally, both of the model can be use since in each model, the level of each factor are individual levels of other factor. To get the data in the approximation of polynomials, an experimental design must be conducted properly. The method of least square method is used to determine the parameters in the polynomials once the data was obtained. Then the response surface analysis conducted by using fitted surface.

Central Composite Design (CCD) is one of the most popular design for fitting a second-order model. It was introduced by Box and Wilson. The design consists of factorial points, central points and axial points. Whereas, the factorial design is from 2^q design and 2^{q-k}. Through a sequential experimentation, the CCD was developed. When lack of fit occur in the first-model, axial point was added to quadratic terms with more center points to construct CCD. The distance *a* of the axial and the number of center points, n_c, at the origin runs from the design center are two parameters in the CCD design. The center contains the details about the curvature of the surface and efficient estimation of the quadratic terms from additional axial points can be obtain if the curvature is notable.

Since the aim is to find the fit for the second-order model using CCD, the ANOVA and regression analysis was used for the response variable. In this research, the optimization condition for extraction of *Aquilaria Subintegra* by using RSM is under the second-order model.

Aquilaria is a tree that is also known as agarwood or gaharu. This type of tree when attacked by fungus, able to produce agarwood resins that is highly aromatic that can be used as a main component in perfume and the main uses of aquilaria subintegra is in medicinal which will be discuss later. Aquilaria belong to the family of Thymelaceaceae and also known with more than 15 species [5]. The tree is able to grow up into 40 m tall and the diameter is range from 0.6 to 2.5 m [6]. This tree can grow in many types of environment such as well-drained slopes, sandy and rocky places. Aquilaria subintegra are commonly found in some part at Thailand.

In medicinal field, aquilaria plants could give many benefits. Different studies have been done to explore the medicinal capability of this plant. Aquilaria subintegra could be used to treat Alzheimer's disease due to the extract of the plant leaves [7]. This plant also has been used as a traditional medicine used to treat aches, anaphylaxis, inflammation and muscular pains also it proven to have hepatoprotective and anti-inflammatory activity [8]. It also has potential as an anticancer and antidepression activity in the tree extract [9]. Furthermore, the leaves also have capability in anti-diabetic activity [10].

This medicinal benefits from the plants was all due to the presence of phytochemicals which act as health supplement. Phytochemicals such as 2-(2- phenylethyl) chromenes, lignans, diterpenoids and flavonoid glycosides were found in this plant [8]. In the leaves extract, there are presence of the chemical constituents of alkaloids, tannins, saponins and terpenoids [9]. This shows that the aquilaria plants have antipyretic compound in it. Antipyretic is a drug to soothing down fever, migraine, sore throat and many more. In the aquilaria plant, there also antipyretic and analgesic compound which called acetaminophen or in other name 4'-hydroxyacetanilide [11]. 4'-hydroxyacetanilide is in a form of white crystal and has a molecular weight of 151.06 g/mol. This compound also usually found in the synthetic drugs. Excessive dosage of acetaminophen may bring harm to internal organs. This antipyretic compounds were needed to be analyze on the effect of drying temperature of the plant and one if the core component in this research [11].

II. METHODOLOGY

A. Experimental Data of Extraction of Aquilaria Leaves

Experimental data of extraction of the Aquilaria leaves were obtained from the previous research by Siti Khairun Nissa' on "Determination of Acetaminophen Compound from *Aquilaria Sp.* Leaves by Hydrodistillation and its Antibactrobial Activity on *E. Coli*" [12]. The data are consisting of a range of drying temperature and different type of *Aquilaria* leaves such as *Aquilaria Malaccencis*, *Aquilaria Subintegra* and *Aquilaria Crassna*. The data for *Aquilaria Malaccencis* and *Aquilaria Crassna* serve as variable in the software. The software used for the optimization is Design Expert Software version 10.0.1 and Central Composite Design (CCD) were selected as the design in the software. The data were then inserted into the software to study the effect of the drying temperature on extraction of *Aquilaria Subintegra* leaves.

Table 2.1: Experimental data of extraction of the Aquilaria leaves

Sample	Temperature (ºC)	Mass (g)	Concentration (µg/mL)
Aq. Malaccencis	30	6	4.12357
	40	6	0.81565
	50	6	1.00602
	60	6	1.05685
	70	6	1.26613
Aq. Subintegra	30	6	1.18288
	40	6	2.71380
	50	6	3.30969
	60	6	9.81144
	70	6	4.44353
Aq. Crassna	30	6	1.44665
	40	6	2.89285
	50	6	3.13581
	60	6	15.60389
	70	6	4.18213

B. Response Surface Methodology (RSM) Analysis

In this study, face-centered Central Composite Design (CCD) has been selected as experimental design. The numeric factor for analysis was input to the software which is 2 referring to the two independent variable which is drying temperature and type of *Aquilaria* leaves. The variable value was input as shown as table 2.2.

Table 2.2: Criteria for optimization for extraction of compound in terms of concentration

Variables	Target	Lower limit	Upper limit
Temperature (ºC)	Minimize	30	70
Type of leaves	Is target = 0	-1	1
Concentration of compound (µg/mL)	Maximize	1.0062	4.44353

Where type,
Aq. Malaccencis= -1
Aq. Subintegra = 0
Aq. Crassna = 1

The procedure is continued by setting the experimental response to 1 and the process response which is the concentration of compound were input in the software as shown in table 2.3

Table 2.3: Experimental values of response variables for central composite design

Run	Temperature (ºC)	Type	Concentration of Antipyretic compound (µg/mL)
1	50	0	3.30969
2	50	0	3.30969
3	70	0	4.44353
4	50	0	3.30969
5	50	0	3.30969
6	50	1	3.13581
7	70	1	4.18213
8	30	0	1.18288
9	50	-1	1.00602
10	30	1	1.44665
11	50	0	3.30969
12	70	-1	1.26613
13	30	-1	4.12357

Then, the quadratic order and polynomial model was selected to represent second-order polynomial model also, the transformation was selected to none for typical responses. The result for the optimization responses was analyzed.

III. RESULTS AND DISCUSSION

A. *The effects of temperature on the concentration of Antipyretic compound*

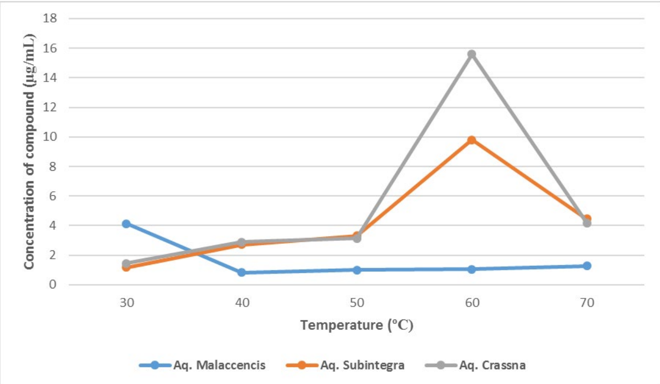

Figure 3.1: Effect of temperature on concentration of Antipyretic compound at different type of leaves

In figure 3.1, the graph shows the effect of temperature on concentration of compound at different type of leaves. From the figure, the graph shows the concentration increase as temperature increase until 60ºC then drop at 70ºC for Aq. Subintegra and Aq. Crassna leaves. As for Aq. Malaccencis the concentration decrease and remain almost constant. The increasing and decreasing of the graph indicates the limit of the drying temperature of the Aq. Subintegra and Aq. Crassna leaves which is 60ºC. As the temperature of the drying increase to 70ºC, the heat destroys the enzyme action present in the plant cell [13]. As for the Aq. Malaccencis, it must happen due to the Aq. Malaccencis nature to unable to yield high amount of the antipyretic compound by increasing the temperature.

B. *Second order polynomial equation*

The quadratic model was chosen due to value of adjusted R^2 of quadratic was slightly smaller than 2FI. Based on the result it was discovered that relationship between temperature and type of leaves towards the concentration of the compound were fitted to second-order polynomial as shown in Eq. 5 below

$$Y = 2.29699 + 7.84118\times10^{-3} A - 3.10077 B + 0.069912 AB - 1.83145\times10^{-4} A^2 - 0.66903 B^2 \quad \text{(Eq. 5)}$$

Where;
Y = Concentration of Antipyretic compound (µg/mL)
A = Temperature (ºC)
B = Type of Aquilaria leaves

The acceptability of the model was evaluated on the value of coefficient of determination (R^2). The closer the value of the R^2 to 1, the closer the predicted response to actual response. In this analysis, the value was 0.6623 and the value can be considered high which indicate the predicted value of concentration of compound was close to the actual value. High value of R^2 show that significance of the model was high.

C. *Statistical analysis*

To test the model fitted quality, the analysis of variance (ANOVA) was used. ANOVA was used to identify the significant effects of the relationship between the independent variable and generated response which was compatible with the second order polynomial fitting data. The significant of the model was known by observing the F-test for confidence of 95% and lack of fit test. F-test was used in ANOVA to compare the fits of different models, to test the overall significance for regression model, to test the quality of means and to test specific regression terms [14]. The high number of F-value and small number of p-value indicates that the model was significant. For significant lack of fit, it must be ($p>0.05$) to show the model was significant. Table 3.1 shows the data for ANOVA for response surface quadratic model.

The model F-Value of 2.75 implies the model is not significant relative to the noise. There is a 11.02% chance that a F-Value this large could occur due to noise. Values of Prob>F less than 0.05 indicate model terms are significant. In this case AB is a significant model term. Values greater than 0.1000 indicate the model terms are not significant. The high value of R^2 which is 0.6623 and smaller value of adjusted R^2 which is 0.4211 was compared to show the model was sufficient.

Table 3.1: ANOVA for Response Surface Quadratic Model

Source		Sum of square	DF	Mean square	F-value	Prob>F
Model		11.74	5	2.35	2.75	0.1102 Not Significant
	A: Temperature (ºC)	1.64	1	1.64	1.92	0.2084
	B: Type of leaves	0.94	1	0.94	1.09	0.3304
	AB	7.82	1	7.82	9.15	0.0193
	A^2	0.015	1	0.015	0.017	0.8990
	B^2	1.24	1	1.24	1.45	0.2683
Residual		5.99	7	0.86		
	Lack of fit	5.99	3	2.00		
	Pure error	0.000	4	0.000		
	Correction total	17.73	12			
	R^2	0.6623	Adjusted R^2	0.4211		

D. *Graphical presentation of model equation*

The relationship between drying temperature and type of the leaves to the response of concentration of the compound can be shown in the contour plot on x-y plane and three dimensional response surface plot based on the model equation. Figure 3.2 and figure 3.3 shows the contour plot and three dimensional plot respectively. R1 indicates the concentration of compound in µg/mL.

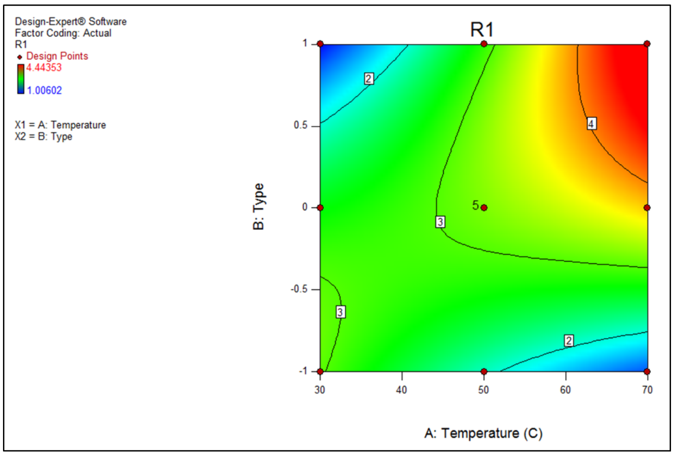

Figure 3.2: Contour plot for concentration of compound

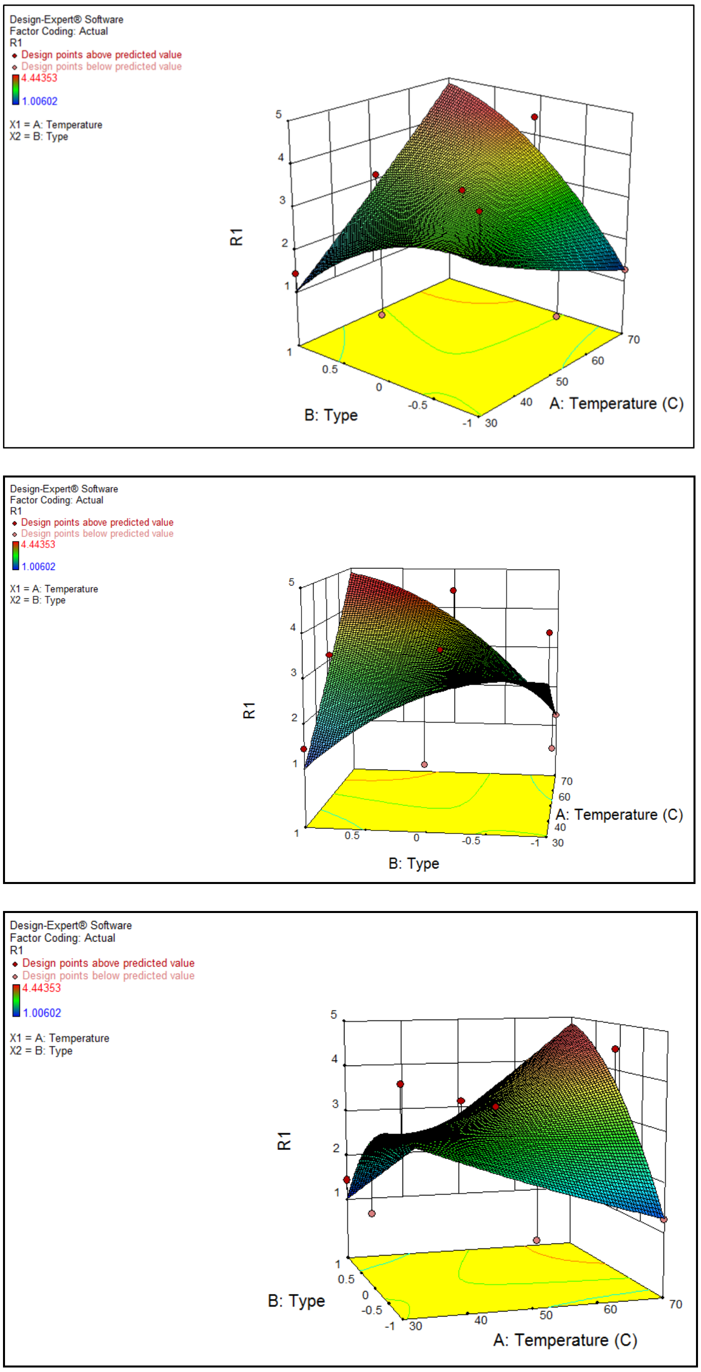

Figure 3.3 (a), (b) and (c) (From top, middle and bottom respectively): Different view of three dimensional response surface plot of concentration of compound

Models are used to predict in order to generate response surface graph and contour plots. There was notable relationship between drying temperature and type of leaves on the response surface graphs and contour plots as variation of process conditions as shown in figure 3.2 and 3.3. As illustrated in figure 3.2 and 3.3, type -1 (Aq. Malaccencis) show the decreasing yield of concentration of the compound as the temperature increase. Meanwhile, type 0 (Aq. Subintegra) and type 1 (Aq. Crassna) show the increasing yield of concentration of the compound as the temperature increase. The yield of concentration of the compound in type 1 leaves is higher than type 0 leaves as the temperature increase.

E. Optimization

Optimization of the drying temperature of Aq. Subintegra leaves by concentration of antipyretic properties was performed to determine the desired criteria for the response. By utilized the desirability function method, only 1 solution was generated by the software since the optimization was focused on Aq. Subintegra leaves type only. Table 3.2 shows the optimum condition of the process variable with predicted values of response generated by the software. The closer the desirability to 1 the better the solution and since there was only 1 solution thus the only solution was the best one. Figure 3.4 shows the solution generated by the software according to the solution.

Table 3.2: Desirable solutions for optimized process of extraction

Number	Temperature (ºC)	Type of leaves	Concentration of compound (µg/mL)	Desirability
1	30	0	2.697	0.789

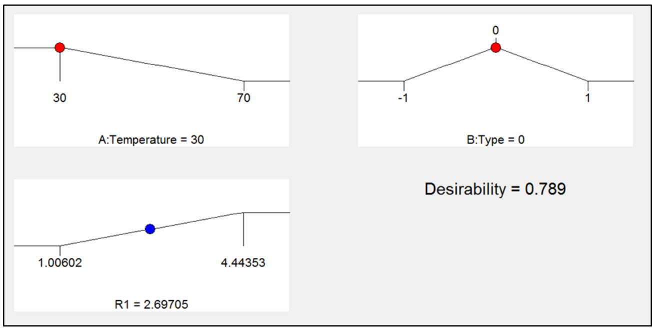

Figure 3.4: The solution generated based on the model selected

Based on the figure 3.4, the desirability was 0.789 and the value was high enough to 1. Since the study was focused on Aq. Subintegra leaves there only 1 solution available. Different result can be presented if the study was focused on all the types of the leaves and the solution generate will be more diverse.

F. Model validation

Since the validation experiment cannot be run to validate the optimum condition due to the time constrain. The model was validate by comparing the model validation of the study by past researchers. This model will be compared with to research which by Izwan Hafidzi, 2017 on "Response Surface Methodology (RSM) Analysis of Oil Removal by using Tecoma Leaves" and by Fatin Nurainaa, 2016 on "Response Surface Methodology (RSM) Analysis of Aquilaria Leaves for Optimum Drying Conditions".

In Izwan Hafidzi study, the optimization was performed to find the optimum condition of the percentage oil removal on the effect of pre-treatment process on Tecoma leaves and volume of extracted oil. Meanwhile in Fatin Nurainaa study, the optimization was performed to find the optimum condition of the percent water moisture removal on the effect of the drying temperature and pressure of the leaves. Table 3.3 and 3.4 shows the model validation of Izwan Hafidzi and Fatin Nurainaa study respectively.

Table 3.3: Model validation of Izwan Hafidzi study on "Response Surface Methodology (RSM) Analysis of Oil Removal by using Tecoma Leaves"

Model desirability	Pre-treatment (pH)	Volume of waste oil (mL)	Oil removal (%)		Error (%)
			Predicted	Actual	
1	5	6	95.23	97.2	2.03

Table 3.4: Model validation of Fatin Nurainaa study on "Response Surface Methodology (RSM) Analysis of Aquilaria Leaves for Optimum Drying Conditions"

Model desirability	Temperature (ºC)	Pressure (mbar)	Moisture removal (%)		Error (%)
			Predicted	Actual	
1	46.47	-600	63.3702	62.0719	2.05

As shown in table 3.3, the optimum condition of oil removal was on 5 pH and 6mL volume of waste oil which predicted to yield 95.23% of oil removal. After undergo the validation experiment, the actual value of oil removal was 97.2%. Thus the error was calculated which was 2.03%. For table 3.4, the optimum condition of water moisture removal was at 46.47 ºC temperature of drying and -600 mbar pressure of drying which predicted to yield 63.3702% of moisture removal. After undergo the validation experiment, the actual value of moisture removal was 62.0719%. The error calculated was 2.05%. Based on both of the data, the error of the current study could be taken the average of the error of both past research which was

$(2.03 + 2.05)/2 = 2.04\%$

Thus, the model validation can be draw as table 3.5

Table 3.5: Model validation

Model desirability	Temperature (ºC)	Type of leaves	Concentration of compound (µg/mL)		Error (%)
			Predicted	Actual	
1	30	0	2.697	-	2.04

IV. CONCLUSION

The purpose of this study is to find the optimum condition of the effect of drying temperature towards the extraction of antipyretic compound in the Aquilaria Subintegra leaves by using RSM. The concentration of the extraction of antipyretic compound was predicted by using second order polynomial model. The model F-value of 2.75 and Prob>F of 0.1102 indicate that the model is suitable to use. The high value of R^2 which is 0.6623 and smaller value of adjusted R^2 which is 0.4211 was compared to show the model was sufficient. The predicted optimum of drying temperature was 30ºC. The predicted value of concentration of the compound on Aquilaria leaves was 2.697 µg/mL and the error calculated was based on the previous study by taking the average of the studies which is 2.04%. There is no actual value in this study since there are no validation experiment was run to prove the optimum value. In conclusion, the end results show that the response surface methodology is succeeded in determining the optimum drying temperature of Aquilaria Subintegra.

However, there were few recommendations and suggestion that can be apply to improve for the future study which were by adding more variable and parameters such as the drying pressure, volume of solvent and time of the extraction to ensure the result obtained are more diverse and flexible to find the optimum condition for the extraction process. Then, the validation of experiment should be run to obtained the actual data so that the actual error can be obtained and the accuracy of the simulation can be reliable. Lastly, compare the result with all types of the leaves since in this study it was only focused on Aquilaria Subintegra leaves only.

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