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COMPUTER-AIDED OPTIMIZATION OF PHYTOCHEMICAL EXTRACTION: BOX-BEHNKEN DESIGN-RESPONSE SURFACE METHODOLOGY (BBD-RSM) APPROACH TO *Mimusops Elengi* L. LEAVES FLAVONOIDS

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ABSTRACT

Tanjung (*Mimusops elengi* L.) leaves contain flavonoids that exhibit antimicrobial, antifungal, and antioxidant properties. The extraction conditions must be optimized to obtain an extract with the highest flavonoid content. This study was conducted to determine the optimal extraction conditions using Response Surface Methodology (RSM). The RSM study consisted of stage I, which involved a one-factor-one-time approach to screen factors influencing the extraction and determine their levels, and stage II, which utilized the Box-Behnken Design three-level-three-factor to obtain a regression equation model. The obtained regression equation model was then tested using the Lack of Fit test, individual and simultaneous regression parameter tests, and residual assumption tests. The results of the RSM optimization were verified under optimal conditions and analyzed using a one-sample t-test at a 95% confidence level. The research results indicate that the regression equation model follows a second-order model, namely $Y = 1.1623 + 0.0978X_1 + 0.052X_1X_3 - 0.0873X_2X_3 - 0.0111(X_1)^2 - 0.0915(X_2)^2 - 0.1145(X_3)^2$. The optimum extraction conditions were a particle size of 354 µm, ethanol concentration of 68%, and solvent-solid ratio of 8.5 mL/g. Verification of the optimum conditions yielded a total flavonoid content of 1.20 ± 0.03% w/w ($n=3$), which was not significantly different from the predicted result of 1.19% w/w ($p > 0.05$).

Keywords: *Mimusops elengi* L., Response Surface Methodology (RSM), Box-Behnken Design, total flavonoid

INTRODUCTION

Tanjung (*Mimusops elengi* L.) belongs to the Sapotaceae family and is an evergreen tree. Historically, Tanjung has primarily been utilized as a shade tree planted in parks and along roadsides, although it offers several health benefits. Empirically, infusion of its leaves is used to treat headaches and cleanse wounds. A decoction of the bark and flowers can alleviate fever and diarrhea, while an infusion of Tanjung flowers is used as an antitoxin (Jerold, Soundhararajan and Srinivasan, 2022; Srivastava *et al.*, 2024).

Preclinical studies have indicated that Tanjung exhibits analgesic, antibiotic, anti-hyperlipidemic, anti-inflammatory, antimicrobial, antioxidant, antipyretic, cytotoxic, gingival bleeding, and hypotensive activities (Baliga *et al.*, 2011). These activities are attributed to its phytochemical content. Screening results revealed that Tanjung contains compounds such as flavonoids, phenolics, tannins, saponins,

steroids, glycosides, and triterpenes (Morikawa *et al.*, 2018; Baky, Elsaid, and Farag, 2022; Ipit and Desnita, 2015). Among these compound classes, flavonoids have been reported to exhibit in vitro antifungal activity (Ipit and Desnita, 2015; Ramamoorthy *et al.*, 2023). Total flavonoids and phenolics are positively correlated with antioxidant effects (Muflihah, Gollavelli and Ling, 2021).

Extraction factors can influence the quantity of the extracted compounds. Optimizing these factors can enhance the efficiency of the extraction process (Zhang, Lin and Ye 2018; al Ubeed *et al.* 2022). Some critical factors affecting extraction efficiency include the extraction method, solvent type and concentration, powder particle size, extraction time and temperature, solvent-solid ratio, and extraction pH (Feudjio *et al.*, 2022).

Extraction condition optimization can be achieved using Response Surface Methodology (RSM). RSM is a mathematical and statistical technique used in experimental design and modeling. The regression equation model obtained can be used to evaluate the effects of variables, optimize response conditions, and predict optimum responses (Wu *et al.*, 2015; Yolmeh and Jafari, 2017). BBD is a commonly used design in the RSM. BBD is a second-order design, rotatable or nearly rotatable, using a three-level fractional factorial design (Tian *et al.*, 2017). It has many advantages, including block usage, lack of model fit detection, the ability to build sequential designs, and estimation of the parameters of the quadratic model. The most important advantage over other designs is that it avoids performing experiments under extreme conditions, such as using the lowest or highest levels of all factors simultaneously, which could be expensive or impossible to test (Iwundu and Cosmos, 2022). BBD has been implemented to optimize the extraction of bioactive components, including alkaloids (Teng and Choi, 2014), phenolics (Juntachote *et al.*, 2006; Sai-Ut *et al.*, 2023), flavonoids (Yu *et al.*, 2019; Shangguan *et al.*, 2023), polysaccharides (Kan *et al.*, 2015; Cai *et al.*, 2019), and oleoresins (Dewi, Khasanah and Kawiji, 2012; Hanif, Widyasanti and Putri, 2021).

RESEARCH METHODS

Equipment and Materials

The equipment included a moisture balance, milling machine (Retsch, Germany), sieving machine (Retac Mitamura, Japan),analytical balance, vortex mixer, ultrasonicator, chromatography instrument, thin-layer chromatography (TLC) scanner with UV 254 nm and UV 366 nm, and spectrophotometer (Hitachi U-2900, Japan). The materials includes *Mimusops elengi* L. leaves (collected from Gandok area, Wedomartani, Ngemplak, Sleman), distilled water (General Labora, Indonesia), ethanol technical grade 70% and 96% (General Labora, Indonesia), standard of rutin (Fluka Biochemika, Swiss) given by Department of Biology Pharmacy, Faculty of Pharmacy UGM, ethanol p.a (E. Merck, Jerman), cellulose plates (E. Merck, Jerman), glacial acetic acid p.a (E. Merck, Jerman), citroborate (E. Merck, Jerman), aluminium chloride p.a (E.Merck, Jerman), sodium acetate p.a (E. Merck, Jerman) and methanol p.a (E.Merck, Jerman).

Research Procedure

1. Sample preparation and determination

Fresh Tanjung leaves are wet-sorted, dried, and dry-sorted to separate unwanted parts. They were oven-dried for 72 hours at 50°C. The dried leaves were subsequently powdered and sieved through mesh sizes of 30, 35, 40, 45, and 50. The drying shrinkage of the herbal material was determined using a moisture balance. Approximately 1 g of the herbal material was placed in the apparatus, and the

temperature was set at 105°C. The percentage of moisture content was displayed on the screen. The plant material was identified at the Department of Pharmaceutical Biology, Faculty of Pharmacy, Universitas Gadjah Mada (certificate number BF/6/Ident/I/2016).

2. Flavonoid identification using thin layer chromatography

Powdered Tanjung leaves (1.000 ± 0.001 g) were extracted with 10 mL of 96% ethanol (HPLC grade) in 15 mL conical tubes through 1-hour maceration at 25 ± 2°C with vortex mixing (5 min at 0, 30, and 60 min). The extract was filtered through Whatman No. 1 paper, quantitatively transferred to a 10 mL volumetric flask, and adjusted to the volume with ethanol. For the standard, rutin (10.00 ± 0.05 mg) was dissolved in methanol via ultrasonication (5 min) and diluted to 10 mL to prepare a 1 mg/mL stock solution, from which working standards (5-35 µg/mL) were prepared and stored at 4°C in amber vials. The TLC system used was as follows: **stationary phase:** cellulose, **mobile phase:** 15% acetic acid, **elution distance:** 8 cm, **spotting volume:** rutin (0.5 µL); Tanjung leaf extract (2 µL), **spray reagent:** citroborate (heated at 105°C for 5 minutes), and **detection:** visible light and UV 366 nm.

3. Determination of total flavonoid content

The total flavonoid content in each experiment was quantified using a modified $AlCl_3$ colorimetric method adapted from the Indonesian Herbal Pharmacopoeia (2016). A rutin (Fluka Biochemika, ≥95% purity) standard stock solution (1 mg/mL) was prepared by dissolving 10.00 ± 0.05 mg of rutin in 96% ethanol and diluting it to 10 mL in a volumetric flask. Serial dilutions were prepared to obtain standard solutions ranging from to 5-35 µg/mL. For analysis, 0.5 mL aliquots of both sample extracts and standard solutions were mixed with 1.5 mL ethanol (96%, analytical grade), 0.1 mL 10% aluminum chloride ($AlCl_3$) solution, 0.1 mL 1 M sodium acetate buffer (pH 4.5), and 2.8 mL distilled water. The mixtures were vortexed (30 sec) and incubated at 25 ± 1°C for 30 minutes in the dark. Absorbance was measured at λmax 418.5 nm against a reagent blank (prepared without $AlCl_3$) using a UV-Vis spectrophotometer (Hitachi U-2900) with 1 cm quartz cuvettes. All measurements were performed in triplicates.

4. Response surface design

The first independent variable was the particle size of the powder with the following design: powder sizes (X_1) of 595 µm (30 mesh), 500 µm (35 mesh), 400 µm (40 mesh), 354 µm (45 mesh), and 297 µm (50 mesh); ethanol concentration (X_2) 70%; and solvent-solid ratio (X_3) 10 mL/g. The second design was as follows: ethanol concentrations (X_2) of 50, 60, 70, 80, and 90%; fine powder size (X_1) of 40 Mesh (400 µm); and solvent-solid ratio (X_3) of 10 mL/g. The last design was as follows: solvent-solid ratios (X_3) of 6, 8, 10, 12, and 14 mL/g; fine powder size (X_1) of 40 mesh (400 µm); and ethanol concentration (X_2) of 70%. Each design was performed duplo. The Box-Behnken Design was employed to determine the regression equation model in RSM. After determining the levels of each independent variable, the next step was to follow the design of the Box-Behnken Design. The Box-Behnken Design used three factors with three levels, as shown in **Table I** and **Table II.**

Table I. Experimental ranges and codes of independent factors in the RSM

Independent variables	Units	Factors	Variable range		
			-1	0	1
Particle size	µm	X_1	30	40	50
Ethanol concentration	%v/v	X_2	60	70	80
Solvent-solid ratio	mL/g	X_3	6	8	10

Table II. Details of experimental design in the Box-Behnken design

Run	X_1	X_2	X_3	Total flavonoid content (%w/w)*
1	-1	-1	0	0,88 ± 0,07
2	1	-1	0	1,06 ± 0,03
3	-1	1	0	0,85 ± 0,02
4	1	1	0	1,06 ± 0,03
5	-1	0	-1	0,87 ± 0,01
6	1	0	-1	0,96 ± 0,06
7	-1	0	1	0,81 ± 0,05
8	1	0	1	1,11 ± 0,09
9	0	-1	-1	0,93 ± 0,06
10	0	1	-1	1,01 ± 0,03
11	0	-1	1	1,08 ± 0,02
12	0	1	1	0,81 ± 0,03
13	0	0	0	1,16 ± 0,01
14	0	0	0	1,15 ± 0,07
15	0	0	0	1,18 ± 0,02

**obtained by the experiment*

Data Analysis

The total flavonoid content data from the one-factor-one-time experiment were statistically analyzed with a 95% confidence level using SPSS 21 software. The statistical tests included normality and variance homogeneity tests. Normality was assessed using the Shapiro-Wilk test, and Levene's test was employed to analyze variance homogeneity. If the obtained data are normally distributed ($p > 0.05$) or not normally distributed ($p < 0.05$) and exhibit homogeneous variance ($p > 0.05$), the data are analyzed using the One-way ANOVA test to determine if there are significant differences among treatment groups. If there was a significant difference ($p < 0.05$), *post hoc* analysis was conducted using the Tukey test to identify significantly different groups.

For the Box-Behnken design, the total flavonoid content data were analyzed using Matlab2015a software. The determination and testing of the first-order model were conducted using simultaneous, individual, and lack-of-fit testing. Simultaneous, individual, and lack-of-fit testing were performed to identify the coefficients that significantly affected the model. A good model must satisfy the residual assumptions, including identical, independent, and normality assumptions (Jarantow, Pisors and Chiu, 2023). The optimization results were verified using a one-sample t-test to identify differences in average values between the two groups. A significance value greater than 0.05 ($p > 0.05$) indicated no significant difference in the average values between the two groups.

Statistical analysis and optimisation

Best fitted model of response can be achieved by highlighting these statistical parameters, including the adjusted multiple correlation coefficients (adjusted R^2), multiple correlation coefficients (R^2), coefficient of variation (CV%), and lack of fit. This statistical approach was used to summarize the results obtained under all experimental conditions, with a confidence interval of 95% set to test the significant effect of the factors and their interactions. The optimal extraction conditions were selected based on the condition of achieving the highest total flavonoid content in Tanjung leaves using the desirability function approach in Design Expert software. Te ftted polynomial equation was expressed in the form of three-dimensional surface plots in order to illustrate the relationship between responses and the experimental variables used.

Verification of models

The optimal conditions for the extraction of flavonoids from Tanjung leaves, in terms of raw material particle size, extraction time, and ethanol concentration, were determined by comparing the actual experimental values with the predicted values from the final response regression equations. In addition, a few random extraction conditions were prepared to validate the models. This action is of utmost importance to confirm the adequacy of the final reduced models.

RESULTS AND DISCUSSION

Preliminary phytochemical screening

TLC profiling was conducted to qualitatively analyze the compound composition of the extract. Rutin was used as the reference standard because it is a flavonoid glycoside with polarity characteristics that match those of the flavonoids present in Tanjung leaves. The stationary phase consisted of cellulose plates, which are ideal for separating different glycosides from one another, distinguishing glycosides from their aglycones and resolving less polar aglycones (Ren, Chen and Shen, 2022) Cellulose is particularly effective in separating flavonol glycosides, flavon aglycones, and flavanones (Waksmundzka-Hajnos, Sherma and Kowalska, 2008; Feng, Hao and Li, 2017). The mobile phase employed was 15% acetic acid, chosen because its polarity is compatible with the rutin standard and provides optimal elution strength for clear spot separation.

This optimized TLC system enabled effective compound visualization under both visible light and UV 366 nm after derivatization with the citroborate reagent. As depicted in **Figure 1**, the chromatographic profile of the extract showed distinct spots at hRf values of 25, 38, 52, 75, and 80, while the rutin standard appeared at an hRf value of 54. Under UV 366 nm (prior to derivatization), these extract spots exhibited the following colors: yellow, yellow, dark purple, yellow, and yellow.

Detection was performed using both UV 366 nm observation and a citroborate spray reagent. The citroborate reagent is known to produce yellow to yellowish-green fluorescence upon reacting with flavonoids (Muflihah, Haryoto and Indrayudha, 2020). After spraying, all the extract spots exhibited a uniform yellow color. The rutin spot (initially yellow under UV 366 nm) exhibited an enhanced yellow fluorescence intensity. These findings confirmed the presence of flavonoids in the Tanjung leaf extract. The multiple spots suggest that the extract contains several flavonoid derivatives with varying polarities, as evidenced by their distinct hRf values.

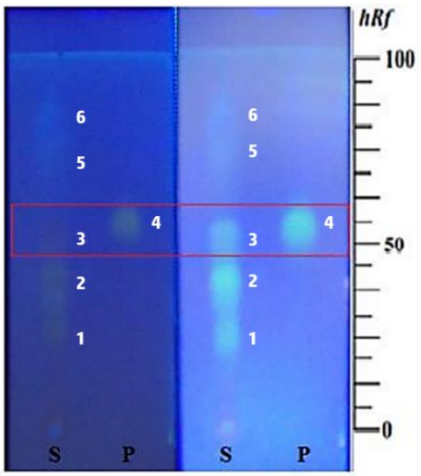

Figure 1. Thin layer chromatography profile (S : Tanjung leaves extract (200 µg); P : rutin standard (0.5 µg) illuminated using UV 366 nm (right: before spraying and left: after spraying).

The fluorescence behavior of flavonoids can be correlated with their hydroxyl group positions at C-3 and C-5. Rutin, a flavonol compound containing a free hydroxyl group at C-5 and a substituted hydroxyl group at C-3, characteristically displays purple fluorescence (De, Jo and Kim, 2022). However, the observed yellow coloration of rutin under UV 366 nm prior to spraying may be attributed to the alkaline pH conditions of the cellulose plates resulting from storage factors. This phenomenon is consistent with established findings that flavonoid hydroxyl groups develop a yellow coloration when exposed to ammonia vapor or sodium ions (Luiza Koop *et al.*, 2022).

The chromatographic profile of the extract revealed yellow spots at hRf 25, 38, 75, and 80, suggesting the presence of flavonols with free C-5 hydroxyl groups. A distinct dark purple spot at hRf 52 potentially represents flavones with free C-5 hydroxyls or flavonols containing both free C-5 and substituted C-3 hydroxyl groups (Nahari *et al.*, 2019). These fluorescence characteristics collectively indicate that the Tanjung leaf extract contains both flavonol and flavone compounds. Consequently, the spectrophotometric determination of total flavonoid content using Method 2 from the Indonesian Herbal Pharmacopoeia is analytically justified, as this method effectively detects compounds through C-5 hydroxyl group interactions.

Determination of total flavonoid content

The total flavonoid content was quantitatively measured using spectrophotometry based on Method 2 from the Indonesian Herbal Pharmacopoeia (2013), which is a modified version of the protocol developed by (Chang *et al.*, 2002). This method uses aluminum chloride ($AlCl_3$) and sodium acetate ($C_2H_3NaO_2$) as complexing reagents. Quantification is based on the formation of stable complexes between $AlCl_3$ and flavonoids containing hydroxyl groups at the keto positions C-4 and C-3 or C-5 of the C-ring and C-3', C-4' or C-5' positions of the B-ring (Chang *et al.*, 2002). Flavonoids that react with $AlCl_3$ at the C-3 and C-5 positions, including flavonols such as rutin, quercetin, quercitrin, and myricetin, exhibit maximum absorption at 415-440 nm (Hudz *et al.*, 2017).

For quantification, a linear regression equation was established using rutin as the standard compound. A calibration curve was prepared using rutin standard solutions at concentrations of 5, 10, 15, 20, 25, 30, and 35 µg/mL, showing a maximum absorbance at 418.5 nm. The resulting standard curve yielded the linear equation: y = 0.0249x - 0.0265, with a coefficient of determination (R^2) of 0.9983 and a correlation coefficient (r) of 0.9992, demonstrating excellent linearity in the tested concentration range. This calibration curve was subsequently used to calculate the total flavonoid content in each running test.

Fitting the model

The regression model analysis of the experimental data was conducted using the Matlab R2015a software. The independent and response variables were analyzed using a quadratic model (according to the second-order model). Based on the analysis from Matlab R2015a software, the equation model obtained from the relationship between total flavonoid content and the independent variables (X_1, X_2, X_3) is as follows:

$$Y = 1.1623 + 0.0978X_1 - 0.0291X_2 + 0.0074X_3 + 0.008X_1X_2 + 0.052X_1X_3 - 0.0873X_2X_3 - 0.0111X_1^2 - 0.0915X_2^2 - 0.1145X_3^2$$

where X1 is the particle size, X_2 is the ethanol concentration, and X_3 is the solvent-to-solid ratio.

Based on the software calculation, the coefficients influencing the model included the linear form of particle size (X_1), the interaction between particle size (X_1) and solvent-solid ratio (X_3), the interaction between ethanol concentration (X_2) and solvent-solid ratio

(X_3), the quadratic forms of particle size (X_1), ethanol concentration (X_2) and solvent-solid ratio (X_3). Non-significant coefficients were removed, resulting in the following refined model:

$$Y = 1.1623 + 0.0978X_1 + 0.052\ X_1X_3 - 0.0873\ X_2X_3 - 0.0111X_1^2 - 0.0915X_2^2 - 0.1145X_3^2$$

The obtained R^2 value was 0.976, indicating that the variables— ethanol concentration (X_2), particle size (X_1), and solvent-solid ratio (X_3)— accounted for 97.6% of the variation in total flavonoid content. The remaining 2.4% (error) is attributed to variables not included in the model. The adjusted R^2 value of 0.933 is a modified version of R^2 that accounts for multiple predictors, providing a more reliable measure of the model fit.

The adequacy of the model was further assessed using an analysis of variance (ANOVA) of the experimental data. The significance of the model was evaluated using the F-value and p-value. Because the p-values for both the linear and nonlinear terms were < 0.05, it can be concluded that the model is statistically significant and well-fitted, whether in its linear or nonlinear form..

The lack of fit indicates the adequacy of the model. The *p*-value for the lack of fit was **0.1088**. Since the *p*-value is > **0.05**, it can be concluded that the model is suitable for determining the total flavonoid content. A good model must satisfy the residual assumptions of independent and identically distributed noise (IIDN), that is, it should meet the requirements of homoscedasticity (equal variance), independence, and normal distribution. Testing the homoscedasticity assumption aimed to examine whether the residuals of the model exhibited consistent variance (uniform spread). Based on **Figure 2**, the residuals were randomly scattered and did not form any discernible pattern. Thus, it can be concluded that the homoscedasticity assumption is satisfied. Testing the independence assumption aimed to assess whether there was any dependency between residuals at specific observation times.

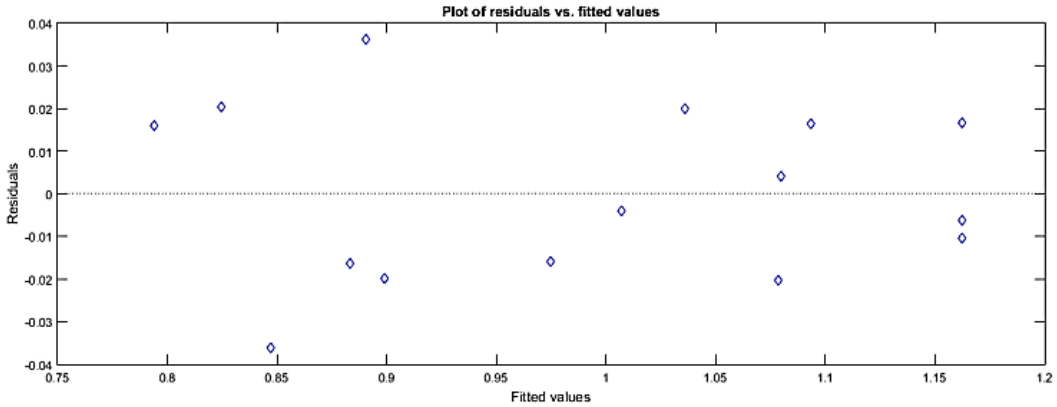

Figure 2. Scatter plot of calculated residuals versus fitted value

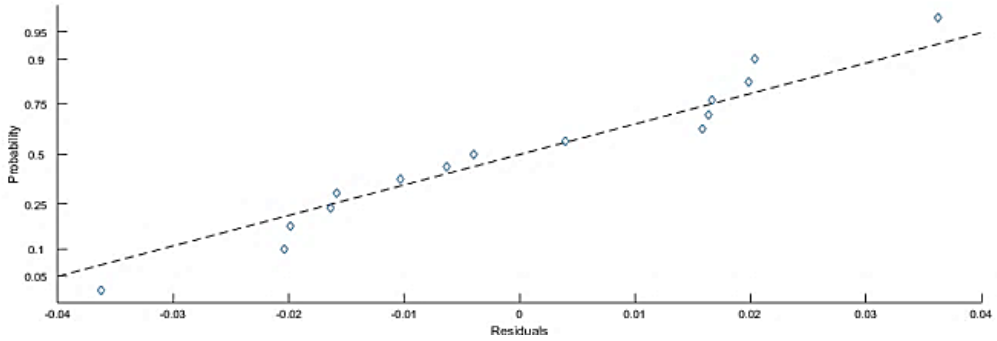

Figure 3. Plot of probability versus residuals in normality test of residuals

The residuals meet the independence assumption if the **Autocorrelation Function (ACF)** values fall within the interval **±2/√n**. For this model, the residuals satisfy the independence assumption because the ACF values lie within the interval **±1.033**, where *n=15*. The normality assumption test for residuals aims to assess the model deviations. The residuals meet the assumption if the plotted points closely follow a straight reference line. As shown in **Figure 3**, the residuals satisfy this assumption because the points align well with or closely approximate a straight line. Based on the tests for homoscedasticity, independence, and normality, it can be concluded that the model satisfies all residual assumptions. Therefore, the model can be considered valid and reliable for determining the optimal factors.

Analysis of response surface

Canonical analysis was used to determine the optimal state of the system. The optimal response value can be either the maximum or minimum. The eigenvalues of matrix were λi = [−0.1590, −0.1028, −0.0552]. Because all three eigenvalues are **negative**, the response surface exhibits a **maximum** characteristic.

One way to represent the response surface model is by generating **contour** and **3D surface plots** based on the three influencing factors. The results are illustrated in a three-dimensional form, with one factor held constant. Optimization using Matlab R2015a software, considering the **interaction between particle size (X_1)** and **solvent-solid ratio (X_3)**, yielded the following equation:

$$Y = 1.106 + 0.0978X_1 - 0.00734X_3 + 0.052X_1X_3 - 0.1042{X_1}^2 - 0.1075{X_3}^2$$

where X_1, X_2, and X_3 are the particle size, ethanol concentration, and solvent-solid ratio.

Contour plot in **Figure 4** illustrates the interaction between **particle size** and **solvent-solid ratio** on total flavonoid content. The interaction has a **positive effect** on the response informing that the **increasing particle size** leads to **higher total flavonoid content**. Similarly, **increasing the solvent-solid ratio** enhances flavonoid extraction. The optimal ranges for the particle size and solvent-solid ratio were found to–354-400 µm and 7-9.5 mL/g, respectively. The stationary point (0.4901, 0.0842, 1.1296) represents the optimal response for the interaction between particle size (X_1) and solvent-solid ratio (X_3) on total flavonoid content. After conversion to real values, the optimal conditions were 354 µm (particle size); 8.17 mL/g (solvent-solid ratio) with predicted total flavonoid content 1.13 % w/w.

Subsequently**,** the interaction between **the ethanol concentration (X_2)** and **solvent-solid ratio (X_3)** was explored and represented by the following equation:

$$Y = 1.094 - 0.0291X_2 + 0.0074X_3 - 0.0873X_2X_3 - 0.0829{X_2}^2 - 0.106{X_3}^2$$

The analysis revealed a **negative interaction effect** between ethanol concentration and the solvent-solid ratio on flavonoid yield, indicating that a **higher particle size** and a **higher solvent-solid ratio** resulted in **a lower total flavonoid content**. The optimum ranges were **60-76%** and **7-9.5 mL/g.** The stationary point **(-0.2472, 0.1364, 1.0981)** corresponds to the following real-value optima: **ethanol concentration: 67.53%**, **solvent-solid ratio: 8.27 mL/g**, and **predicted total flavonoid content: 1.1 % w/w.**

At the end of the optimization stage, the combined effect of particle size (X_1), ethanol concentration (X_2), and solvent-solid ratio (X_3) was established.

$$Y = 1.1623 + 0.0978X_1 + 0.052X_1X_3 - 0.0873X_2X_3 - 0.0111X_1^2 - 0.0915X_2^2 - 0.1145X_3^2$$

The model obtained the optimal extraction conditions, as **Table III.**

- Particle size: ≈354 µm
- Ethanol concentration: 67.55%
- Solvent-solid ratio: 8.46 mL/g

Under these circumstances, the regression model predicted a total flavonoid content of 1.19% w/w. Experimental verification of the optimized parameters yielded a flavonoid content of 1.20 ± 0.03% w/w. A one-sample t-test was conducted to evaluate the difference between the experimental means and predicted values. The results showed no statistically significant difference ($p > 0.05$), confirming the model validity for predicting the total flavonoid yield under the specified extraction conditions.

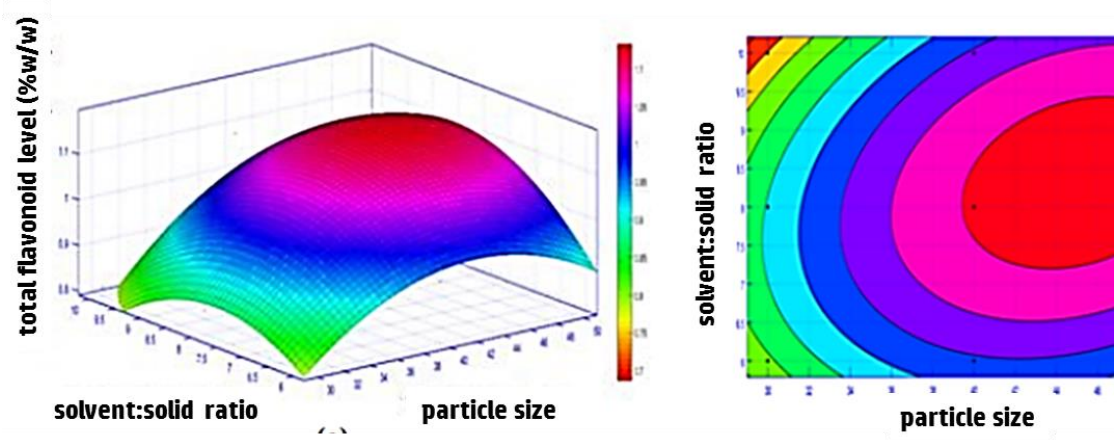

Figure 4. 3D graphic of total flavonoid level optimisation based on particle size and solvent-solid ratio (a) plot surface and (b) contour plot

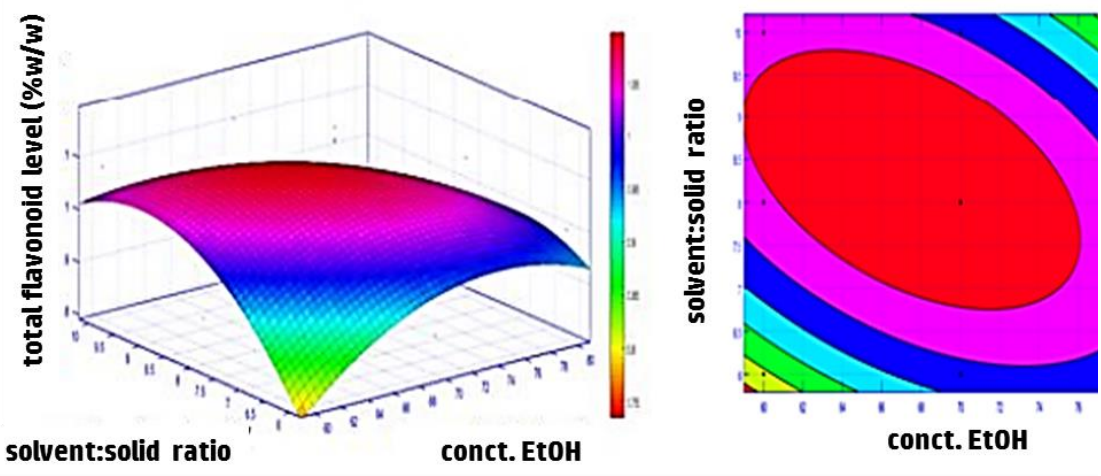

Figure 5. 3D graphic of total flavonoid level optimisation based on particle size and ethanol level (a) plot surface and (b) contour plot

Table III. Comparing the experimental result with the predicted result of total flavonoid content at optimum condition

Independent variables	Optimum value	Total flavonoid content (%w/w)	
		Experimental	Prediction
Particle size	354 µm	1.20 ± 0.03 (n=3)	1.19
Ethanol concentration	68 %v/v		
Solvent-solid ratio	8.5 mL/g		

CONCLUSION

Response surface methodology (RSM) and a design called Box-Behnken Design (BBD) were successfully developed to determine the optimum process parameters and the second order polynomial models for predicting responses were obtained. The best combination of dried powder particle size, ethanol concentration, and solvent-solid ratio were found to be 354 µm, 67.55% ethanol, and 8.46 mL/g, respectively, which rendered a mean total flavonoid content of from experimental run and thus indicated good antioxidant activities from the leaves of *Mimusops elengi* (Tanjung).

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