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Optimization on turmeric extraction to obtain curcuminoid with low-cost operation technique

Abil Fadila*, Efri Mardawati, Desy Nurliasari, Roni Kastaman, Selly Harnesa Putri

Department of Agricultural Industrial Technology, Faculty of Agricultural Industrial Technology, Universitas Padjadjaran, Bandung, Indonesia

KEYWORDS

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Percolation

Response surface methodology

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ABSTRACT

Turmeric (*Curcuma longa L.*) has become one of the potential plants to be developed due to its numerous benefits from the active ingredient, curcuminoid. Curcuminoid has antibacterial, antioxidant, and antihepatotoxic properties that can enhance the absorption of vitamins A, D, E, and K. The process to obtain curcuminoid can be carried out through an extraction process using a solvent. This research aimed to optimize the extraction process of curcuminoid from turmeric at a lower cost. The response surface methodology with a central composite design was used in this study to optimize the concentration of ethanol solvent (50-90%, v/v) and the flow rate of ethanol solvent (20-40 mL/minute) on the yield and curcuminoid. The research results indicate that the optimum conditions for the percolation extraction process (ethanol concentration = 90% and ethanol flow rate = 20 mL/minute) result in a yield value of 22.75% (w/w) and curcuminoid content of 13.54% (w/v). The curcuminoid was characterized based on several parameters, including water content of 11.15% (w/w) and antioxidant activity of 98.39% (w/v). The research concludes that the optimum results of the process conditions demonstrate the percolation extraction method with the variables of ethanol concentration and minimal ethanol solvent flow rate, which require lower costs while yielding optimum yield and curcuminoid content. Therefore, this can be applied in further research on curcumin production or its application in the food and non-food industries.

Introduction

The biodiversity in Indonesia is extensively utilized for food crops, medicinal plants, and industrial plants, including turmeric (*Curcuma longa L.*). Turmeric has been used in traditional medicine for various ailments such as anti-inflammatory, allergies, and antibacterial purposes. The rhizome is a commonly used part of the turmeric plant for various productions. Several chemical studies have shown that the chemical composition of turmeric includes fat oil (4.4-12.7%), essential oil (4.2-14%), and curcuminoid compounds (60-70%) (Simanjuntak, 2012).

Curcuminoids are classified as phenolic compounds with two symmetric phenolic rings and are connected by a heptadiene chain. Curcuminoids have benefits in treating stomachaches, diarrhea, asthma, headaches, leukorrhea, irregular menstruation, and as an expectorant (Sitepu, 2015). This is the basis for further research and exploration of using turmeric plants that produce curcuminoid compounds. Curcuminoids are obtained through the extraction process. Curcuminoid compounds can be extracted through several methods, such as Soxhlet extraction, maceration, and percolation (Wahyuningtyas et al., 2017). Curcuminoids are extracted using organic solvents, such as hexane, acetone, and ethanol (Dai et al., 2013).

In the study by Wahyuningtyas et al. (2017), the extraction of curcuminoids using maceration with 96% ethanol solvent obtained the highest curcuminoid content compared to solutions of methanol, acetone, and isopropanol, yielding a curcuminoid content ranging from 1.90%. Meanwhile, in the study by Paryanto and Srijanto (2016), percolation extraction was conducted to extract curcuminoids from *temulawak* (Curcuma xanthorrhiza) using temperature, solvent flow rate, and ethanol solvent concentration as the

*Corresponding author
E-mail address: ssuhartini@ub.ac.id

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studied variables. The study concluded that the optimum extraction was achieved with a concentration of ethanol-water solvent of 100:0, resulting in a curcuminoid concentration of 10.7%. According to Wientarsih and Prasetyo (2016), the maceration extraction method has the advantage of simple equipment and procedures. However, this method requires a relatively long extraction time and may yield less satisfactory results. On the other hand, the percolation method ensures that the sample is always supplied with fresh solvent, facilitating complete absorption when it comes into direct contact with the sample. The liability of this method is that if the sample in the percolator is not homogeneous, the solvent may have difficulty reaching all areas (Abdel-mageid et al., 2018).

Literature studies have shown that the extraction process of curcuminoids using percolation is influenced by the solvent concentration and solvent flow rate. Therefore, optimizing the extraction process conditions by minimizing the input of independent variables is necessary to achieve the optimum yield and curcuminoid content at a lower cost (Azmir et al., 2013). Based on these data, the response surface methodology (RSM) is used to optimize the extraction conditions of curcuminoids from turmeric. RSM is a combined statistical and mathematical technique used to develop, improve, and optimize processes, where several independent factors influence the response. This research aimed to optimize the extraction conditions of curcuminoids from turmeric at a lower cost and to identify the curcuminoid compounds through water content analysis and antioxidant activity.

Research Methods

Materials and tools

Turmeric was bought from the Kosambi Traditional Market. The turmeric was obtained directly from the harvest by separating from the leaves, stems, and flowers, thus the rhizome or the main part of turmeric is obtained for research. The materials used in the extraction of curcuminoids are 96% ethanol and distilled water. The materials used in the analysis phase are methanol PA, PA grade ethanol, DPPH solution, curcumin (in which there is a curcuminoid) Merck standard, and other supporting chemicals.

The tools used in the raw material preparation stage are an oven, blender, and 40 mesh sieve. The tool used in the extraction of curcuminoids is a percolator. The tools used in the concentration and drying stages are rotary vapor and the Merck Binder FD56 blower oven. The tools used in the analysis are the Ultraviolet-Visible spectrophotometer Merck DLAB Indonesia, magnetic stirrer, aluminum foil, measuring flask, measuring cup, Erlenmeyer, micropipette, porcelain cup, aluminum cup, analytical balance Merck Ohaus model PA323 Indonesia, spatula, beaker glass, dropper, chocolate bottles, plastic bottles, stirring rods, funnels, filter paper, cotton and to record calculations using Microsoft Excel and Word software (Christina et al., 2019).

RSM analysis

Research design

The research design used for RSM optimization was a centralized composite design (CCD). The factors used in this study were solvent concentration (%) and solvent flow rate (mL/min). The response variables determined were curcuminoid content (%) and extract yield (%). The levels of each factor used in this study after preliminary experiments were carried out are as follows:

1) Solvent concentration factor
a. Solvent concentration = 50% ($X1_1$ =1)
b. Solvent concentration = 90% ($X1_2$ =1)

2) Solvent flow rate factor
a. Solvent flow rate = 20 mL/min ($X2_1$ =1)
b. Solvent flow rate = 40 mL/min ($X2_2$ =1)

The levels of the two factors can be seen in Table 1, and the RSM research design is shown in Table 2.

Table 1. Level of solvent concentration factor and solvent flow rate

Variable	Unit	*Low* (-1)	*High* (+1)	0 (central point)	(-α)	(+α)
Ethanol solvent concentration	%	50	90	70	41.7157	98.2843
Solvent flow rate	mL/min	20	40	30	15.8579	44.1421

Table 2. Optimization treatment variations

Run	Treatment Variables		Response	
	Ethanol solvent concentration (%)	Solvent flow rate (mL/min)	Curcuminoids (%)	Yield (%)
1	70	44.1421	Y_1	Y_2
2	50	20	Y_1	Y_2
3	70	15.8579	Y_1	Y_2
4	98.2843	30	Y_1	Y_2
5	90	40	Y_1	Y_2
6	70	15.8579	Y_1	Y_2
7	50	20	Y_1	Y_2
8	41.7157	30	Y_1	Y_2
9	90	20	Y_1	Y_2
10	70	44.1421	Y_1	Y_2
11	70	30	Y_1	Y_2
12	90	40	Y_1	Y_2
13	98.2843	30	Y_1	Y_2
14	70	30	Y_1	Y_2
15	90	20	Y_1	Y_2
16	50	40	Y_1	Y_2
17	41.7157	30	Y_1	Y_2
18	50	40	Y_1	Y_2
19	70	30	Y_1	Y_2

Raw material preparation

This stage consists of preparing fresh turmeric raw materials, washing, drying using an oven, size reduction, then the final stage of refining using a blender with a mesh size of 40. The turmeric powder was used as the sample in the extraction, which has been weighed as much as 100 g for 19 trials or runs.

Extraction

The percolation extraction process to obtain turmeric extract was carried out with ethanol as a solvent according to the procedure. About 100 g of turmeric powder was soaked or macerated in a beaker with ethanol solvent and then covered with plastic wrap; thus, no steam came out for four hours. Next, the macerated sample was inserted into the designated percolator and allowed to stand for 24 hours. This step could dry the sample and trigger the ethanol solvent to be above the turmeric sample (about a few cm thick). The percolation extract process was carried out after 24 hours, and then the faucet was opened according to the flow rate (as in Table 2) at a temperature of 30 ^{o}C. Then, the extract was evaporated using a rotary evaporator to allow the evaporation of ethanol solvent. The concentrated extract was then analyzed for curcuminoids, yield, etc. The treatment was carried out 19 times or run according to the procedure (Euterpio et al., 2012; Sahne et al., 2016).

Observation analysis

Each process is further tested using optimum process conditions. The test uses quantitative analysis to calculate the extract yield by comparing the product's with the mass of the initial raw material (Rezki et al., 2015). The water content was analyzed by calculating the initial sample weight with the weight of the sample after drying (SNI 3532-2021). The analysis of solvent concentration on curcuminoid extraction assays and analysis of antioxidants was done by methods related to the reagent diphenyl picryl hydrazyl (DPPH) indicated in the percentage of inhibition (Doldolova et al., 2021). Then, making a standard curve to analyze the levels of curcumin with the equation y = a+bx (Kadam et al., 2018). The levels of curcuminoids was tested using a UV-Visible Spectrophotometer.

Data processing

The research data obtained in the form of curcuminoids and extract yields were processed and analyzed using Design Expert software 13.0.5.0 trial version. Data were entered in a 2-factor centered composite design with curcuminoid responses and yields. The treatment

factor consisted of solvent concentration and solvent flow rate. Table 2 shows the optimization treatment data on the turmeric extraction process.

Water content analysis **(SNI 3532-2021)**

Water content analysis is an analysis that aims to determine the amount of water contained in a material. The principle is the evaporation of water using heat energy. Moisture content is calculated based on weight loss at a heating temperature of 105°C. Checking the moisture content is done by heating the container in the oven at a temperature of 105°C for one hour, then cooled in a desiccator for half an hour and weighed. Turmeric is weighed as much as two grams in a container whose empty weight is known. Then, the container containing the sample is weighed and heated at a temperature of 105°C for 24 hours. The sample was tested back and then cooled in a desiccator for half an hour until a constant weight is obtained (SNI 3532-2021). The formula is as follows in Equation 1.

$$\text{Water Content} = \frac{b_1 - b_2}{b_3}\ x\ 100\% \quad \text{.....(1)}$$

Where:

b_0 = weight of empty cup (g)

b_1 = weight of sample and cup before drying (g)

b_2 = weight of sample and cup after drying (g)

b_3 = weight of turmeric sample (g) = b_1 - b_0

Antioxidant test analysis **(Doldolova et al., 2021)**

To prepare 0.6 mM DPPH (2.2-diphenyl-1–picrylhydrazy) solution, 0.012 g of DPPH solution was dissolved with 50 mL of methanol, homogenized with a *magnetic stirrer*, and then put into a dark bottle. The absorbance was measured with a UV-Vis spectrophotometer to obtain the maximum wavelength. The control solution was prepared in 3000 L of methanol and added with 1000 L of DPPH solution. Then, the solution was shaken until homogeneous with a magnetic stirrer.

Preparation of the first test solution mother liquor with 10 mg of turmeric extract dissolved in 10 mL of methanol = 10 mg/10 mL = 1 mg/mL = 1000 g/mL = 1000 ppm. Series solutions were made with concentrations of 50 ppm, 100 ppm, 150 ppm, and 200 ppm. A 200 ppm solution was prepared with 2000 L of the mother liquor added with 8000 L of methanol. Then take 2000 L of 200 ppm series solution to which 2000 L of DPPH solution is added. A 150 ppm solution was prepared with 1500 L of the mother liquor added with a volume of 8500 L of methanol. Then take 2000 L of 150 ppm series solution to which 2000 L of DPPH solution is added. A 100 ppm solution was prepared with 1000 L of the mother liquor added with 9000 L of methanol. Then take 2000 L of 100 ppm series solution to which 2000 L of DPPH solution is added. A 50 ppm solution was prepared with 500 L of the mother liquor added to 9500 L of methanol. Then take 2000 L of 50 ppm series solution to which 2000 L of DPPH solution is added.

All control and turmeric extract solutions were homogenized using a magnetic stirrer and incubated at 37 ^{o}C for 30 minutes in the dark (covered by aluminum foil). This was done because the DPPH radical is easily degraded by light; this process was carried out in duplicate to ensure consistency in the results. The absorbance was measured using a wavelength of 517 nm. After getting the absorbance value, the inhibition was calculated using Equation 2 below.

$$\%\text{Inhibition} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}}\ x\ 100 \quad \text{.....(2)}$$

According to Molyneux (2004), the antioxidant activity is excessive if it has an IC_{50} value of <50 ppm, strong with an IC_{50} value of 50-100 ppm, moderate with an IC_{50} value of 100-250 ppm, and weak with an IC_{50} value of > 250-500 ppm. (Molyneux, 2004). After obtaining the % of inhibition activity, the IC_{50} value is calculated using the linear regression in Equation 3.

$$Y = a + bx \quad \text{.....(3)}$$

Where, x is the concentration of extract (ppm) as abscissa, y is the value of % inhibition (antioxidant) as ordinate, a: slope, b: intercept.

Results and Discussion

The optimization of extracting turmeric

Optimization was conducted in the turmeric extraction process with several factors, such as the solvent concentration and the solvent flow rate, using the percolation method to obtain the optimum curcuminoid's level. The calculation results were analyzed using Design Expert 13.0.5.0 software with the central composite design (CCD) model type.

Curcuminoids response analysis

The highest level of curcuminoids was obtained at a solvent concentration of 98% and a solvent flow rate of 30 mL/minute, which was 13.87% (Table 3). While, the lowest level of curcuminoids

(6.12%) was obtained at a solvent concentration of 41.7157% and a solvent flow rate of 30 mL/minute. The data processing results using Design Expert 13.0.5.0 obtained an ANOVA analysis of variance with a value of p = 0.0001 (p <0.05), which represents that the model is significant. The p-value at the solvent concentration was 0.0001, and the solvent flow rate was 0.0002. Thus, these two factors significantly affected the curcuminoid response. According to Myers et al. (2016), the model should have a significant effect when the F value obtained is high and the p-value is low. The selected quadratic model regression equation for the vanillin content response is shown in Equation 4.

$$\text{Curcuminoids} = -6.79717 + (0.359889 \times \text{solvent concentration}) + (0.140298 \times \text{solvent flow rate}) - (0.001120 \times \text{solvent concentration} \times \text{solvent flow rate}) - 0.001484 \times \text{solvent concentration}^2 - 0.001959 \times \text{solvent flow rate}^2 \quad (4)$$

Based on the equation above, the solvent concentration significantly affects the outcome yield of curcuminoids, which is indicated by a positive value of +0.359889, an increase in the level of curcuminoids. This proves that there is a significant effect of the concentration of the solvent used on the outcome yield of curcuminoids. The higher the concentration of polar solvents added, the higher the curcuminoid levels. Also, the higher the solvent concentration used, the maximum level of curcuminoids will be obtained. In addition, the solvent used is ethanol, which works quite optimal compared to other hydrocarbon solvents. The polarity of ethanol increases as its concentration in water decreases (Fadhilah et al., 2021).

A study by Rezki et al. (2015) showed that curcuminoids diluted with ethanol solvent with a concentration of 96% for 180 minutes produced the highest curcumin of 1.78-2.61%, the process used was the maceration method. The research has proven that a solvent with a relatively high polar property may speed up the reactions that occur in the extraction process. It can be seen from the powdered turmeric sample mixed with high-concentration solvents. It dissolved easier and faster compared to organic solvents, which have low concentrations. Thus, the curcuminoid pigments could be dissolved in the solvents with polar properties and high concentrations.

Table 3. Curcuminoid response data and yield obtained from the combination of treatment with solvent concentration and solvent flow rate

Run	Treatment Variables		Response	
	Ethanol solvent concentration (%)	Solvent flow rate (mL/min)	Curcuminoids (%)	Yield (%)
1	70	44.1421	9.99	14.05
2	50	20	8.24	13.56
3	70	15.8579	11.33	18.39
4	98.2843	30	13.59	23.54
5	90	40	11.16	19.55
6	70	15.8579	11.64	18.56
7	50	20	9.04	13.145
8	41.7157	30	6.88	6,136
9	90	20	13.54	22.75
10	70	44.1421	10.69	14.51
11	70	30	11	16.969
12	90	40	12.02	18.46
13	98.2843	30	13.87	22.85
14	70	30	11.16	17.52
15	90	20	13.36	22.35
16	50	40	7.62	10,197
17	41.7157	30	6.12	7.105
18	50	40	7.73	9.727
19	70	30	11.49	16.047

As for the effect of the solvent flow rate, it can be seen from the equation that it has a significant effect on curcuminoid levels, with a marked positive increase of +0.140298. The flow rate of the solvent is critical and influences the yield of curcuminoids. The solvent flow rate in low conditions will produce high curcuminoid levels because the absorption process is relatively stable and optimal. A low flow rate will maximize the absorption process because the bioactive compounds will extract the curcuminoids optimally with an adequate stable pressure given by the working solvent (Fu et al., 2008). The effect of the solvent flow rate must be considered because it is directly related to the mixing of organic solvents to obtain the extraction results. Thus, it must be in a stable condition. It should not be too fast because the flow rate will potentially produce a small amount of curcuminoid and not an optimal result. It is similar to the displacement of organic solvents with the turmeric sample under high pressure; when the speed is too high, the extraction results are uneven.

In the study of Paryanto and Srijanto (2016), it was concluded that the optimum extraction was influenced by a 100:0 ethanol-water solvent concentration or using a 90% concentration and a solvent flow rate of 40 mL/minute, which resulted in a curcuminoid concentration of 10.7%. Based on this study, neither high nor low solvent flow rates have a significant effect. However, based on current research, a low solvent flow rate may provide a high level of curcuminoids.

Based on the graph in Figure 1, it is known that the data for each treatment combination is spread around a straight line. These results indicate that the residues of the curcuminoid response follow a normal distribution, which is scattered not far from a straight line (moderate scattering).

The three-dimensional curve in Figure 2 describes the condition of the effect of solvent concentration on the yield of curcuminoids. It shows that there are 5 regions with different colors. The darker the red, the higher the curcuminoids level. In contrast, the darker the blue, the lower the curcuminoids produced. This indicates an optimal curcuminoid level at the peak before finally decreasing. The position of the curcuminoid level is seen constantly at the green to yellow point, indicating a significant influence of solvent concentration on curcuminoid levels (Hayakawa et al., 2011).

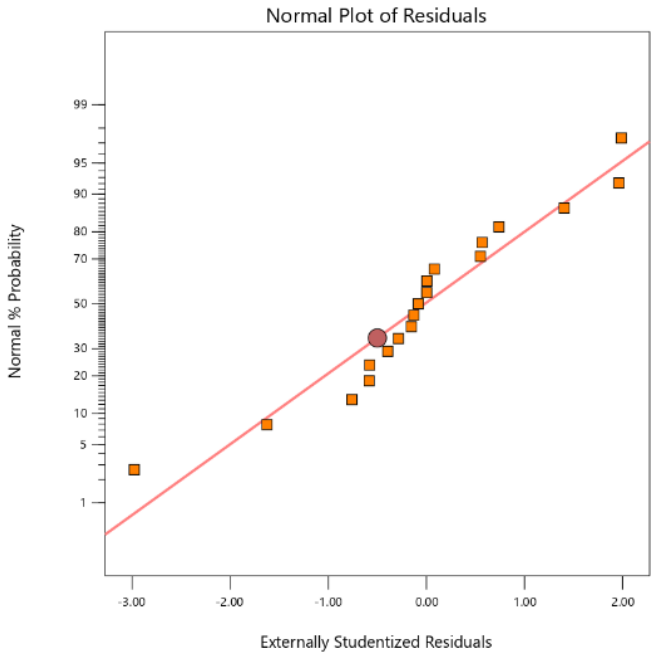

Figure 1. Normal graph plot of residual curcuminoid

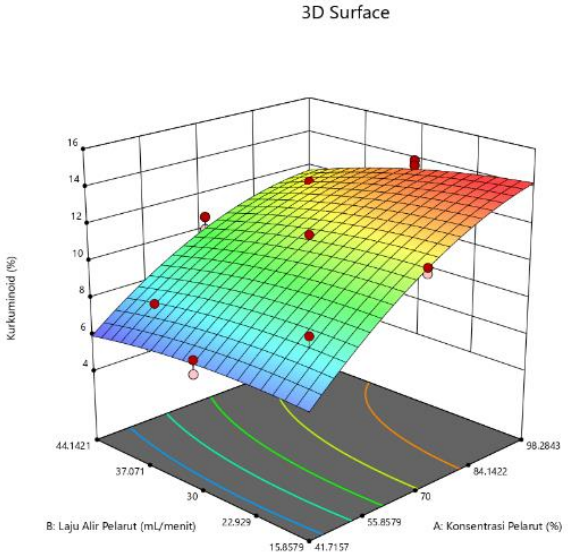

Figure 2. Three-dimensional response surface curve for the relationship between the solvent concentration and solvent flow rate on the curcuminoid response

Yield analysis response

The highest yield was obtained at a solvent concentration of 98% and a solvent flow rate of 30 mL/minute, which was equal to 23.54% (Table 3). While, the lowest yield (6,136%) was obtained at a solvent concentration of 41.7157% and a solvent flow rate of 30 mL/minute. The data processing results show an ANOVA with a p-value of 0.0001 ($p < 0.05$), representing that the model is significant. The p-value of the solvent concentration was 0.0001, and the solvent flow rate was 0.0001. Thus, these two factors had a significant effect on the yield response. The selected quadratic model regression equation for the yield response is shown in Equation (5).

$$\text{Yield} = -7.88944 + (0.555083 \times \text{solvent concentration}) - (0.093195 \times \text{solvent flow rate}) - (0.000193 \times \text{solvent concentration} \times \text{solvent flow rate}) - 0.002063 \times \text{solvent concentration}^2 - 0.000902 \times \text{solvent flow}^2 \quad \ldots\ldots\ldots\ldots (5)$$

Based on the equation above, the solvent concentration has a significant effect on the yield, marked by a positive value of +0.555083, an increase in the yield's value. The higher the concentration of polar solvents added, the higher the yield. In summary, there is a significant influence of the solvent concentration on the yield of turmeric extract. When the solvent concentration is high enough, the extraction process occurring between the solvent and the simplicia could be optimal. This is because the polar nature of the solvent may speed up the process, and the extraction yield could be high and optimal.

According to Wahyuningtyas et al. (2017), the extract yield obtained was 14.90% when using a 96% ethanol concentration. Rezki et al. (2015) concluded that a 96% ethanol concentration could result the extract yield of 12% and only 8.8% of extract yield when using 50% ethanol concentration. Based on those two studies, it is proven that the higher the concentration of the solvent used, the higher the polarity. In addition, mixing it with powdered simplicia may enhance absorption, hence maximize thing extraction process as the bioactive compounds could easily and optimally decompose.

Figure 3 shows that the data for each treatment combination is distributed in a straight line. These results indicate that the residuals of the yield response follow a normal distribution that is scattered not far from a straight line (moderate scattering) (Jiang et al., 2021). The three-dimensional curve in Figure 4 describes the condition of the effect of solvent concentration on the yield's value. It is known that there are 5 regions with different colors. The darker the red, the higher the yield. In contrast, the darker the blue, the lower the yield. This shows an optimal yield value at the peak before finally decreasing. The position of the yield value is seen constantly at the green point towards the yellow one.

The effect of the solvent flow rate is not too significant. It can be seen from the yield equation for a negative solvent flow rate value of -0.093195. On the other hand, the solvent flow rate

affects the yield, but it was not significantly visible from the three-dimensional graphs and curves. A low flow rate will maximize the absorption process because the bioactive compounds will be extracted optimally with a sufficient stable pressure given by the working solvent (Islamadina et al., 2020). The equilibrium constant in the extraction process will cause contact between the simplicia and water, attracting more secondary metabolites. This is because the longer the extraction time, the more compounds could be extracted. It can be seen that when the solvent flow rate is 20 mL/minute, it will produce a fairly high extract yield due to optimal absorption. Alos, the bioactive compounds are maximally released during the process.

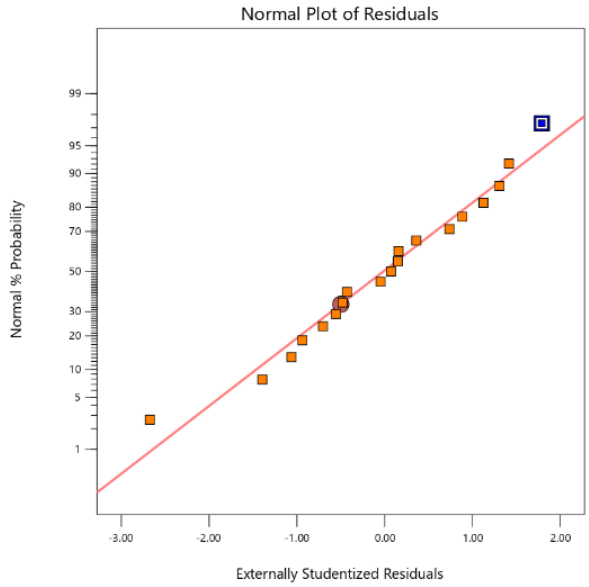

Figure 3. Normal graph plot of residual yield

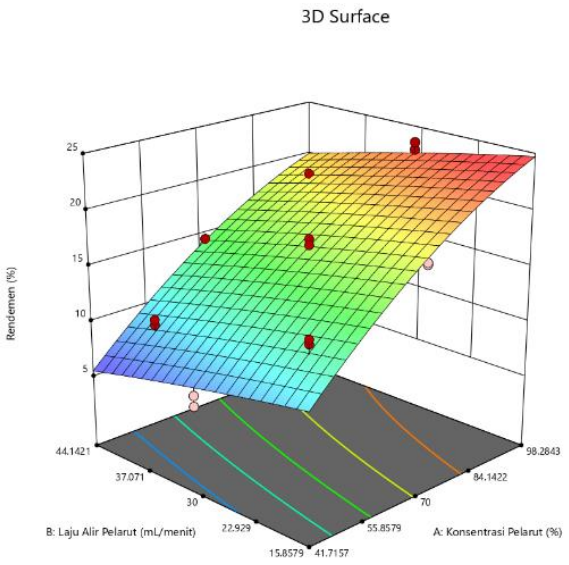

Figure 4. Three-dimensional response surface curve for the relationship between the solvent concentration and solvent flow rate on the yield response

Table 4. The optimal solution for curcuminoid response and yield from combining the solvent concentration factor and the computational solvent flow rate

Parameter	Prediction Standard
Ethanol Solvent Concentration (%)	90
Solvent Flow Rate (mL/min)	20
Curcuminoid Content (%)	13.575
Yield (%)	22.787
Desirability	0.959
Information	*Selected*

Table 5. Curcuminoid validation results and yield

Run 9					
Parameter	**Prediction (*)**	**Verification Results (**)**	**Difference**	**Deviation (%)**	**Validation Comparison (%)**
Curcuminoid Content (%)	13.575	13.54	0.035	0.25783	99.74
Yield (%)	22.787	22.75	0.037	0.16237	99.84

Note : *Results from Design Expert 13.0.5.0
**Validation result data

Optimal solution for curcuminoid response and yield

The optimization was conducted to optimize the response value in the form of curcuminoids and yield based on predetermined limited factors, namely solvent concentration and solvent flow rate. The ideal value for curcuminoid response and yield is chosen optimally because the higher the curcuminoid value, the more results are obtained. The optimal solution in Table 4 shows that combining treatments with a solvent concentration of 90% and a solvent flow rate of 20 mL/minute will produce 13.575% curcuminoids and 22.787% yield. The desirability value (accuracy) is 0.959, or close to 1, indicating the program's ability to achieve the desired optimum solution.

Optimal condition validation

The validation of curcuminoid level and yield was carried out to ensure that the predicted results of the optimal solution for each variable were in accordance with the actual results. The validation was conducted according to the optimal treatment combination of ethyl acetate solvent volume and extraction time, namely 90% and 20 mL/minute, respectively. The validation results obtained curcuminoids of 13.54% and a yield of 22.75%. The validation results were then compared with the optimal solution results obtained from Design Expert 13.0.5.0, as shown in Table 5. These results are in accordance because the validation results are quite similar to the predicted results, with a difference of less than 5%. According to Sugiono (2015), the gap in measuring accuracy between the validation and the predicted values must be less than or equal to 5%. The comparison resulted in 99.84% yield and 99.74% curcuminoids. This condition indicates that the reliability is perfect as the range of validation values is > 0.90. These values proved that the predicted results are a somewhat accurate model to determine the optimal yield and curcuminoids with a solvent concentration of 90% and a solvent flow rate of 20 mL/minute. The following are validation value categories according to Cahyani et al. (2016):

- Perfect reliability if the validation value > 0.90
- High reliability if the validation value is between 0.70-0.90
- Moderate reliability if the validation value is between 0.50 – 0.70.
- Low reliability if the validation value < 0.50, then low reliability

The data above proves that the solvent concentration and flow rate significantly affects the yield value and curcuminoid content produced (Martins et al., 2013). The results also show that the percolation method was very effective in producing optimum yield and curcuminoids with lower costs. This is because the process can minimize the solvent used after optimizing the extraction process conditions. Thus, the extraction method and the minimized independent variables may produce optimum results at a low cost. This could potentially be applied to an industrial scale

because it brings in more profit with inexpensive but high-quality raw materials.

Analysis of turmeric content in optimum conditions

a. Water content analysis

One of the chemical characteristics which are contained in turmeric rhizome, especially the powder, is water. The National Standardization Agency has issued SNI 01-354-2004, which sets up a standard for water content in turmeric. The regulation states that water content in spices must be <12% (%w/w); therefore, a water content test is conducted on the sample.

The turmeric powder sample were dried at a temperature of 105°C. This study resulted in a 11.15% ± 0.00 water content, which means it meets the turmeric water criteria content according to national standards. Excessive water content in traditional medicines could accelerate microbial growth and may ease the occurrence of hydrolysis (Pham et al., 2015). Such a process could decrease the quality of the medicines. The aim of figuring out the water content is to determine the maximum limit or range of water content in the material. This is related to the purity and presence of contaminants in the simplicia. Thus, removing water content up to a certain amount is useful to extend the material's durability when stored (Handayani et al., 2017).

b. Antioxidant Test

The antioxidant activity of turmeric is due to the curcuminoid compounds and essential oils content. Curcuminoids belong to the class of phenolic compounds, which makes the simplicia part with potent antioxidants. The method used in this test is DPPH (2,2-diphenyl-1–picrylhydrazyl) solution. The DPPH method was chosen because it is a rapid, easy, simple method and requires only a small number of samples (Doldolova et al., 2021; Martins et al., 2013).

The principle of antioxidant testing using the DPPH method is that there should be a change in the intensity of the purple color, comparable to the DPPH solution's concentration. This change is caused by the binding of free radicals due to the reaction of DPPH molecules with hydrogen atoms released by sample compound molecules to form 2,2-diphenyl-1-picrylhidrazy compounds. Such a mechanism resulted in a color change from purple to yellow. This color change may cause a change in the absorbance reading process when using a UV-Visible spectrophotometer. The process helps discover free radicals binding activity, expressed by the IC_{50} value. The smaller the IC_{50} value, the higher the free radicals' binding activity. It proves that the simplicia part of turmeric has strong antioxidant activity (Somporn et al., 2011).

The antioxidant activity from the optimum combination of 90% ethanol concentration and flow rate of 20 mL/minute was an IC_{50} value of 98.39, as shown in Table 6. The IC_{50} value shows the strength level of antioxidant activity based on free radicals' inhibition of 50% (Zhang et al., 2018). Antioxidant activity is overpowering if the IC_{50} value is < 50 ppm, strong if the IC_{50} value is between 50 – 100 ppm, moderate if the IC_{50} value is between 100 – 250 ppm, and weak if the IC_{50} value is> 250 – 500 ppm (Molyneux, 2004). Therefore, the results indicated that turmeric powder is categorized as having strong antioxidant activity. This comes from the turmeric rhizome and contains curcuminoids and curcumin. The relationship between turmeric extract and inhibition value in the antioxidant activity is shown in Figure 5.

The above curve was obtained using linear regression in the Microsoft Excel 2019 data processing program. The y coefficient in the linear equation was 50, which is the IC_{50} coefficient. Whereas, the x coefficient in this linear equation is the extract concentration which value will be sought. The x value was obtained from the concentration needed to reduce 50% of DPPH radical activity. The value of R^2 describes the linearity of concentration to % inhibition. The R^2 values close to +1 (positive value) show a good correlation between the sample's concentration and % inhibition.

Table 6. Antioxidant activity test results on turmeric extract (*Curcuma longa)*

No	Concentration (ppm)	Average Absorbance	Inhibition (%)	IC_{50} (ppm)
1	50	0.901	55.77	98.39
2	100	0.748	62.46	
3	150	0.658	66.95	
4	200	0.590	70.37	
	Blank	1.991		

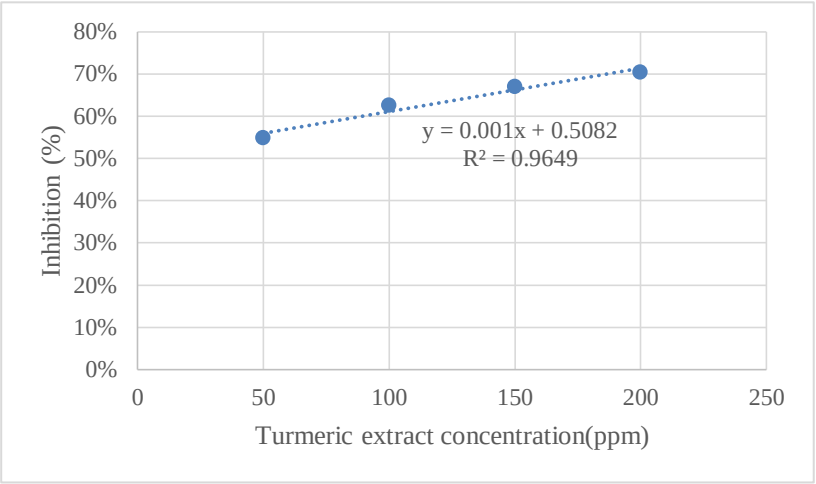

Figure 5. Concentration and % inhibition relationship curve in turmeric extract

The antioxidant activity formed from the turmeric rhizome sample shows a strong antioxidant that can reduce DPPH radicals. One of the compounds that affect the antioxidant activity is the presence of curcuminoid and curcumin compounds. The value of antioxidant activity in the simplicial part reaches 98.39 ppm and is categorized as a strong activity. The curcumin compounds found in turmeric are bioactive compounds and belong to the phenol group, where these compounds have the potential to produce antioxidants and could bind free radicals (Yulianto et al., 2019; Pham et al., 2015).

Conclusion

The optimal solution is obtained by combining the treatment with a solvent concentration of 90% and a solvent flow rate of 20 mL/minute with a standard prediction of 13.575% curcuminoids and 22.787% extract yield. The results of the optimal solution validation resulted in curcuminoids and extract yields of 99.74% and 99.84%, respectively. This condition showed perfect reliability because the validation values' range was > 0.90. Thus, this resulted in low expenditure by minimizing independent variables, yet it produced maximum output. The turmeric has a water content of 11.15% ± 0.00, which follows the SNI 01-354-2004standards of <12% (%w/w). As for the antioxidant activity of curcuminoids, it produces a value of 98.39 ppm and is categorized as a strong activity. This means that the antioxidant activity in turmeric helps prevent cell damage due to oxidative stress.

Declarations

Conflict of interests The authors declare no competing interests.

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