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EFFECTS OF EXTRACTION CONDITION ON EXTRACTED CURCUMIN FROM *Curcuma xanthorrhiza* RHIZOME: A META-ANALYSIS

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ABSTRACT

Curcumin is a chemical found in the *Curcuma xanthorrhiza* rhizome that has a variety of pharmacological properties. However, it is unknown whether the extraction conditions have a significant effect on the extracted curcumin from *C. xanthorrhiza*. In this research, the effects of extraction conditions (i.e., particle size, extraction solvents, and extraction methods) on curcumin content from *C. xanthorrhiza* rhizome were studied by doing a meta-analysis. An article search was performed in ScienceDirect, Garuda Portal, Google Scholar, and the IPB Repository database. Research data produced during the identification and selection phases were then classified, processed, and entered into the meta-analysis statistical program and then analyzed. The maximum curcumin was obtained using extraction conditions with n-hexane as a solvent, 100 mesh for particle size, and Soxhlet for the extraction method. Particle size and extraction method correlate and influence curcumin content; however, solvent extraction does not. This review will highlight how the effectiveness of the extraction of curcumin compounds from *C. xanthorrhiza* rhizome so that it can be used for extraction at the industrial level.

Keywords: Meta-Analysis, Curcumin, Solvent, Particle Size, Extraction Method.

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INTRODUCTION

Curcuma xanthorrhiza is a plant species growing wild in the forests of Java, Maluku, and Kalimantan islands of Indonesia. It grows upright with a stem height of about 2 m, green or dark brown, each having 2-9 leaves with an elongated round lanceolate shape, as well as 31-84 cm long and 10-18 cm wide. *C. xanthorrhiza* rhizome contains secondary metabolites, namely curcumin which have pharmacological potential as anti-cancer, anti-inflammatory, antioxidant, hepatoprotective, anti-inflammatory, hepatoprotective, antihyperglycemic, antiplatelet, antihypertensive, antidiabetic, and antibacterial activities.[1,2] Furthermore, it is known to have medicinal properties because it contains curcumin of around 24.70 – 54.09 mg/ g extract.[3] Curcumin is an important molecule that can be utilized as a medicine and functional food.[4,5] There has been research to optimize the curcumin extraction technique for obtaining high levels of active ingredient extracts, and diverse findings have been reached. Therefore, data analysis techniques are needed in evaluating these differences. One of which is meta-analysis used to quantitatively analyze data obtained from similar primary studies by determining the effect size.[6] The effect size is a measure related to the magnitude of the impact, the difference, and the relationship between the variables involved. Meta-analysis techniques are used as a tool in answering new questions based on the old data. Furthermore, a meta-analysis was carried out to strengthen the accuracy and provide consideration regarding different aspects and problems from similar studies, therefore, it is a suggestion for further research.[7] Previous studies regarding the optimization of solvent use, particle size, and extraction methods

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in the extraction process of curcumin active compounds from *C. xanthorrhiza* have been quite numerous, and the results obtained were mixed up. As a result, more research and data analysis are required to draw conclusions about the efficacy of each element in curcumin content. The aim of this research was to use a meta-analysis of extraction conditions such as particle size, extraction solvent, and extraction method to find the most optimal extraction of the curcumin compound of *C. xanthorrhiza* by examining the efficiency of solvents, particle size, and extraction processes to be employed, this study should provide recommendations for selecting the optimal approach for extracting curcumin compounds. Furthermore, it is also useful as a reference in developing theories related to the curcumin extraction method from *C. xanthorrhiza* rhizome.

EXPERIMENTAL

General Procedure

This research was conducted using a meta-analysis method, which is a quantitative test that systematically combines previous studies' results in generating conclusions.[8] This method was carried out in 2 stages, namely searching and processing data, as well as analysis. The data collection and processing were carried out in 3 steps, namely identification, selection, and abstraction. Identification is a step in collecting the articles which are included in the meta-analysis. The articles used are in the form of research reports conducted around the world, as published in online journals over the span of the last 15 years. The research population included all studies with the dependent variable of curcumin content and associated with the use of solvents, particle size, and extraction method as the independent factors. Identification is carried out manually based on the inclusion criteria using the ScienceDirect search engine by the keywords of "*Extraction curcumin*", "*Curcuma xanthorrhiza*", and "Extraction *method*". Manual identification was also carried out using the Garuda Portal, Google Scholar, and IPB Repository using the keywords "curcumin extraction of Curcuma", and "extraction particle size", and based on a list of references in systematically identified research reports. Furthermore, research journals obtained from the identification step were then selected to determine their quality. The selection of research journals was carried out based on screening of titles and abstracts and then analyzed as a whole to determine their suitability with the inclusion and exclusion criteria that had been set. The inclusion criteria used consisted of the curcumin criteria used in the form of rhizomes, aged 10-12 months and reported research data on the curcumin content of Curcuma extracts. The exclusion criteria used were the extraction method, particle size, and the solvent used in the isolation process. Abstraction is a process of quantifying the results of each study and then combined in a meta-analysis. The research data generated at the identification and selection stages were then identified, processed, and inputted into the statistical software used for meta-analysis, and then analyzed.

Data Analysis

Data analysis was carried out using the IBM SPSS 23 program. It was first tested for normality with the Shapiro-Wilk, homogeneity, and Lavene assessment. Furthermore, the analysis was carried out with the One-Way ANOVA test, followed by the Post Hoc Tukey analysis, when the data were normally distributed and homogeneous with a confidence level of 95%. However, when the data was not homogeneous with a significance value of ANOVA <0.05, then the Post hoc Tamhane test was used. Furthermore, to determine the relationship between the choice of solvent, sample particle size, and the extraction method toward Curcumin content of Curcuma extract, an analysis was performed with Pearson correlation and simple linear regression. Simple linear regression analysis is a method for obtaining the relationship between the independent and the dependent variable in the form of a line equation. The simple linear regression equation was used to determine the relationship between the choice of solvent use, sample particle size, and the method of extraction, and whether they affect the coefficient of determination (R^2) and the correlation coefficient (r) obtained. Meanwhile, Pearson correlation was used to determine the closeness strength of the relationship between the three factors.

RESULTS AND DISCUSSION

Meta-analysis is a quantitative analysis that systematically combines the results of previous studies, for generating research conclusions.[8] This method is carried out in two stages, including searching and processing data, as well as analysis. Search and data processing were carried out through a manual

identification using the ScienceDirect search engine, Garuda Portal, Google Scholar, and IPB Repository with a limitation in the form of articles from the last 15 years, and 58 studies were successfully identified. The identification process was followed by examining the title and the abstract. In this process, 14 relevant articles were selected to be included in the meta-analysis of this study. The reason others were excluded was that they did not meet the predetermined inclusion and exclusion criteria. Some of the identified studies were also literature reviews (not original research), which did not support meta-analysis. This process was shown in Fig.-1.

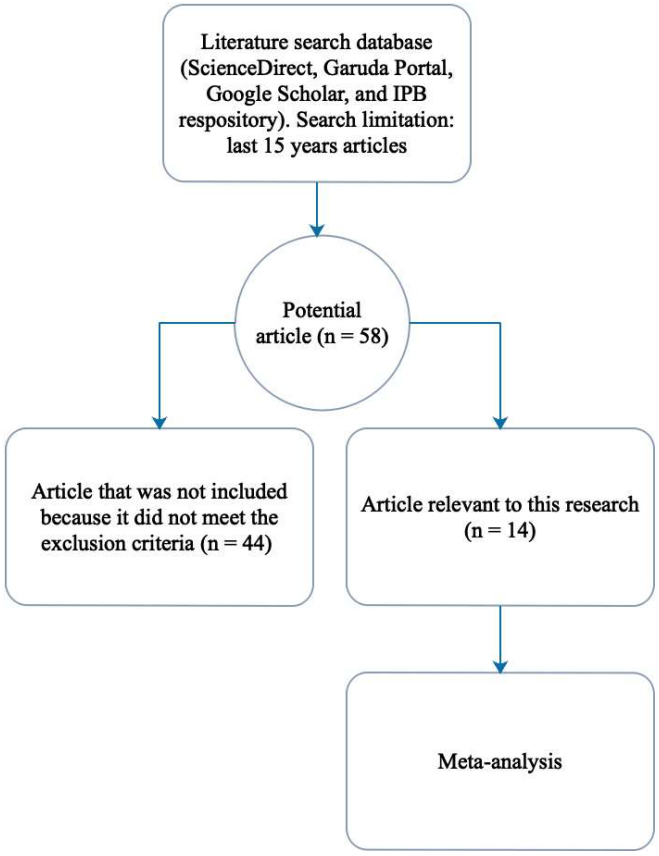

Fig.-1: The Process of Identifying and Selecting Secondary Data

Solvents

Extraction is a series of processes for obtaining certain compounds from a mixture of solid using a suitable solvent. This process is very dependent on the polarity of a compound to be extracted, as well as the choice of solvent. Furthermore, in selecting the solvent to be used, its selectivity, extractability, toxicity, ease of evaporation, and price needs to be considered.[9] The results showed that the highest and lowest mean curcumin content of *C. xanthorrhiza* extract was obtained with the use of n-hexane (16.044 ± 14.755) and water solvent (0.301 ± 0.034). The highest curcumin content in n-hexane was not in line with Santoto *et al*. which showed that this solvent extracted the least curcumin compared to ethyl acetate, butanol, and methanol.[10] Furthermore, Jayaprakasha *et al*. stated that the n-hexane solvent is able to extract curcuminoid compounds in the form of curcumin and desmethoxycurcumin, as well as play a role in removing volatile compounds in the extraction process from turmeric.[11] Curcumin is a group of polar polyphenol compounds that have low solubility to water and ether, however, dissolves in organic solvents, such as alcohol.[12] The extraction of curcumin using water as a solvent in Rara showed that the curcuminoid content in the extract increases with the addition of solvent, and decreases due to the precipitation process.[13] Furthermore, Photitirat and Gritsanapan showed that ethanol is an organic solvent that is suitable for separating curcuminoids optimally.[14] This is in line with Popuri and Pagala, which stated that ethanol is the best solvent in curcumin extraction compared to other hydrocarbon solvents.[15] Liu *et al.* also showed that extraction with ethanol produced higher extract yields than acetone solvent.[16] The normality test was conducted to determine the distribution of data in a group or variables. A homogeneity test was performed to ascertain

the homogeneity or variation of data from a population. The normality and homogeneity test in this study was carried out on the curcumin content of Curcuma extracts from several solvent variations using one sample Shapiro-Wilk method and the Lavene test with the IBM SPSS 23. The data tested was said to be normal and homogeneous, when the significance value was greater than 5%.[17] The results of the normality test in Table-1 showed the significant values of the following solvents: water, methanol, ethanol, and n-hexane respectively, namely 0.112, 0, 0.089, and 0. The significant level of water, ethanol, and n-hexane solvents was greater than 5%, which means that data were normally distributed. This suggested that the normality assumption of the data was met. The homogeneity test results as shown in Table-2 gave a significance value of 0.000 ($p < 0.05$), therefore, it was observed that the curcumin content of the *C. xanthorrhiza* extract from the variation of the solvent has different variations. Based on the normality and the homogeneity test, the content from the solvent variation did not meet the homogeneity assumption. Therefore, it is necessary to conduct an ANOVA and Post hoc test with the Tamhane method, when the significant value obtained is less than 0.05.

Table-1: The Results of the Shapiro-Wilk Normality Test for Solvent Variation

Solvent	Statistics	Sig.
Water	0.799	0.112
Methanol	0.000	0.000
Ethanol	0.820	0.089
n-Hexane	0.000	0.000

Table-2: The Results of the Levene Test for Homogeneity of Solvent Variations

df1	df2	Levene statistic	Sig.
3	9	25100.238	0.000

The ANOVA test results in Table-3 showed a significant value of 0.018 ($p < 0.05$), which means that there was an effect of solvent variation on the curcumin content. Table-3 also showed the average value of the content with water, methanol, ethanol, and h-hexane as solvents, respectively 0.301 ± 0.034, 10.509 ± 6.154, 0.400 ± 0.087, and 16.044 ± 14.755. The ANOVA test only proved that there were differences between the four solvents used, and has not yet shown which solvent has the difference. Therefore, a further test (Post hoc) is needed to find out which solvents have differences, for more detailed conclusions to be drawn. The post hoc test used was the Tamhane assessment. The results of the Post hoc test in Table-4 showed that the significant value in all groups was greater than 0.05, which means that there was no significant difference in the curcumin contents between the solvents. ANOVA analysis of one factor in the study showed that the solvent variation had no significant effect on the average difference in the curcumin contents.

Table-3: ANOVA Distribution of Average Curcumin Content of *C. xanthorrhiza* Extract Based on Solvent Variation

Solvent	N	Mean	SD	F (ANOVA)	Sig.
Water	3	0.301	0.034	5.760	0.018
Methanol	2	10.509	6.154		
Ethanol	6	0.400	0.087		
n-Hexane	3	16.044	14.755		

Table-4: The Results of the Post Hoc Tamhane Test for Solvent Variation

Solvent	Mean Difference	Sig.
Water vs Methanol	8.79867	0.937
Water vs Ethanol	0.09900	0.240
Water vs n-Hexane	0.32367	0.978
Methanol vs Ethanol	8.69967	0.939
Methanol vs n-Hexane	8.47500	0.943
Ethanol vs n-Hexane	0.22467	0.995

A simple linear regression test was used to examine the relationship between the solvent variations. A simple linear regression test was performed after the linearity and homogeneity assumption tests were met.

The linearity and homogeneity tests were carried out by determining the distribution of data on the scatter plot (Fig.-2), and the significance value obtained. The homogeneity test showed the location of the data variations that were scattered or irregular. This means that the data on a solvent variation on curcumin content was homogeneous.[18] The results of the simple linear regression test in Table-5 showed a 0.026 coefficient of determination (R^2), which means that the contribution of the solvent variation to the curcumin content was 2.6%, while the rest was caused by other factors. The relationship between solvent variation was expressed in the equation Y = 3.334 - 0.624 X, where Y = the curcumin content of Curcuma extract and X = the solvent variation. This means that without the solvent variation, the curcumin content of the Curcuma extracts increases constantly by 3.334. Table-5 showed that the significance value = 0.595> p (0.05), which means that the linear regression model between the variation of the solvent was insignificant. The results in Table-5 showed the value of the Pearson correlation coefficient (r) between the solvent and the curcumin content of r = -0.163. While the significance value (2-tailed) between the solvent and the curcumin content was 0.595> 0.05, which means that there was no significant correlation between water, methanol, ethanol, and n-hexane solvents. The correlation analysis and simple linear regression in this study showed that solvent variation did not affect the curcumin content. One-factor ANOVA in this study showed that the variation of solvent did not significantly influence the average difference in the curcumin content. This hypothesis is supported by the results of the Pearson correlation test and simple linear regression which showed that there is no significant correlation and linear relationship between the two variables tested (p > 0.05). According to Sepahpour *et al.* the use of solvents that are in accordance with the polarity properties of a compound in the extraction process has an effect on the resulting phytochemical compounds.[19] The coefficient of determination (R^2) obtained showed that the contribution of solvent variation to the curcumin content was only 2.6%, while the remaining was caused by other factors.

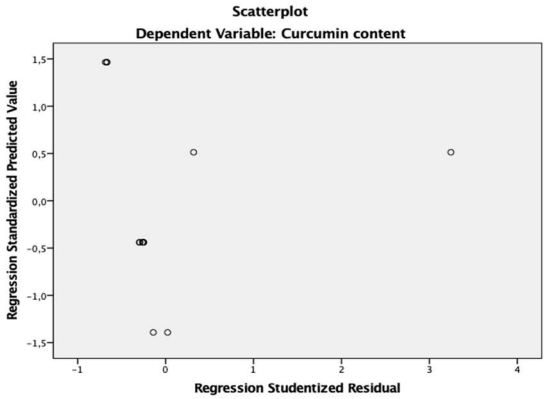

Fig.-2: Scatter Plot of Solvent Variation

Table-5: Results of Correlation Analysis and Simple Linear Regression of Solvent Variation

Variable	r	R^2	Line equation	Sig.
Solvent	-0.163a	0.026	Y = 3,334 – 0,624 X	0.595

Nurcholis *et al*. stated that each dry powder has a different cell wall thickness.[20] Therefore, the ability of the solvent to penetrate the cell wall, and extract the active compound was also different. This was related to the mechanism of the active compound extraction process in *Curcuma*. During the immersion of the material in the solvent, a washing process occurs, where the permeability of the cell wall increases as the solvent enters the cell wall. Therefore, the cell swells and eventually breaks. Cell damage causes the compounds contained in the cell wall to be released and enter the solvent through the diffusion process. The presence of a concentration gradient causes compounds that have the same solubility to dissolve and be drawn out by the solvent from the cell wall. This process stops when the solvent is saturated and no longer has a concentration gradient.[21]

Particle Size

The process of reducing the particle size in the extraction process is one of the factors influencing the isolation of active plant compounds. According to Sukaryo, the degree of powder fineness and the level of

solvent concentration affect the extraction process of a curcumin compound.[22] Particle size reduction is an extraction process that aims to increase the surface area. The larger surface of the material will increase the interaction between the particle and the solvent, therefore, increasing the number of compounds extracted in the diffusion process. The results showed that the highest and lowest curcumin content was obtained by *C. xanthorrhiza* extract at a particle size of 100 mesh 0.593 ± 0.125 and 60 mesh 0.208 ± 0.645. This was in accordance with the opinion of Sembiring *et al.* which stated that, the curcumin content obtained using these measures, has greater and finer powder.[23] Sembiring *et al*. also showed that the curcumin content obtained during the 4-hour extraction process at a size of 40 mesh was higher than that of 60 and 80 mesh.[23] Zhang *et al.* stated that the right material size affects the effectiveness and duration of extraction.[24] The smaller the particle size of a material due to the reduction process causes more cell damage. Therefore, expanding the contact surface of the solvent and the sample, since the yield produced was more. The normality test was conducted to determine the distribution of data in a group or variables. A homogeneity test was conducted to determine the homogeneity or variation of data from a population. The normality and homogeneity tests in this study were carried out on the curcumin content data with variations in particle size using the Shapiro-Wilk method and the Lavene test with IBM SPSS 23. The data tested was said to be normal and homogeneous, when the significance value was greater than 5%.[17] The normality test results in Table-6 indicated the significance value of the sample particle sizes of 60, 80, and 100 mesh respectively as 0.557, 0.493, and 0.377. The significant number obtained from each particle size was greater than 5%, which means the data was normally distributed. This suggested that the normality assumption of the data was met. The results of the homogeneity test in Table-7 showed that the significance value obtained was 0.565 (p> 0.05), therefore, it was stated that the curcumin content from the sample particle size has the same or homogeneous variations. Based on the normality and homogeneity tests that have been carried out, it was stated that the curcumin content from the variation in particle size met the normality and homogeneity assumptions. Therefore, it is necessary to carry out an ANOVA test, followed by the Post hoc assessment with the Tukey method, when the significance value obtained is smaller than 0.05.

Table-6: The Results of the Shapiro-Wilk Normality Test for Variation in Particle Size

Particle size (mesh)	Statistic	Sig.
60	0.924	0.557
80	0.912	0.493
100	0.907	0.377

Table-7: The Results of the Levene Test Homogeneity of Particle Size Variations

df1	df2	Levene statistic	Sig.
2	12	0.598	0.565

The ANOVA test results in Table-8 showed a significance value of 0.002 (p <0.05), which means that there was an effect of variation in particle size of the curcumin content. Table-8 also indicated the average curcumin content with a particle size of 60, 80, and 100 mesh, respectively as 0.208 ± 0.645, 0.361 ± 0.077, and 0.593 ± 0.125. ANOVA testing only proved that there was a difference between the three particle sizes used, however, it has not shown which particle has the difference. Therefore, a further test (Post hoc) is needed to determine the particle sizes that have differences for more detailed conclusions to be drawn. The post hoc test used was the Tukey assessment.

Table-8: ANOVA Distribution of Average Curcumin Content of *C. xanthorrhiza* Extract Based on Variations in Particle Size

Particle size (mesh)	N	Mean	SD	F (ANOVA)	Sig.
60	4	0.208	0.645	11.609	0.002
80	4	0.361	0.077		
100	7	0.593	0.125		

Based on Table-9, the Post hoc test results there was a significant difference in the average curcumin content of Curcuma extract particle sizes of 60 and 100 mesh. This was evidenced by the significance value of each (0.001), which was smaller than p (0.05). However, there was no significant difference between the particle size of 60 and 80 mesh (p = 0.147) and that of 80 with 100 mesh (p = 0.369), because they were p (0.05).

The ANOVA analysis of one factor stated that the variation in particle size had a significant effect on the average difference in the curcumin content at 60 and 80 mesh.

Table-9: Tukey Post hoc Test Results for Particle Size Variation

Particle size (mesh)	Mean difference	Sig.
60 vs 80	0.187750	0.147
60 vs 100	0.389393*	0.001
80 vs 100	0.201643	0.072

*The mean difference is significant at the 0,05 level

A simple linear regression test was used to examine the relationship between particle size variations. This was carried out after the linearity and homogeneity assumption tests were met. The linearity and homogeneity tests were performed by determining the data distribution on the scatter plot and the significance value obtained. The homogeneity test in Fig.-3 showed the location of data variations that were spread or irregular, both above and below the Y-axis.[18] The results of the simple linear regression test in Table-10 showed the coefficient of determination (R^2) by 0.659. This means that the variation contribution in particle size of the curcumin content was 65.9%, while the rest was caused by other factors. The relationship between the variation in particle size on curcumin content was expressed by the equation Y = 0.010 + 0.195 X, where Y = the curcumin content of *Curcuma* extract, while X = the variation in particle size. This means that without any variation in particle size, the curcumin content increase constantly by 1.348. Table 10 showed that the significance value = 0.000 <p (0.05), which means that the linear regression model between the variation in particle size on curcumin content was significant. The results in Table-10 showed that the value of the Pearson correlation coefficient (r) between the particle size and curcumin content was r = 0.812, This means that there was a strong relationship between the variation in particle size and the curcumin content. A positive value on the correlation coefficient indicates that the variation of the extraction method is directly proportional to the curcumin content, meaning that the larger the particle size, the more the content is obtained. Furthermore, a significance value (2-tailed) was obtained between the particle size and the curcumin content of 0.000 <0.05, which means that there was a significant correlation between the particle size of 60, 80, and 100 mesh on the content. The correlation analysis and simple linear regression in this study showed that the variation in particle size affected the curcumin content.

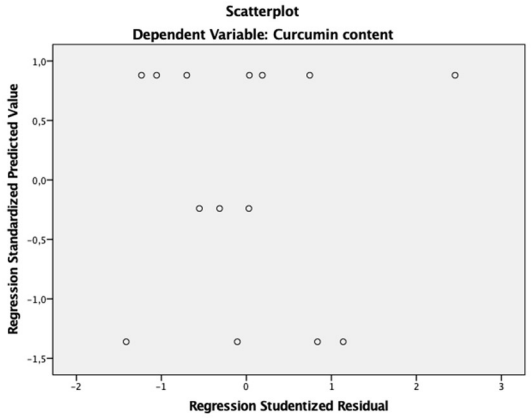

Fig.-3: Scatter Plot of Particle Size Variation

Table-10: Results of Correlation Analysis and Simple Linear Regression of the Variations in Particle Size Effect

Variable	r	R^2	Line equation	Sig.
Particle size	0.812	0.659	Y = 0.010 + 0.195 X	0.000

ANOVA analysis of one factor showed that the variation in particle size had a significant effect on the average difference in curcumin content of *Curcuma* extract at 60 and 80 mesh particle sizes. This was supported by the results of correlation analysis and the simple linear regression which stated that the variation in particle size affects the curcumin content of *C. xanthorrhiza* extract. The coefficient of determination (R^2) obtained, showed that the contribution of the variation in particle size to the content was

only 65.9%, while the remaining was caused by other factors. This was in accordance with the study of Sharma et al., which stated that the extraction time affects the levels of the active components obtained.[25] The longer the extraction time, the increase the contact between the material and the solvent. Therefore, the bioactive components in the solution increase until the saturation point is reached.

Extraction Method

The extraction method used in the isolation process yielded different results. Sasidharan *et al.* stated that qualitative and quantitative studies of bioactive compounds from plants depend on selecting the appropriate extraction method.[26] The results showed that the highest and lowest curcumin levels in the extract were 11.237 ± 1.650 for the Soxhlet method and 0.849 ± 0.324 for the maceration technique, respectively. This was in accordance with the study of Wahyuni et al., which stated that the Soxhlet method is very effective in extracting the active components contained in the sample into the solvent.[27] This effectiveness is influenced by the use of heat in the Soxhlet method which increases the kinetic energy of the solvent. Therefore, the interaction between the solvent and the material is more optimal in extracting the sample. Curcumin is an active compound that is heat resistant. Therefore, it is very good when it is extracted using the Soxhlet method because the help of heat energy aids the isolation process to running well. The normality test was conducted to determine the data distribution in a group or variables. A homogeneity test was conducted to determine the homogeneity or variation of data from a population. The normality and homogeneity tests in this study were carried out on the curcumin content using the extraction method with the Shapiro-Wilk technique and the Lavene test with the IBM SPSS 23. The data tested was stated to be normal and homogeneous, when the significant value was greater than 5%.[17] The results of the normality test shown in Table-11 indicated the value of Soxhlet, sonication, reflux, and maceration methods respectively as 0.245, 0.780, 0.363, and 0.081. The significant level obtained from each method was greater than t5%, which means the data was normally distributed. This suggested that the normality assumption of the data was met. The results of the homogeneity test shown in Table-12 provided a significance value of 0.159 (p> 0.05), therefore, it is stated that the curcumin content from the various extraction methods has the same or homogeneous variations. Based on the normality and homogeneity test that has been carried out, the content from variations in the extraction method met the homogeneity assumption. Therefore, it is necessary to carry out the ANOVA test, followed by the Post hoc assessment with the Tukey method, when the significance value obtained is less than 0.05.

Table-11: The Results of the Shapiro-Wilk Normality Test for Various Extraction Met

Extraction method	Statistic	Sig.
Soxhlet	0.884	0.245
Sonication	0.987	0.780
Reflux	0.893	0.363
Maceration	0.830	0.081

Table-12: The Results of the Levene Homogeneity Test for Variations in the Extraction Method

df1	df2	Levene statistic	Sig.
3	16	1.970	0.159

The ANOVA test results in Table-13 showed that the significance value obtained was 0.000 (p <0.05). Therefore, the variation of the extraction method has an effect on the curcumin content. Table-13 also showed that the average curcumin content of curcuma extracts using the Soxhlet, sonication, reflux, and maceration methods were 11.237 ± 1.650, 3.979 ± 0.025, 2,020 ± 0.122, and 0.849 ± 0.324, respectively. ANOVA testing only proved that there were differences between the four extraction methods used, however, it has not yet shown the exact method with the differences. Therefore, a further test (Post hoc) is needed to determine which extraction methods have differences, in order to draw more detailed conclusions. The post hoc test used was the Tukey assessment. ANOVA analysis of one factor showed that the variation of the extraction method had a significant effect on the average difference in curcumin levels using the Soxhlet method with all the isolation techniques, and the sonication method with the reflux technique. This was supported by the results of correlation analysis and simple linear regression in this study, which stated that the variation of extraction methods has an effect on the curcumin levels.

Table-13: ANOVA Distribution of Average Curcumin Content of *C. xanthorrhiza* Extract Based on Variations in Extraction Method

Extraction method	N	Mean	SD	F (ANOVA)	Sig.
Soxhlet	7	11.237	1.650	137.250	0.000
Sonication	3	3.797	0.025		
Reflux	3	2.020	0.122		
Maceration	7	0.849	0.324		

Based on the Post hoc test results in Table-14, there was a significant difference in the average curcumin content using the Soxhlet technique with sonication, reflux, and maceration methods. This was evidenced by the significance value of 0,000, which was smaller than p (0.05). Similarly, there was a significant difference between sonication and maceration methods with a significance value of 0.003, which was smaller than p (0.05). However, there was no significant difference between the sonication with reflux (p = 0.181), as well as the reflux with maceration methods (p = 0.369), because the significance value was greater than p (0.05). One-factor ANOVA analysis in this study showed that the variation of extraction method had a significant effect on the average difference in curcumin content using the Soxhlet method with all extraction techniques, and the sonication method with the reflux technique.

Table-14: Tukey Post Hoc Test Results of Variations in Extraction Methods

Method	Mean difference	Sig.
Soxhlet Vs Sonication	7.440476*	0.000
Soxhlet Vs Reflux	9.217143*	0.000
Soxhlet Vs Maceration	10.388571*	0.000
Sonication Vs Reflux	1.776667	0.181
Sonication Vs Maceration	2.948095*	0.003
Reflux Vs Maceration	1.171429	0.369

A simple linear regression test was used to examine the relationship between variations in extraction methods on the curcumin content. This was carried out after the linearity and homogeneity assumption tests were met. The linearity and homogeneity tests were carried out by considering data distribution on the scatter plot and the significance value obtained. The homogeneity test in Fig.-4 showed the location of data variations that were spread or irregular, both above and below the Y-axis. This means that the data on variations using the extraction method on curcumin content was homogeneous.[18]

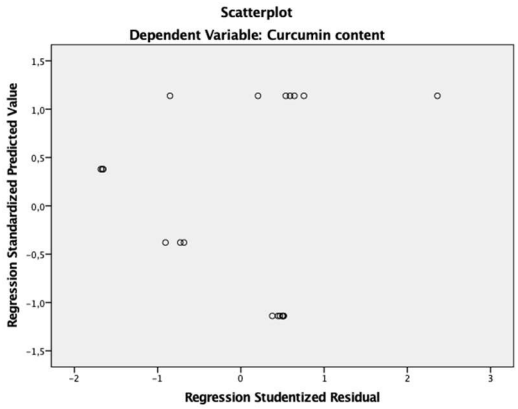

Fig.-4: Scatter Plot of Extraction Method Variation

The simple linear regression test results in Table-15 showed that the coefficient of determination (R^2) was 0.860. This means that the contribution of variation in extraction method to curcumin content was 86%, while the remaining was caused by other factors. The relationship between the variation in extraction method and the curcumin content was expressed by the equation Y = 13,568 - 3,386X, where Y = curcumin content, while X= the variation in extraction method. This means that without any variation in the extraction method, the curcumin content increases constantly by 13.568. Table 15 also showed that the significance

value was 0.000 <p (0.05). This means that the linear regression model between the variation of extraction methods on the content was significant. The results in Table-15 showed that the Pearson correlation coefficient (r) between the extraction method and the curcumin content was r = -0.927. This means that there was a moderate relationship between variations in extraction methods on the curcumin content. The negative value on the correlation coefficient indicates that the variation in extraction methods is inversely related to the content. Furthermore, a significance value (2-tailed) was obtained between the extraction method and the curcumin content of 0.000 <0.05. This means that there was a significant correlation between the Soxhlet, sonication, reflux, and maceration methods on the content. The correlation analysis and simple linear regression in this study stated that the variation of extraction methods had an effect on the curcumin content.

Table-15: Simple Linear Regression Test Results for the Effect of Variations in Extraction Methods

Variable	r	R^2	Line equation	Sig.
Extraction method	0.927	0.860	Y = 13.568 – 3.386 X	0.000

The coefficient of determination (R^2) obtained showed that the contribution of the variation of the extraction method has an influence on the curcumin content by 86%, while the remaining was influenced by other factors. Furthermore, other factors that affected the results of phytochemical levels obtained were extraction time and temperature.[28,29] This was in line with the study of Srijanto *et al.* which stated that the length of contact time between the solvent and the sample increases the number of extracted curcumin until an equilibrium condition is reached, between the concentration of the solvent and the compounds in the material.[30] The dissolved compounds contained in the material have the potential to be lost and damaged when the extraction process takes place past the optimum time.[31]

CONCLUSION

The highest curcumin content in *C. xanthorrhiza* extraction was obtained using n-hexane solvent, 100 mesh particle size, and the Soxhlet method. The effectiveness of these three factors contributed respectively 2.6%, 65.9%, and 86% in the curcumin extraction process. The value obtained in the selection of the solvent did not show a significant effect, however, there was a correlation and influence on the content on variations in particle size and extraction method. Therefore, it is necessary to carry out further research regarding the optimization of curcumin extraction from *C. xanthorrhiza* in various regions. And also to compare and determine which has the most curcumin content by considering the combination of the following factors, namely solvent selection, particle size, and extraction method. In addition, in vitro, and in vivo research is needed regarding the potential of the active compound curcumin in *C. xanthorrhiza*.

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[RJC-6798/2021]