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Phytochemical Screening and Thin Layer Chromatography Analysis of Ethanol Extract of Bangle Rhizome (*Zingiberis cassumunar* Roxb.)

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Abstract: Bangle rhizome (*Zingiber cassumunar* Roxb.) is a part of the plant that is widely used in traditional medicine because its diverse secondary metabolites have great potential in treating various diseases. The aim of this research are to identify the content of curcumin and active compounds found in bangle rhizome (*Zingiber cassumunar* Roxb.) qualitatively and quantitatively using Thin Layer Chromatography (TLC) and UV-Vis Spectrophotometry methods. The maceration method was used to extract the bangle rhizome with 70% ethanol solvent, resulting in an extract yield of 7.92%. The results of the study from qualitative analysis using TLC with 3 different mobile phases, chloroform: ethanol (95:5), chloroform: methanol: glacial acetic acid (94:5:1), and chloroform: n-hexane: methanol (1:1:0.1). It is indicate that the ethanol extract from the bangle rhizome positively contains polyphenolic compounds, terpenoids, and glycosides. Quantitative analysis of curcumin using UV-Vis Spectrophotometry at a wavelength of 437 nm resulted in a linear equation $y = 0.026x + 0.1026$ with an R^2 value of 0.9958 and $r = 0.997898$. The absorbance values from the 3 replicates of the bangle rhizome ethanol extract sample solution obtained in this study were 0.35 nm; 0.38 nm; 0.392 nm, indicating the presence of curcumin in all three replicates of the bangle rhizome ethanol extract solution. Based on the research results, it can be concluded that the ethanol extract of bangle rhizome (*Zingiber cassumunar* Roxb.) positively contains polyphenolic compounds, terpenoids, glycosides, and curcumin.

Keywords: Bangle; Thin Layer Chromatography; Curcumin; UV-Vis Spectrophotometry; *Zingiber cassumunar* Roxb.

1. Introduction

Indonesian have long known and used plants from the *Zingiberaceae* family in various ways, such as in seasoning dishes, food, and beverages, as well as for various medicinal purposes by extracting plant juice, boiling them, making herbal concoctions, or applying them to sore body parts. One of the well-known medicinal plants of the *Zingiberaceae* family in Indonesia is Bangle. (*Zingiber cassumunar* Roxb.) (Mutaqin *et al.*, 2017; Safari and Sinaga, 2022). Bangle (*Zingiber cassumunar* Roxb.) is a plant of the genus *Zingiber* which belongs to the *Zingiberaceae* family. Bangle have long been used in traditional medicine cure various diseases. The main part of Bangle that is often used as a medicine is the rhizome. Bangle rhizome often used as a medicine that has antimicrobe, antioxidant, immunomodulator, antipyretic, constipation, worms, stomach pain, and

gout benefits (Batubara *et al.,* 2018; Nurkhasanah *et al.,* 2017; Rungruang *et al.,* 2021; Suci *et al.,* 2024; Syamsuri and Alang, 2021). Antioxidant and immunomodulatory properties of bangle are provided by active compounds found in bangle rhizomes, such as essential oils containing pinene, caryophyllene, sabinene, and caryophyllene oxide (Nurkhasanah *et al.,* 2017) that could increase phagocytic activity *in vitro* (Setyani *et al.,* 2021). The ethanol extract of bangle rhizome contains curcumin at 0.0175g/100g, an important phytochemical compound with antimicrobial potential against various pathogens (Rungruang *et al.,* 2021). The pharmacological effects of bangle are likely caused by one or more secondary metabolite compounds found in the rhizome of the bangle plant (Setyani *et al.,* 2021).

Choosing the right and appropriate extraction method is crucial to maximize the potential of bangle rhizomes. Factors to consider include the choice of solvent that matches the polarity of the compounds and the extraction method used (Susiloningrum and Sari, 2023). Extraction methods are classified into two types, namely hot and cold. The use of appropriate extraction method will maximize its medicinal potential because it produces bangle rhizome extracts with optimal secondary metabolite content (Daryanti *et al.,* 2023).

Currently, the issue of "*back to nature*" is circulating in Indonesian society. This has triggered rapid developments in bioactive plant-based medicine. Plants utilize secondary metabolites as a means of defending against various adverse conditions, such as extreme temperatures, pests, and climate change, and exhibit bioactivity that can help treat diseases. These secondary metabolites include a variety of compounds, including polyphenols, saponins, flavonoids, quinones, tannins, alkaloids, steroids, and triterpenoids (Wahidah *et al.,* 2021). One initial method to identify and determine the secondary metabolite compounds contained in plants is called phytochemical screening. Phytochemical screening can be done qualitatively or quantitatively based on the desired analysis method. Color reactions using reagents can be used for qualitative phytochemical screening.

The Thin Layer Chromatography (TLC) method is a relatively simple qualitative analysis to identify the chemical compounds in plants. The results of TLC analysis, such as Rf values and spot colors, can provide information about the identity of the contained compounds. Each plant has a unique chromatogram profile that differs from other plants, so it can be used as a reference to ensure the presence of plant content in natural medicines, as well as to avoid the addition of chemicals and drug counterfeiting (Forestryana and Arnida, 2020). Based on this, the research focuses on the phytochemical screening of bangle rhizome (*Zingiber cassumunar* Roxb.). The main objective of this study is to identify the content of curcumin and compounds present in the bangle rhizome (*Zingiber cassumunar* Roxb.) qualitatively and quantitatively using Thin Layer Chromatography and UV-Vis Spectrophotometry methods.

2. Materials and Methods

This research is an experimental study conducted in the Chemistry Laboratory of Universitas Muhammadiyah Magelang in February-March 2024 to identify the phytochemical content in the bangle rhizome.

Material: The main material used in this research is bangle rhizome powder (*Zingiber cassumunar* Roxb.). The chemicals used are aquadest, 70% ethanol, 95% ethanol, chloroform, methanol, glacial acetic acid, n-hexane, $FeCl_3$ 1%, NaCl 2%, Gelatin solution 1%, HCl, H_2SO_4.

Equipment: The equipment used in this research includes Beakers (Pyrex), Measuring Flasks (Pyrex), Test Tubes (Pyrex), Erlenmeyer Flasks (Pyrex), Volumetric Flasks (Pyrex), Micropipettes, Analytical Balance (OHAUS), Glass Funnels (Pyrex), Water Bath (WNB14RING Memmert), Electric Stove (Maspion SH-31), Filter Paper, Aluminium Foil, Test Tube Rack, Capillary Tubes, Porcelain Dishes, Chamber, Silica Gel GF254 (Merck), UV Lamps 254 nm and 366 nm, Cuvette, UV-Vis Spectrophotometer (Cecil CE 1021 UV/VIS Spectrophotometer-E36).

Method

Extraction of Bangle Rhizome

The extraction of bangle rhizome was carried out using the maceration method. Twenty five (25) grams of bangle rhizome powder is macerated for 24 hours with 96% ethanol solvent, with a sample-to-solvent ratio of 1:10. After 24 hours, the filtrate and residue are separated. Then, the powder is remacerated for 24 hours with a sample-to-solvent ratio of 1:5. The filtrate from the maceration and re-maceration is evaporated over a water bath until a thick extract is obtained. Collect the thick extract in a petri dish or a closed bottle.

Qualitative Analysis

Polyphenol Identification

Two hundred (200) mg bangle rhizome powder was heated with 10 ml of water in a boiling water bath for 20 minutes, then the mixture was filtered while still hot. After cooling, 3 drops of $FeCl_3$ were added. If a black-green color is formed, it shows that there are positive polyphenol compounds (Padamani *et al.*, 2020).

Identification of Tannins

Two hundred (200) mg bangle rhizome powder was heated with 10 ml of water in a boiling water bath for 20 minutes, then the mixture was filtered while still hot. Then, 5 ml of filtrate was added with 1 ml of 2% NaCl solution. Use filter paper to filter out any precipitate that may form. The filtrate was added with 5 ml of 1% gelatin solution. If a precipitate is formed, it is positive for tannin (Azzahra *et al.*, 2022).

Saponin Identification

Two hundred (200) mg of bangle rhizome powder was put in a test tube and 10 ml of distilled water was added. The tube was then closed and shaken vigorously for 30 seconds. After that, it is allowed to stand upright for 30 minutes. Add 1-3 drops of dilute HCl if there is froth as high as ± 3 cm from the surface. If the froth does not disappear, it is positive for saponin (Putri *et al.*, 2023).

Terpenoid Identification

Two hundred (200) mg bangle rhizome powder was heated with 10 ml water. Add 2 ml of bangle extract into a test tube, followed by 10 drops of anhydrous acetic acid and 3 drops of concentrated sulfuric acid. If the formation of a red-green solution accompanied by a blue-violet ring indicates positive terpenoids (Puspa *et al.,* 2017).

Identification of Glycosides

Two (2) grams of bangle rhizome powder was put into a glass baker and then added 10 ml of methanol. After that, it was heated to boiling and then filtered. Then added 1 drop of $FeCl_3$ 1% and 1 drop of concentrated H_2SO_4. When a blue or green precipitate forms, it is positive for glycosides (Cahyani *et al.,* 2019).

Identification of Ethanol Extract of Bangle Rhizome by Thin Layer Chromatography (TLC) Method

Silica gel GF_{254} paper with a distance of ± 1 cm from the bottom edge of the plate and a creepage distance of 8 cm. Then the ethanol extract of bangle rhizome was bottled on silica gel GF_{254} paper with a capillary pipe and then eluted with 3 mobile phases, namely:

1. Chloroform: ethanol (95: 5)
2. Chloroform: methanol: glacial acetic acid (94: 5: 1)
3. Chloroform: n-hexane: methanol (1: 1: 0.1)

Observe the stain formed due to UV light with a wavelength of 254 nm.

Quantitative Analysis

Determination of the maximum wavelength of curcumin

Determination of the maximum wavelength using a UV-Vis spectrophotometer with a range of 400 - 600 nm. The test results for curcumin standard solution were obtained at the maximum wavelength of 437 nm. The absorbance of the bangle rhizome ethanol extract sample was measured at that wavelength.

Determination of Standard Curve

Weighing the standard curcumin standard for curcumin is 5 mg, then put into a measuring flask and continued with the addition of 10 ml of ethanol to get a concentration of 100 ppm. The stock solution made was taken 6 mL and diluted with ethanol to a volume of 10 mL so as to obtain a concentration of 60 ppm. Then, a UV-Vis Spectrophotometer with a maximum wavelength of 435 nm was used to conduct the test. Because the concentration is too high, further dilution is carried out again. 2 mL of stock solution is diluted with ethanol to a volume of 10 mL so that a concentration of 12 ppm is obtained. Furthermore, 7 different concentrations were made, namely 10 ppm, 15 ppm, 20 ppm, 25 ppm, 30 ppm, 35 ppm, and 40 ppm. Solution testing was carried out on a UV-Vis spectrophotometer with a maximum wavelength of 435 nm.

Determination of Chemical Compound Content of Ethanol Extract of Bangle Rhizome

A total of 10 mg of ethanol extract of bangle rhizome was put into a measuring flask and continued with the addition of 10 mL of ethanol. The stock solution made was taken 2 mL and diluted with

ethanol to a volume of 10 mL so that a concentration of 200 ppm was obtained. Then 3 replications were made. Measurement of absorbance at 435 nm with a UV-Vis spectrophotometer.

Data Analysis

The linear regression equation can be used to calculate the analysis of curcumin content in bangle rhizomes based on absorbance values obtained from seven different concentrations of curcumin with a linear regression equation: y = a + bx

Flavonoid levels in the sample were calculated using the formula: $\frac{C \; x \; V \; x \; Fp}{W}$ x 100%.

3. Results and Discussion

Phytochemical screening was conducted to determine the secondary metabolite content in the ethanol extract of the bangle rhizome (*Zingiber cassumunar* Roxb.). The extract in the study was obtained through the process of maceration. The maceration method was chosen because it does not require heating thus preventing damage to the compounds to be used, and the necessary equipment is easy to obtain. The solvent used in the maceration process is 70% ethanol. According to Dewi *et al* (2022) and Hakim & Saputri (2020) 70% ethanol as a solvent is more selective, neutral, and has high absorption. In addition, ethanol can be mixed with water at various concentrations and its concentration requires little heating and can dissolve various kinds of active substances while reducing the dissolution of inactive substances. The yield of maceration with 70% ethanol solvent will be higher than maceration with 96% ethanol and 50% ethanol. This study used 25 g of dried bangle rhizome (*Zingiber cassumunar* Roxb.) as simplisia. The extraction yielded a thick extract weighing 1.98 g with a yield of 7.92%. The extract from the maceration process was thick, sticky, reddish-brown in color, and had a distinctive smell.

Table 1. Results of qualitative analysis of chemical compound identification in bangle rhizome (*Zingiber cassumunar* Roxb.)

Compund Group	Reagents	Results		Desc
		Theory	Observation	
Polyphenol	Hot water + $FeCl_3$	Green, Black	Green	(+)
Tannin	Hot water + NaCl 2% + Geltin 1%	Precipitate	No precipitate occurs	(-)
Saponin	Distilled water + HCl diluted	Froth up to 3 cm high	Froth disappears	(-)
Terpenoid	Hot water + Anhydrous Acetic Acid + H_2SO_4 concentrated	Red-Green, Blue Violet Ring	Red with Violet Rings	(+)
Glycoside	Methanol + $FeCl_3$ 1% + H_2SO_4 concentrated	Blue, Green	Deep Green	(+)

Description: (+) : Positive, (-): None/Negative

Identification of chemical compounds contained in the ethanol extract of bangle rhizome (*Zingiber cassumunar* Roxb.) showed that the extract contain polyphenols, terpenoids and glycosides (Table 1). Based on previous research conducted by (Daryanti *et al.*, 2023) and (Susiloningrum & Sari, 2023), bangle rhizomes contain secondary metabolites, namely flavonoids and triterpenoids.

Polyphenol	Tannin	Saponin	Terpenoid	Glycoside

Figure 1. Results of the qualitative analysis of the identification of chemical compounds in the bangle rhizome (*Zingiber cassumunar* Roxb.)

Polyphenols show positive results, indicated by a green color change (Figure 1). According to previous research by (Padamani *et al.*, 2020) the green-black color change indicates the presence of polyphenolic compounds. Terpenoids show a positive result marked by a color change to red accompanied by a violet ring on the surface of the liquid, as shown in Figure 1. This is consistent with the research (Puspa *et al.*, 2017) which states that the formation of a greenish-red solution accompanied by a blue-violet ring indicates the presence of terpenoids.

Glycosides showed positive results as evidenced by a change in color in the solution to solid green (Figure 1). Based on research Nurrosyidah and Ambari (2019) and Rizki *et al* (2023) stated that the *Zingiberaceae* family group positively contains glycosides The results of the identification of chemical compounds in bangle rhizomes showed negative tannins as indicated by the absence of precipitate formation. Based on previous research conducted (Azzahra *et al.*, 2022), identification of chemical compounds in bangle rhizomes showed negative tannin content as evidenced by the absence of precipitate formation. The identification of negative saponin compounds is characterized by the froth disappearing when tested with a dilute HCl reagent.

Confirmation tests using the TLC method were conducted to determine the chemical compound content in the bangle rhizome. The data obtained include Rf values and colored spots or stains on the chromatogram produced from the elution of the TLC plate. This data explains the active compound content suspected to be present in the ethanol extract of the bangle rhizome. (*Zingiber cassumunar* Roxb.). The obtained Rf value can be used to identify active compounds because it shows the differences in compound properties. Stain detection is performed using a UV

lamp with a short wavelength of 254 nm. The principle of UV light observation is that the plate will fluoresce at a short wavelength of 254 nm, while stains are indicated by dark-colored samples. This occurs because the fluorescent indicator on the TLC plate interacts with UV light (Forestryana and Arnida, 2020).

Table 2. Comparison of TLC results of ethanol extract of bangle rhizome (*Zingiber cassumunar* Roxb.)

Mobile Phase	Chloroform : Ethanol			Chloroform : Methanol : Glacial Acetic Acid			Chloroform : n-Hexan : Methanol		
	95 : 5			94 : 5 : 1			1 : 1 : 0,1		
λ=254 nm	Stain	Color	Rf	Stain	Color	Rf	Stain	Color	Rf
Curcumin	1	Y	0.94	1	Y	0.68	1	G	0.31
	2	Y	0.78	2	Y	0.54	2	Y	0.16
	3	Y	0.56				3	Y	0.10
Sampel	1	Y	0.94	1	G	0.68	1	Y	0.31
				2	G	0.54			

Description: Y: Yellow, G: Green

The TLC test for the content of curcumin compounds was conducted with the first mobile phase, which is Chloroform: Ethanol in a 95:5 ratio, resulting in an eluent of the standard curcumin solution with 3 yellow spots (Figure 2) with Rf values of 0.94; 0.78; 0.56 (Table 2). Meanwhile, the eluent of the ethanol extract sample of the bangle rhizome only produced an eluent with 1 yellow spot (Figure 2) with an Rf value of 0.94 (Table 2). The results of the TLC test in this first mobile phase indicate that the ethanol extract of the bangle rhizome positively contains curcumin compounds.

Then, the TLC for the content of curcumin compounds was conducted using the second mobile phase, which is Chloroform: Methanol: Glacial Acetic Acid in a 94:5:1 ratio. This resulted in the elution of the standard curcumin solution, producing 2 yellow spots (Figure 2) with Rf values of 0.68 and 0.54 (Table 2). Meanwhile, the elution of the ethanol extract of the bangle rhizome only produced an elution similar to the standard solution, which was 2 green spots (Figure 2) with Rf values of 0.68 and 0.54 (Table 2). The results of the TLC test in this first mobile phase indicate that the ethanol extract of the bangle rhizome positively contains curcumin compounds.

In the TLC test for the content of curcumin compounds conducted with the third mobile phase, namely Chloroform: n-Hexane: Methanol in a ratio of 1:1:0.1, the eluent of the standard curcumin solution produced 3 spots with 1 green spot (Figure 2) having an Rf value of 0.31 and 2 yellow spots (Figure 2) with Rf values of 0.16 and 0.10 (Table 2). Meanwhile, the eluent of the ethanol extract of the bangle rhizome only produced 1 yellow spot (Figure 2) with an Rf value of 0.31 (Table 2). The results of the TLC test in this first mobile phase indicate that the ethanol extract of the bangle rhizome positively contains curcumin compounds.

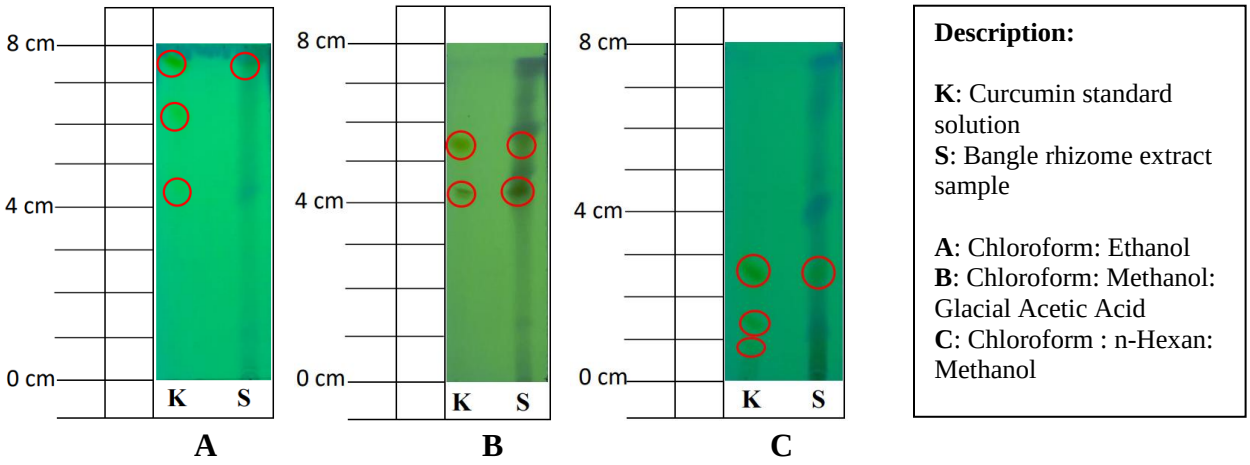

Figure 2. Comparison of KLT identification results of ethanol extract of bangle rhizome (*Zingiber cassumunar* Roxb.)

Based on the TLC test of curcumin content in the ethanol extract of bangle rhizome using 3 different mobile phases, it can be concluded that the bangle rhizome, which is part of the *Zingiberaceae* family, positively contains curcumin compounds, as evidenced by the presence of yellow and green spots on the sample eluent track. Curcumin belongs to the polyphenol group. Curcumin is an active compound that can provide yellow and orange colors and has antioxidant properties that help prevent damage caused by free radicals (Sugiandi *et al.*, 2021).

The determination of the standard linear regression curve for curcumin was performed by scanning the standard solution using UV-Vis Spectrophotometry at the maximum wavelength, which is 435 nm (Rizki *et al.*, 2023). The maximum wavelength obtained in this study is close to the results of previous research, which is 437 nm. The absorbance results from the concentration of the curcumin stock solution were recorded, and then a linear regression curve was created, as shown in Figure 3.

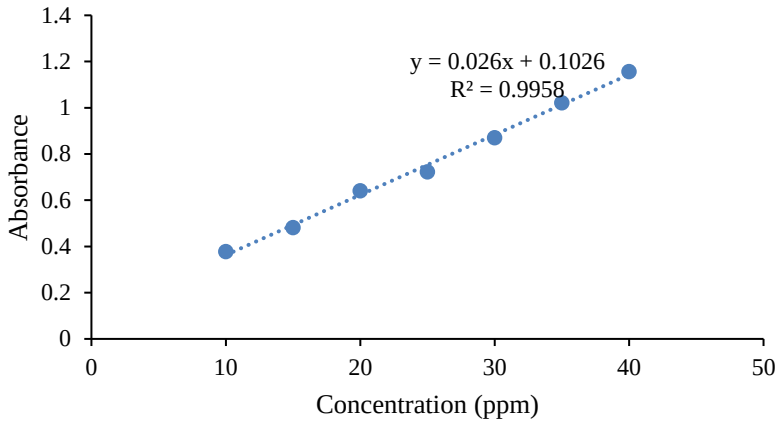

Figure 3. Linear regression curve of the curcumin stock solution at concentrations of 10-40 ppm

Figure 3 shows the results of the linear regression equation from the calibration curve, y = 0.026x + 0.1026, this equation will be used to calculate the curcumin content in the sample. Meanwhile, the linearity relationship on the curve can be seen from the coefficient of determination (R2) value of 0.9958 and the correlation coefficient (r) of 0.997898. The R2 value obtained with a value of 0.9958 means that 99.58% of the concentration affects the absorbance by 99.58%. The r value is a parameter of the linear relationship between concentration and the instrument's response, with an r value approaching +1 or -1 indicating that the relationship has reached an ideal state (Esati *et al.*, 2024). Based on Figure 3, the relationship between curcumin concentration and absorbance is linear; the higher the curcumin concentration, the higher the absorbance obtained.

The measurement of the test sample in the form of a ginger rhizome ethanol extract solution with an initial concentration of 1000 ppm was then diluted 5 times to obtain a ginger rhizome ethanol extract concentration of 200 ppm. The solution was then replicated 3 times. The results of the UV-Vis Spectrophotometry tests obtained are shown in Table 3.

Table 3. Results of UV-Vis Spectrophotometry testing on ethanol extract of bangle rhizome

Replication	Absorbance (nm)	Concentration (ppm)	Substance Content (% $^{b}/_{v}$)	Average Substance Content (%)	Standard Deviation
1	0.35	9.51	0.047		
2	0.38	10.6	0.053	0.051	0.00339
3	0.392	11.13	0.055		

In Table 3, the results of the absorbance measurements of the ethanol extract sample solution of the bangle rhizome at UV wavelengths using a UV-Vis Spectrophotometer with 3 replicates are shown. In the 1st Replication, an absorbance of 0.35 nm was produced with a sample solution concentration of 9.51 ppm, resulting in a curcumin content of 0.047%. In the 2nd Replication, an absorbance of 0.38 nm was produced with a sample solution concentration of 10.6 ppm, resulting in a curcumin content of 0.053%. In the 3rd Replication, an absorbance of 0.392 nm was produced with a sample solution concentration of 11.13 ppm, resulting in a curcumin content of 0.055%. This shows that the absorbance increases as the concentration of the sample solution of ethanol extract of bangle rhizome increases (Esati *et al.,* 2024).

The relationship between the standard curcumin solution and the sample solution of ethanol extract from the bangle rhizome is that the standard curcumin solution has an absorbance wavelength range of 0.378 nm - 1.156 nm (Figure 2), while the sample solution of ethanol extract from the bangle rhizome has absorbance wavelengths of 0.35 nm; 0.38 nm; 0.392 nm (Table 3). Therefore, the absorbance wavelengths of the sample solution that fall within the range of the standard curcumin solution are 0.38 nm and 0.392 nm.

Standard deviation (SD) of measurement is necessary to determine the accuracy of a result because a more precise method is used when the SD value of the measurement is smaller. The result of the SD value calculation from the data of 3 sample replications obtained is 0.00339. The SD value from this study is considered accurate because the smaller the standard deviation, the smaller the coefficient of variation (Trinovita *et al.,* 2020).

The determination of the absorbance of the standard solution is in accordance with Beer-Lambert's law, which states that the concentration of a substance in a sample is directly proportional to its absorbance. If the absorbance value in the sample is high, then the concentration value will also be high according to Beer-Lambert's law (Syamsul *et al.,* 2019). In the results of this study, the obtained absorbance values fall within an acceptable absorbance range. A good absorbance range is between 0.2 and 0.8 nm (Trinovita *et al.,* 2020). Based on Table 3, the absorbance values of the ethanol extract sample solution of the bangle rhizome in three consecutive replicates obtained in this study are 0.35 nm; 0.38 nm; and 0.392 nm. Therefore, it can be said that the bangle rhizome contains curcumin compounds. The results obtained from the ethanol extract of the bangle rhizome contain an average curcumin content of 0.051%.

4. Conclusion

Based on the research results using the quantitative analysis method, it shows that the ethanol extract of bangle rhizome positively contains polyphenolic compounds, terpenoids, glycosides, and curcumin. Meanwhile, the quantitative analysis of curcumin using UV-Vis spectrophotometry resulted in curcumin levels from three replicates of the ethanol extract solution of bangle rhizome samples obtained in this study, which were 0.047%; 0.053%; 0.055%, respectively. Therefore, it can be concluded that curcumin is present in all three replicates of the ethanol extract solution of bangle rhizome.

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