

Qualitative and Quantitative Analysis of Compounds in Kencur Extract Using TLC and UV-Vis Spectrophotometry

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Abstract

Kencur (*Kaempferia galanga* L.) is one of the plants in the Zingiberaceae family (gingers) that is often used by people in Asia, including Indonesia. Kencur is also widely used as a traditional medicine because it contains active compounds that are beneficial for treatment. This research aims to conduct a qualitative and quantitative analysis of secondary metabolites in Kencur extract samples. Qualitative analysis was conducted using thin layer chromatography with three variations of the mobile phase: Chloroform : Methanol (95:5), Chloroform : Methanol : Glacial Acetic Acid (94:5:1), Chloroform : n-Hexane : Methanol (1:1:0.1), and the stationary phase Silica Gel GF254. Meanwhile, the quantitative analysis was conducted using UV-Vis spectrophotometry at a wavelength of 429.5 nm. The research results show that Kencur contains compounds such as tannins, glycosides, and terpenoids. This research also identifies the presence of curcumin, which has antioxidant effects and other benefits. The results indicate that 2 out of 3 experiments showed positive results, with Kencur containing curcumin at levels of 0.4593% and 0.4393%. The methods used were qualitative methods through thin-layer chromatography and quantitative analysis of curcumin levels in the samples using UV-Vis spectrophotometry.

Keywords: *Kaempferia galanga* L., TLC, UV-Vis Spectrophotometry, Curcumin

INTRODUCTION

Indonesia is known for its biodiversity, particularly its natural resources, which include plants that can be used as traditional medicine. One of the plants that is widely cultivated is Kencur. Kencur (*Kaempferia galanga* L.) is one of the plants in the Zingiberaceae family (gingers) that is often used by people in Asia, including Indonesia. Kencur thrives in lowlands, in mountains with loose soil and not too much water. The rhizome has a specific aroma. The flesh of the fruit is

white and its outer skin is brown. Kencur grows and develops during a specific season, which is the rainy season (Nurmala & Zahara, 2022).

Kencur is widely used by the community as a cooking spice. In addition, Kencur has also been studied and utilized as medicine. Empirically, Kencur can be effective in treating stomach inflammation, ear inflammation, influenza, colds, headaches, coughs, diarrhea, irregular menstruation, tired eyes, and sprains (Subaryanti dkk., 2020). The rhizome or

rhizome of Kencur also contains essential oils and alkaloids that are used as stimulants (Rohman dkk., 2021). Based on previous research findings, Kencur has activities as an anti-inflammatory, analgesic (Silalahi, 2019), nematicide, mosquito repellent, larvicide, vasorelaxant, sedative, antineoplastic, antimicrobial, antioxidant, anti-allergic, and wound healer (Umar, 2011). The biological function is closely related to the active compounds found in the Kencur rhizome itself.

The chemical content found in Kencur includes essential oil at 2.4-2.9%, which consists of ethyl para-methoxy cinnamate (30%), camphor, borneol, cineole, and pentadecan. Ethyl para-methoxy cinnamate in Kencur is a derivative compound of cinnamate (Khotimah dkk., 2023). Similar research on the phytochemical screening results of ethanol extract from Kencur rhizomes detected the presence of flavonoids, polyphenols, tannins, steroids, monoterpenes, and sesquiterpenes (Umar, 2011). In addition to those compounds, one of the derivatives from plants that has antioxidant effects is curcumin. Curcumin is one of the most recommended active compounds to be developed for the therapy of various diseases (Suprihatin dkk., 2020). Curcumin is more soluble in ethyl acetate, ethanol, acetonitrile, acetone, methanol, and chloroform (Rahmat dkk., 2023).

Therefore, this research aims to identify the compounds or secondary metabolites contained in the extract of Kencur rhizome both qualitatively and quantitatively.

RESEARCH METHOD

Tools and Materials

Tools – glassware (Pyrex), Silica gel plates GF254 (TLC Silica Gel 60 F254), thin layer chromatography chamber (Local), micropipette (DLab), UV lamp (Local), UV-Vis spectrophotometer; cuvette (Cecil), analytical balance (AND), 96% ethanol, methanol (Supelco), Kencur simplicia, chloroform (Supelco), n-hexane, distilled water, curcumin standard, reagents (FeCl₃, 2% NaCl, 1% gelatin, HCl, anhydrous acetic acid, glacial acetic acid) (Brataco).

Procedure

Sampling and Extraction Using the Maceration Method

The sample of Kencur powder (*Kaempferia galanga L*) was purchased in the city of Magelang, Central Java. The sample was extracted with 96% ethanol in a ratio of 1:10. A sample weighing 25 mg was dissolved in 250 ml of 96% ethanol and then macerated for 24 hours. The liquid extract obtained is filtered and then evaporated over a water bath until a thick extract is obtained that meets the desired consistency.

Identification of Active Compounds

1. Identification of Polyphenols

Two hundred mg of the extracted sample, then heat it with 10 ml of distilled water. Filter it and take the filtrate, then place it into a test tube, adding 3 drops of FeCl₃. If the positive sample contains polyphenols, a color change to blue-green occurs.

2. Identification of Tannins

Two hundred mg of the extract sample is then heated with 10 ml of distilled water for 30 minutes. It is filtered, and 5 ml of the filtrate is taken, then 1 ml of 2% NaCl solution or 5 ml of 1% Gelatin is added. If a precipitate forms, it indicates the presence of tannins.

3. Identification of Saponins

Two hundred mg of extract sample in a test tube, and 10 ml of distilled water. Close and shake vigorously for 30 seconds, then let the tube stand upright for 30 minutes. If bubbles rise to a height of approximately 3 cm from the surface and do not disappear after being treated with dilute HCl, it indicates a positive presence of saponins.

4. Identification of Terpenoids

Place 2 ml of the extract into a test tube, then dissolve it with 5 ml of chloroform. Add 10 drops of anhydrous acetic acid and 3 drops of concentrated sulfuric acid. The formation of a red-green

or violet-blue solution indicates the presence of terpenoids.

5. Identification of Glycosides

Two grams sample of the extract and add 10 ml of methanol, boil, and filter. Add glacial acetic acid containing 1 drop of 1% FeCl₃, then add concentrated sulfuric acid. If a chemical reaction occurs, it indicates the presence of cardiac glycosides/steroids.

Standard Solution

The standard used in thin layer chromatography is curcumin.

Identification of Compounds using Thin Layer Chromatography

The sample and standard solutions are spotted on the TLC plate with the mobile and stationary phases as shown in Table 1. Thin Layer Chromatography Mobile Phase System

Table 1. Thin Layer Chromatography Mobile Phase System

Standards	Curcumin	Curcumin	Curcumin
Mobile Phase	Chloroform: Methanol (95:5)	Chloroform: Methanol: Glacial Acetic Acid (94:5:1)	Chloroform: n-Hexane: Methanol (1:1:0,1)
Stationary Phase	Silica gel GF ₂₅₄	Silica gel GF ₂₅₄	Silica gel GF ₂₅₄

The identification of compounds is carried out qualitatively by comparing the spots formed on the TLC plate of the sample with the spots of the standard solution observed under UV light at 254 nm.

Calculation of the Retention Factor Value in Thin Layer Chromatography

The spots formed on the TLC plate are placed under UV light with a wavelength of 254 nm and a ruler is used to facilitate measurement. Calculate the *R_f* value using the following equation (Adnina, 2018).

$$R_f = \frac{\text{Distance traveled by the component}}{\text{Distance traveled by the solvent}}$$

Determination of Maximum Wavelength

A standard solution of curcumin at a concentration of 1000 ppm in ethanol p.a. was measured for its absorbance and maximum wavelength using a UV-Vis spectrophotometer in the range of approximately 400 – 500 nm.

Determination of the Standard Curve

Dilute the standard solution of curcumin at 1000 ppm by taking 0.1 ml, placing it in a 10 ml volumetric flask, and adding ethanol up to the mark. Then measure the absorbance value at a wavelength of 400 – 500 nm. If the values of absorbance are not suitable, perform a dilution again until five variations of concentration are obtained, namely 10, 8, 6, 4, and 2 ppm. Measure the absorbance values of each of these solution concentrations at the maximum wavelength of curcumin, which is between 400 and 500 nm. If the absorbance range of 0.2 to 0.8 is already suitable, create a standard curve until you obtain the linear regression equation.

Calculation of Curcumin Content in Samples

The samples were prepared in two replicates, each by weighing 10 mg of the galanga extract sample, and dissolving it in methanol in a 10 ml volumetric flask. Perform absorbance measurements using a UV-Vis spectrophotometer. If the absorbance value exceeds 0.2 – 0.8, then dilution should be carried out.

$$\frac{\text{Concentration (ppm)} \times \text{Vol} \times \text{Dilution Factor}}{\text{Sample mass (mg)}} \times 100\%$$

The curcumin content in the sample can be determined from the results of the linear regression equation obtained from the standard curve, then insert the absorbance value of the sample into the equation $y = a + bx$. The concentration obtained represents the sample concentration. The amount of curcumin can be calculated using the following equation.

RESEARCH RESULT AND DISCUSSION

Sample Extraction by Maceration

Extraction is the process of selectively transferring compounds between two immiscible solvents, typically an organic solvent and water, or from a solid to a liquid. The solvent used in this experiment is 96% ethanol because it is widely available in the market at a lower cost. Additionally, 96% ethanol yields a higher percentage of extract compared to water (Shary & Mahfur, 2023). Ethanol is a

polar solvent and is considered very effective for preliminary extraction.

This experiment was conducted using the maceration extraction method. Maceration is the process of soaking materials in a suitable solvent to extract active compounds, using low heat or no heat at all. The maceration method was chosen because the procedures and equipment used are simple. Maceration in this experiment was carried out using a sample of 25 grams of powdered Kencur rhizome, soaked in 250 mL of 96% ethanol and left to stand for 24 hours before being filtered. The purpose of the filtration was to separate the Kencur residue from the Kencur filtrate. Subsequently, a second maceration was performed using 125 mL of solvent, left to stand for 24 hours, and then filtered again. The filtered extract can be evaporated using a dish with the help of a water bath until a thick extract of Kencur is obtained. The extracted data can be seen in Table 2.

Extraction Yield Result

Table 2. Extraction Yield result

Initial Weight	Final Weight	Weight Percentage
25 gram	0,911 gram	0,03%

The yield produced by this method tends to be low because several factors can affect the effectiveness of extraction. According to research conducted by Meloan (1999), extraction is influenced by the extent of surface area interaction with the solvent, the duration of sample exposure to the solvent, the volume of solvent used, and the distribution coefficient. (Yulianti dkk.,2021)

Identification of Compound Content

The extract obtained from maceration was subsequently subjected to qualitative identification of metabolite compounds, including tests for polyphenols, tannins, saponins, glycosides, and terpenoids. The results of the identification of secondary metabolite compounds in the ginger extract can be seen in Table 3. Result of Compound Content Identification.

Table 3. Result of Compound Content Identification

Compound	Polyphenol (sample + 3 drops of FeCl ₃)	Tannin (sample + NaCl + Gelatin)	Saponin (sample + HCl)	Glycoside (sample + Methanol + Acetic acid)	Terpenoid (sample + Chloroform + Sulfuric acid)
Result					
Descriptions	Negative, there is no change to green-blue color.	Positive, a precipitate is formed.	Negative, no bubbles are formed	Positive, a chemical reaction occurs with a color change to a	Positive, there is a color change to red-green or violet-blue.

From the data, it is known that positive results were shown in the tests for tannins, glycosides, and terpenoids. Meanwhile, negative results were indicated in the tests for polyphenols and saponins. The results of this identification can be influenced by the properties of the chemical compounds and the solvents used.

A positive result for tannins is indicated by the formation of a precipitate, which occurs because tannins precipitate proteins in gelatin. Tannins react with gelatin to form a copolymer that is insoluble in water. This reaction becomes more sensitive when NaCl is added to enhance the salting out of tannin-gelatin, making the resulting precipitate more stable. The saponin test did not show positive results as no foam was formed. This is supported by research conducted by Rivai (2019), which indicated that the curcumin compound in the ethanol fraction yielded negative results (Harrizul Rivai dkk., 2019). The results of the glycoside test also showed positive results, indicated by a chemical reaction where the color changed from bright to dark. The terpenoid test showed positive results as well, marked by the presence of a purple color. Test the terpenoid using the Liebermann–Bouchard method, which involves identifying terpenoids with

chloroform, acetic anhydride, and sulfuric acid reagents.

The purple color is caused by changes in conjugation within the molecules, resulting in the formation of complex compounds that produce the purple hue (Novia Fransiska dkk., 2021).

Identification of Compounds using Thin Layer Chromatography

The extract sample of Kencur was spotted on a TLC plate and compared with the spots of the standard solution. The stationary phase used is silica gel GF254, while the mobile phase consists of a mixture of two to three solvents in a specific ratio. The solvent mixture used as the mobile phase is employed to optimize the process in chromatography and enhance the selectivity of the analyte results. The use of non-polar chloroform in each moving phase mixture is employed to isolate curcumin. Meanwhile, silica gel, as the stationary phase, is polar, to facilitate the separation of curcumin through this stationary phase. After performing TLC, the spots that are formed are visualized with ultraviolet light at 254 nm, so the spots will appear on the plate where the compounds absorb the light that hits specific areas (Namir dkk., 2019). The results of the qualitative analysis of curcumin compounds using thin layer

chromatography can be seen in Image 1. Result off Compound Identification Using TLC.

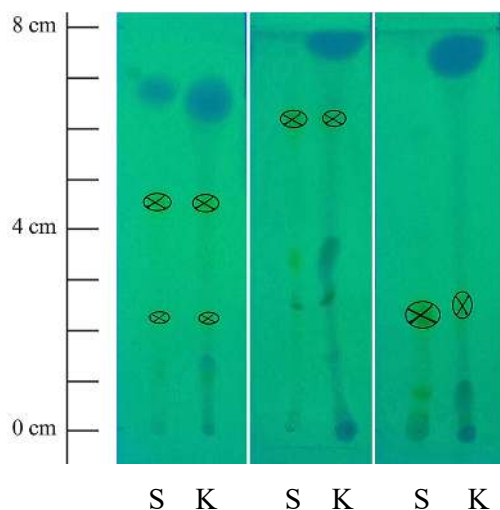


Image 1. Result of Compound Identification Using TLC

Description:

S : Standards of Curcumin

K : Sample extract of Kencur rhizome

Mobile phase

I. Chloroform:Methanol (95:5)

II. Chloroform:Methanol:Glacial Acetic Acid (94:5:1)

III. Chloroform:n-Hexane:Methanol (1:1:0,1)

The stain spots obtained in this experiment show the presence of yellow spots that are aligned between the standard and the sample at points 2.3 and 4.5 during the first movement phase. In the second movement phase, there is also an aligned yellow spot at point 6.2. However, in the third movement phase, there are yellow spots at different points, specifically at the standard at point 2.3 and the sample at point

2.5. In both results of the TLC, it shows a positive outcome that Kencur contains curcumin compounds because the parallel spots have the same color, suggesting the presence of curcumin. However, one of the results shows a negative outcome.

The R_f value at that sample point still meets the requirements where, for compound identification, the R_f value of the sample is the same as or close to the R_f value of the standard. This is likely because the plate with negative results has a lower concentration of curcumin compared to the other two plates, making the curcumin spots less visible or not reaching the detection limit of TLC. In addition, curcumin is poorly soluble in n-hexane, which is a non-polar hydrocarbon solvent, resulting in a lack of interaction between curcumin molecules and the solvent (Jayuska & Rahmalia, 2019). According to the research by Muhafidzah (2018), the fraction of Kencur rhizome has weak antioxidant activity, so the compound is not attracted to low polarity levels such as n-Hexane (Muhafidzah dkk., 2018). Another factor may also be due to using a type of solvent with different polarity levels. The results of the r_f value calculations for the spots formed in the TLC can be seen in Table 4. The R_f values for the spots formed during the movement phases of Variations II and III.

Table 4. The R_f values for the spots formed during the movement phases of Variations II and III.

	II	III
Standar	Rf ₁ 0,287	Rf ₁ 0,1
	Rf ₂ 0,412	Rf ₂ 0,287
	Rf₃ 0,775	
Sample	Rf ₁ 0,175	Rf ₁ 0,125
	Rf ₂ 0,437	Rf ₂ 0,312
	Rf₃ 0,775	Rf ₃ 0,987
	Rf ₄ 1	
	Rf ₅ 0,312	

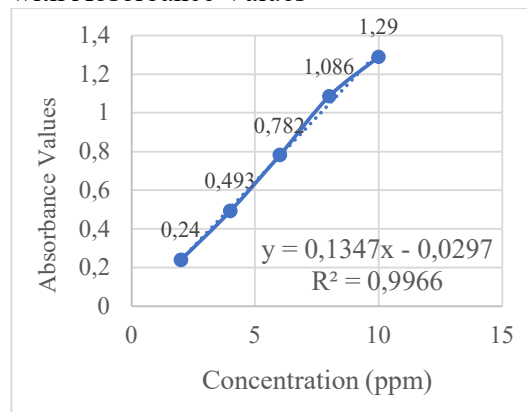
After the plate was developed with the mobile phase and observed under ultraviolet light at 254 nm, the spots that appeared could be measured for their R_f values. The R_f values for the spots with the eluent of chloroform: methanol: glacial acetic acid were 0.287; 0.412; 0.775 for the standards and 0.175; 0.437; 0.775; 1; 0.312 for the sample of Kencur. Meanwhile, with the eluent of chloroform: n-hexane: methanol, the R_f values for the standards were 0.1; 0.287 and for the sample were 0.125; 0.312; 0.987.

Quantitative Analysis of Curcumin Levels in Samples

The maximum absorbance wavelength of the standard curcumin solution using UV-Vis spectrophotometry with ethanol as the solvent was obtained at a maximum wavelength (λ_{max}) of 429.5 nm. This wavelength is used to establish the standard curve for curcumin, which can then be utilized to determine the total concentration of curcumin compounds in the sample extract. The curve formed based

on absorbance values and concentration can be seen in Image 2. Curve of Curcumin Compound with Absorbance Values

Image 2. Curve of Curcumin Compound with Absorbance Values



Description:

II Chloroform : Methanol : Glacial Acetic Acid (94:5:1)

III Chloroform : n-Hexane : Methanol (1:1:0,1)

The determination of the standard calibration curve for curcumin at a concentration of 1000 ppm was then followed by dilution to five different concentrations of 10, 8, 6, 4, and 2 ppm. The obtained absorbance values are 1.290; 1.086; 0.782; 0.493; 0.240. In determining the standard calibration curve, the resulting linear regression equation is $y = 0.1347x - 0.0297$ with an R value of 0.9982. Nilai linearitas yang baik adalah $0,99 \leq R \leq 1$ (Chandra & Rivai, 2016). The R value obtained in this experiment falls within that range, so it can be said to have good linearity.

The Kencur sample was prepared with two replicates at a concentration of

1000 ppm and its absorbance was measured at the maximum wavelength of 429.5 nm. The absorbance values obtained were 0.589 for replicate I and 0.562 for replicate II. Both absorbance values fall within the range of 0.2 – 0.8 or within the standard curve range that was created, which is 0.24 – 1.29. The absorbance values of the sample were calculated against the standard curve, resulting in a concentration of 4.593 for replicate I and 4.392 for replicate II. These two values represent the concentration of curcumin contained in the sample. The amount of curcumin contained in the Kencur sample can be seen in Table 5.

Tabel 5. The level of Curcumin in the Sample

Sample Rate	Standard Deviation
4,593	0,142128
4,392	

Based on the results of the quantitative analysis of the ginger extract sample, the curcumin content found in the ginger rhizome extract samples was 0.4593% and 0.4392%, with an average concentration of 0.4492%. Both results indicate that the concentration of curcumin compounds contained in the ginger extract sample is relatively low compared to several Plants such as Java ginger has 27.19% (Rosidi dkk, 2014).

CONCLUSION

Based on the data obtained from this experiment, it can be concluded that the

qualitative analysis of the secondary metabolite content in the extract of Kencur rhizome includes compounds such as tannins, glycosides, terpenoids, and curcumin. Meanwhile, the quantitative analysis of the curcumin content in the Kencur rhizome extract is 0.4593% and 0.4393%.

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