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Phytochemical Analysis and Flavonoid Content of Lempuyang Wangi (*Zingiber aromaticum* Val.) Rhizomes using Various Solvent Extractions

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Abstract: Lempuyang Wangi (*Zingiber Aromaticum Val.*) is a medicinal plant widely used in traditional medicine in Southeast Asia. This study aimed to extract and analyze the secondary metabolites from the rhizomes of Lempuyang Wangi using the maceration method with three different solvents: n-hexane, ethyl acetate, and 96% ethanol. The extraction yielded varying percentages depending on the solvent used, with the ethyl acetate extract having the highest yield at 16.26%. Phytochemical screening identified the presence of alkaloids, phenolics, and flavonoids in all extracts, with the ethanol and ethyl acetate extracts showing a broader range of compounds. The highest total flavonoid content was found in the ethanol extract, followed by ethyl acetate and n-hexane, indicating ethanol's superior ability to extract flavonoids. These findings demonstrate the influence of solvent polarity on extraction efficiency and compound diversity, highlighting ethanol as the most effective solvent for extracting flavonoids from Lempuyang Wangi. The measurement of total flavonoid content showed that the ethanol extract had the highest concentration, at 24.273 mg QE/g. These results provide a foundation for further research and potential applications in utilizing Lempuyang Wangi extracts for health benefits.

Keywords: extraction, maceration, phytochemical, secondary metabolites, *Zingiber aromaticum*

Abstrak: *Lempuyang Wangi (Zingiber Aromaticum Val.) merupakan tanaman obat yang banyak digunakan dalam pengobatan tradisional di Asia Tenggara. Penelitian ini bertujuan untuk mengekstrak dan menganalisis metabolit sekunder rimpang Lempuyang Wangi menggunakan metode maserasi dengan tiga pelarut berbeda: n-heksana, etil asetat, dan etanol 96%. Ekstraksi menghasilkan persentase yang bervariasi tergantung pada pelarut yang digunakan, dengan ekstrak etil asetat memiliki rendemen tertinggi yaitu 16,26%. Skrining fitokimia mengidentifikasi keberadaan alkaloid, fenolik, dan flavonoid di semua ekstrak, sedangkan ekstrak etanol dan etil asetat menunjukkan senyawa yang lebih beragam. Kandungan total flavonoid tertinggi terdapat pada ekstrak etanol, disusul etil asetat dan n-heksana, yang menunjukkan kemampuan etanol yang unggul dalam mengekstraksi flavonoid. Temuan ini menunjukkan pengaruh polaritas pelarut terhadap efisiensi ekstraksi dan keanekaragaman senyawa, sehingga etanol menjadi pelarut paling efektif untuk mengekstraksi flavonoid dari Lempuyang Wangi. Pengukuran kandungan flavonoid total menunjukkan bahwa ekstrak etanol mempunyai konsentrasi tertinggi yaitu sebesar 24,273 mg QE/g. Hasil ini memberikan landasan untuk penelitian lebih lanjut dan potensi penerapan pemanfaatan ekstrak Lempuyang Wangi untuk manfaat kesehatan.*

Kata kunci: *ekstraksi, fitokimia, maserasi, metabolit sekunder, Zingiber aromaticum* Val.

INTRODUCTION

Lempuyang Wangi, scientifically known as *Zingiber Aromaticum Val*., is a species within the *Zingiberaceae* family commonly found in tropical regions such as Southeast Asia. This plant can be found in lowland areas or on hillside slopes (Koga *et al*. 2016). Lempuyang Wangi is well-known and proven as a herbal plant that can be used in traditional medicine. The most utilized part of this plant is its rhizome. In Indonesia, Lempuyang Wangi rhizomes are typically used as herbal remedies to treat various ailments such as inflammation, fever, diarrhea, bacterial infections, stomach cramps, poisoning, body allergies, digestive disorders, sprains, as an antirheumatic agent, and as a diuretic (Silva *et al*. 2018). The plant has a distinctive aromatic scent, which is due to the complex mixture of monoterpenoids and sesquiterpenoids found in the essential oils of the rhizome (Okamoto *et al.* 2011). The Lempuyang Wangi plant has thick, knobby

rhizomes that grow beneath the soil surface. The plant also has thin green leaves that sprout from the rhizomes, with lengths ranging from 25 to 35 cm. The flowers of this plant are green when young and change to white or red as they mature (Jalil *et al.* 2015). The rhizomes of Lempuyang Wangi are commonly used in traditional medicine to treat various ailments, such as inflammation, fever, diarrhea, bacterial infections, stomach cramps, poisoning, body allergies, digestive disorders, sprains, anti-rheumatic agents, and as a diuretic (Silva *et al*. 2018). The plant contains secondary metabolites, such as polyphenols, terpenes, and alkaloids (Wahyuni *et al*. 2013).

In addition to its use in traditional medicine, Lempuyang Wangi has also attracted scientific interest due to its secondary metabolite content, which has the potential to provide health benefits. Secondary metabolites such as flavonoids, alkaloids, and terpenoids are known to possess various biological activities, including antioxidant, anti-inflammatory, antimicrobial, and anticancer properties (Mukherjee *et al*. 2024). With the growing interest in natural medicines and phytopharmaceuticals, in-depth research on the chemical composition and biological activities of Lempuyang Wangi has become highly relevant. The choice of extraction method and solvent type significantly influences the efficiency of isolating bioactive compounds from this plant, which in turn impacts its potential applications in health products and pharmaceuticals. Therefore, this study not only focuses on the identification of secondary metabolites but also explores how solvent variation can affect the yield and composition of the extracts, providing broader insights into the phytochemical characteristics of Lempuyang Wangi.

Secondary metabolites are compounds produced by organisms such as plants, fungi, or bacteria. These compounds are generated through the organism's secondary metabolic processes. Secondary metabolites are not essential for the growth and development of plants (Putri *et al*. 2023). However, the production of secondary metabolites by plants serves as a defense mechanism against environmental conditions, such as temperature fluctuations, climate changes, pest disturbances, and plant diseases (Dalimunthe & Rachmawan 2017). These compounds also play a role in attracting insects to aid in the pollination of plants (Julianto 2019). Secondary metabolites in plants can also be beneficial for humans, being used in medicines, cosmetics, food colorants, paint pigments, and more (Teoh 2016). Based on their structure, secondary metabolites are classified into three main groups: alkaloids, terpenoids, and phenolics (Vora & Pednekar 2017).

Phytochemical screening is a qualitative analysis conducted to identify the content of secondary metabolites in a plant extract, which may have medicinal properties. Various studies have been carried out using both qualitative and quantitative methods to analyze plant extracts for the detection of secondary metabolites such as alkaloids, terpenoids, and phenolics (Yuniar *et al*. 2023). In the quantitative method, phytochemical screening is performed by analyzing plant extracts using UV-Vis spectrophotometry, thin-layer chromatography (TLC), high-performance liquid chromatography (HPLC), gas chromatography (GC), and mass spectrometry (MS). In the qualitative method, phytochemical screening is conducted by detecting changes in color or form in a solution using various reagents (Roghini & Vijayalakshmi 2018).

MATERIALS AND METHOD

Extraction

The extraction of Lempuyang Wangi was carried out using the maceration extraction method. The extraction was conducted using a range of solvents from non-polar to polar, specifically n-hexane, ethyl acetate, and 96% ethanol. Rhizome powder of Lempuyang Wangi, weighing 50 grams for each solvent, was used. The powder was then macerated using the respective solvents. The maceration process involved replacing the solvent every 24 hours for 3 days. The resulting extracts were concentrated using a rotary evaporator at 40°C. The concentrated extracts were then placed into pre-weighed vials. The vials were loosely capped to allow any remaining solvent to evaporate naturally. Once all the solvent had evaporated, the vials were weighed again to determine the percentage yield of the extracts.

Phytochemical Screening

Phytochemical screening was carried out using various reagents as group detectors. Each extract obtained from the maceration process was taken in the amount of 5 mg and dissolved in 5 mL of 96% ethanol. Phytochemical screening was conducted to test for the presence of several compounds as follows:

Alkaloid Test

1 mL of the sample extract was pipetted into a test tube, and then two drops of Dragendroff reagent were added. The mixture was homogenized and left to stand for 30 minutes. A color change to orange indicates a positive test result.

Phenolic Test

1 mL of the sample extract was pipetted into a test tube, followed by the addition of 1 mL of Folin-Ciocalteu reagent. The mixture was homogenized and left to stand for 30 minutes. A color change to green or bluish green indicates a positive test result.

Flavonoid Test

1 mL of the sample extract was pipetted into a test tube, then 2 mg of Mg powder and three drops of concentrated HCl were added. The mixture was

homogenized, and the color change was observed. A color change to red, yellow, or orange indicates a positive test result.

Steroid or Terpenoid Test
1 mL of the sample extract was pipetted into a test tube, followed by the addition of two drops of $ChCl_3$ and three drops of Liebermann-Burchard reagent. The mixture was homogenized, and the color change was observed. A color change from reddish blue to greenish blue indicates the presence of steroids, while a color change from red to brown indicates the presence of terpenoids.

Saponin Test
1 mL of the sample extract was pipetted into a test tube, followed by the addition of hot distilled water. The mixture was homogenized for 10 seconds. The test result is considered positive if the foam is formed within 30 minutes and persists after the addition of one drop of 2N HCl.

Tannin Test
1 mL of the sample extract was pipetted into a test tube, followed by the addition of two drops of 3% $FeCl_3$ solution. The mixture was homogenized, and the color change was observed. A color change to dark blue or greenish black indicates a positive test result.

Total Flavonoid Content
The first step in flavonoid content testing involves preparing a quercetin solution. A total of 100 mg of quercetin is weighed and dissolved in 96% ethanol to reach a final volume of 100 mL. Then, 1 mL of the quercetin solution is pipetted into a test tube and 1 mL of 2% $AlCl_3$ and 8 mL of 5% CH_3COOH are added. The mixture is then homogenized and allowed to stand for 30 minutes at room temperature. The maximum wavelength is measured in the range of 400–450 nm. A blank solution, consisting of 1 mL of 96% ethanol, 1 mL of 2% $AlCl_3$, and 8 mL of 5% CH_3COOH, is used during absorbance measurements.

Next, a quercetin standard solution is prepared with concentrations of 20, 40, 60, 80, and 100 ppm. Each solution is pipetted 1 mL and placed into separate test tubes. Then, 1 mL of 2% $AlCl_3$ and 8 mL of 5% CH_3COOH are added to each test tube. Each solution is homogenized and allowed to stand for 30 minutes at room temperature. The absorbance is measured at the previously determined maximum wavelength, using the same blank as before. The absorbance values obtained are used to create a standard curve for sample measurement.

The next step is measuring the flavonoid content in the sample extracts. In this procedure, extracts from ethanol, ethyl acetate, and n-hexane solvents are tested. Each extract is weighed at 1.2 mg and dissolved in 20 mL of 96% ethanol. Then, 1 mL of the sample solution is pipetted into a test tube, and 1 mL of 2% $AlCl_3$ and 8 mL of 5% CH_3COOH are added. The mixture is then homogenized and allowed to stand for 30 minutes at room temperature. The absorbance is measured at the predetermined wavelength using the same blank as before. The absorbance values obtained are then processed to calculate the total flavonoid content.

RESULTS AND DISCUSSION

Extraction

The extraction of rhizomes from the Lempuyang Wangi plant in this study used the maceration method. This method was chosen because it is relatively easy to perform and does not require high costs. Additionally, the maceration method was selected to preserve secondary metabolites, such as flavonoids, which are prone to degradation at high temperatures. In this study, the maceration extraction was carried out by soaking the samples using three different solvents with non-polar, semi-polar, and polar properties. The solvents used were n-hexane, ethyl acetate, and ethanol. This variation of solvents aims to observe the distribution of secondary metabolites contained in the Lempuyang Wangi plant. The maceration was conducted with solvent replacement every 24 hours for 3 days to maximize the extraction of secondary metabolites from the plant. Furthermore, the extraction process considered the saturation level of the solvent in extracting these compounds. The yield percentage obtained from the extraction process is presented in Table 1.

Based on the obtained percent yield values, maceration extraction of the Lempuyang Wangi sample with semi-polar and polar solvents yields a higher percentage compared to non-polar solvents. This indicates that the Lempuyang Wangi extract contains more semi-polar to polar compounds than non-polar compounds. In maceration extraction with n-hexane, van der Waals interactions occur between n-hexane and non-polar compounds. This happens because n-hexane, which only has a methyl group interactions, and hydrogen bonding. Ethyl acetate has a polar ester group (COO) as well as a methyl group (CH_3), allowing it to dissolve both non-polar (CH_3), can dissolve non-polar compounds such as steroids,

Table 1. Results of maceration extraction of lempuyang wangi rhizomes with solvent variation

Extract	Simplisia Mass (g)	Extract Mass (g)	Yield (%)
with n-Hexane	50.108	4.258	8.50
with Ethyl Acetate	50.172	8.158	16.26
with 96% Ethanol	50.024	7.146	14.29

terpenoids, essential oils, sterols, lipids, and fats. In contrast, extraction with ethyl acetate involves van der Waals interactions, dipole-dipole and polar compounds. Extraction with ethanol involves hydrogen bonding between ethanol and polar compounds. The structure of ethanol, which has a hydroxyl group (-OH), facilitates hydrogen bonding with polar compounds such as phenols, flavonoids, tannins, and alkaloids.

Phytochemical Screening

Phytochemical screening of the Lempuyang Wangi extracts was conducted through various tests using different reagents to detect secondary metabolite groups. This testing aims to identify the types of secondary metabolites present in the Lempuyang Wangi extracts. The results of the phytochemical screening for each rhizome extract of Lempuyang Wangi are presented in Table 2.

The phytochemical screening results table shows the presence of various bioactive compounds in plant extracts obtained using three different solvents: *n*-hexane, ethyl acetate, and 96% ethanol. In the n-hexane extract, alkaloids, phenolics, and flavonoids were found. The ethyl acetate extract showed the presence of alkaloids, phenolics, flavonoids, and tannins. The 96% ethanol extract showed results like the ethyl acetate extract, with alkaloids, phenolics, flavonoids, tannins, and saponins detected. These results indicate that the type of solvent used affects the types of bioactive compounds that can be extracted from the plant, with 96% ethanol and ethyl acetate solvents showing a broader extraction capability compared to *n*-hexane.

Total Flavonoids Content

The total flavonoid content test is conducted to determine the total flavonoid compounds in each extract obtained. Total flavonoid content measurement is often used in phytochemical research to evaluate the biological potential of plants, particularly in the context of health (Chandra *et al*. 2014). One common method for measuring total flavonoid content is the colorimetric method using aluminum chloride ($AlCl_3$) reagent in an acidic environment. This method is based on the formation of a complex between flavonoids and aluminum chloride, which produces a yellow color that can be measured for absorbance using a spectrophotometer at wavelengths in the range of 400-450 nm. Total flavonoid content measurement typically uses compounds such as quercetin, rutin, catechin, and kaempferol as measurement standards (Nasution *et al*. 2022). In the reaction, Al^{3+} ions from $AlCl_3$ react with hydroxyl groups on flavonoid compounds in an acidic environment. This reaction results in the formation of a stable complex compound. The formation of this complex with Al^{3+} ions cause a change in the absorption spectrum, which indicates the presence of flavonoid compounds (Pękal & Pyrzynska 2014). The reaction that occurs in the measurement of total flavonoid content is shown in Figure 1.

The total flavonoid content measurement is calculated using Equation 1.

$$\text{Total flavonoid content} = \frac{V(mL) \times C(mg/mL)}{Extract\ Mass\ (g)} \times Fp \quad ...(1)$$

Where V is the volume of the solvent, C is the concentration of flavonoids in the sample, and Fp is the dilution factor. The concentration of flavonoids can be calculated using Equation 2.

$$C = \frac{absorbance(sample) - \text{intercept}}{slope} \quad ...(2)$$

Table 2. Phytochemical screening results

Extract	Compound Testing						
	Alkaloids	**Phenolics**	**Flavonoids**	**Saponins**	**Tannins**	**Steroids**	**Terpenoids**
with *n*-Hexane	+	+	+	-	-	-	-
with Ethyl Acetate	+	+	+	-	+	-	+
with 96% Ethanol	+	+	+	-	+	-	+

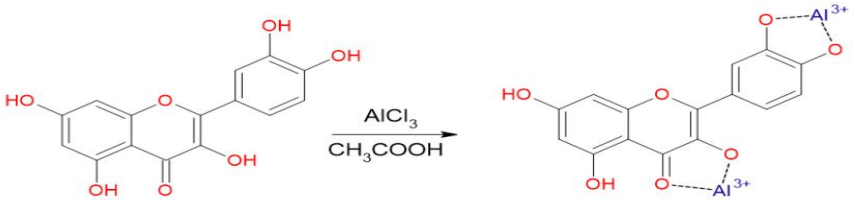

Figure 1. Reaction in the measurement of total flavonoid content

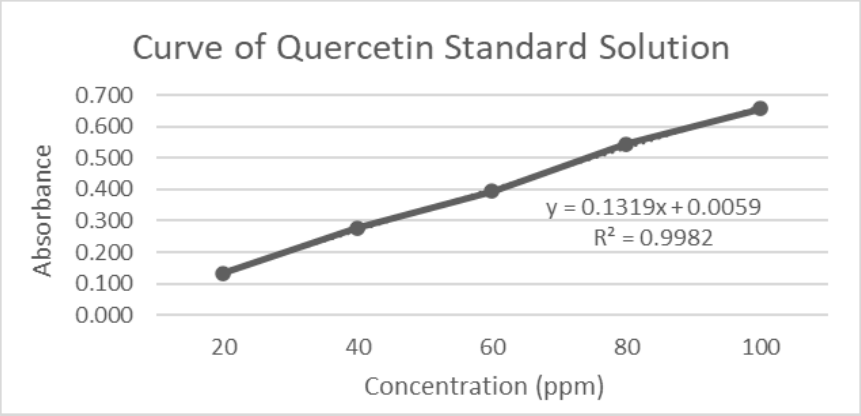

Figure 2. Curve of quercetin standard solution

Table 3. Data on total flavonoid content (TFC) test results

Extract	Total Flavonoids Content (mgQE/g)
with *n*-Hexane	7.890
with Ethyl Acetate	10.063
with 96% Ethanol	24.273

In this test, quercetin is used as the standard solution. The selection of quercetin is due to its classification as a flavonoid compound with hydroxyl groups adjacent to flavone and flavonol. The total flavonoid content measurement is performed by reacting the sample with aluminum chloride ($AlCl_3$) and acetic acid (CH_3COOH) solutions. The $AlCl_3$ solution is used as a complexing agent that reacts with the hydroxyl groups (-OH) on the flavonoid compounds. The CH_3COOH solution is used as a solvent and provides an acidic environment during the reaction process. In this test, quercetin is dissolved in 96% ethanol. Referring to the use of quercetin as the standard solution, the total flavonoid content measurement in this study is expressed in mg QE/g, where mg QE stands for milligram Quercetin Equivalents. This indicates that the resulting value is the amount of flavonoid equivalent to milligrams of quercetin per gram of sample. Absorbance measurement is carried out using a UV-Vis spectrophotometer in the range of 400-450 nm. In the measurements conducted, a maximum wavelength of 413.5 nm was obtained. The calibration curve for the quercetin standard solution obtained is shown in Figure 2.

The total flavonoid content values in the Lempuyang Wangi rhizome extract are summarized in Table 3. Based on the data in Table 3, it was found that the highest total flavonoid content is present in the extract with 96% ethanol as the solvent, while the lowest flavonoid content is found in the extract with n-hexane as the solvent. This is consistent with the previous explanation that extraction with ethanol will bind more flavonoid compounds compared to ethyl acetate or n-hexane. The total flavonoid content obtained shows higher results compared to the study conducted by Sunarto *et al*. 2020. In that study, the total flavonoid content in the Lempuyang Wangi rhizome extract using ethanol as the solvent was 9.924 mg QE/g (Sunarto *et al*. 2020).

CONCLUSION

This study successfully extracted secondary metabolites from the rhizomes of the Lempuyang Wangi plant using the maceration method with three different solvents: n-hexane, ethyl acetate, and 96% ethanol. The yield percentages varied among the solvents, with the highest yield obtained using ethyl acetate and 96% ethanol. The variation in solvent polarity influenced the types and amounts of compounds extracted, as reflected in the yield percentages and phytochemical screening results. Phytochemical screening revealed the presence of various bioactive compounds such as alkaloids, phenolics, and flavonoids in all extracts, with ethyl acetate and ethanol extracts showing a broader range of compounds compared to n-hexane. The total flavonoid content was highest in the ethanol extract, followed by the ethyl acetate and n-hexane extracts, demonstrating ethanol's superior ability to extract flavonoid compounds. Overall, the findings indicate that the choice of solvent significantly affects the efficiency of extraction, and the types of bioactive compounds obtained. The use of ethanol as a solvent resulted in the highest total flavonoid content, making it the most effective solvent for extracting flavonoids from Lempuyang Wangi rhizomes. These results contribute valuable insights for further research and applications in utilizing Lempuyang Wangi plant extracts for their potential health benefits.

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