

EFFECT OF EXTRACTION, RATIO, AND SOLVENT CONCENTRATION ON TOTAL FLAVONOID CONTENT AND ANTIOXIDANT ACTIVITY OF SINGKEL (*PREMNA SERRATIFOLIA* LINN.) USING DPPH METHOD

**PENGARUH EKSTRAKSI, RASIO DAN KONSENTRASI PELARUT TERHADAP
KANDUNGAN TOTAL FLAVONOID DAN AKTIVITAS ANTIOKSIDAN SINGKEL
(*PREMNA SERRATIFOLIA* LINN.) DENGAN METODE DPPH**

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ABSTRAK

Singkel (Premna serratifolia Linn.) adalah tanaman obat yang banyak ditemukan di daerah tropis, termasuk Indonesia, yang mengandung flavonoid, alkaloid, polifenol, dan triterpenoid. Flavonoid pada daun singkel bertindak sebagai antioksidan yang dapat mencegah stress oksidatif penyebab penyakit degeneratif dan kronis seperti kanker, arthritis, dan kardiovaskular. Penelitian ini bertujuan untuk mengetahui pengaruh kadar flavonoid total dan nilai IC_{50} pada daun singkel berdasarkan metode ekstraksi, konsentrasi pelarut, dan rasio ekstrak dengan pelarut. Metode yang digunakan meliputi maserasi, sokletasi, dan MAE (Microwave-Assisted Extraction) dengan konsentrasi pelarut etanol 60%, 70%, 80%, dan 96%, serta rasio 1:10, 1:20, dan 1:30. Aktivitas antioksidan diukur menggunakan metode DPPH (2,2-diphenyl-1-picrylhydrazyl) dengan spektrofotometri UV-Vis. Hasil penelitian menunjukkan bahwa metode sokletasi menghasilkan kadar flavonoid total tertinggi yaitu $24,56 \pm 1,81$ mg QE/g dengan nilai IC_{50} $20,31 \pm 1,58$ μ g/mL. Konsentrasi pelarut 96% memberikan hasil terbaik dengan kadar flavonoid total $26,12 \pm 0,06$ mg QE/g dan nilai IC_{50} $15,51 \pm 2,17$ μ g/mL. Pada pengujian rasio pelarut, rasio 1:20 menunjukkan kadar flavonoid total tertinggi sebesar $19,37 \pm 0,5$ mg QE/g, sementara rasio 1:10 memberikan nilai IC_{50} terbaik yaitu $11,99 \pm 1,02$ μ g/mL. Kesimpulannya, metode ekstraksi, konsentrasi pelarut, dan rasio pelarut mempengaruhi secara signifikan kadar flavonoid dan aktivitas antioksidan daun singkel.

Kata kunci: *Premna serratifolia* L.; flavonoid total; DPPH; IC_{50} .

ABSTRACT

Singkel (Premna serratifolia Linn.) is a medicinal plant commonly found in tropical regions, including Indonesia, which contains flavonoids, alkaloids, polyphenols, and triterpenoids. Flavonoids in singkel leaves act as antioxidants that can prevent oxidative stress, which is a causative factor of degenerative and chronic diseases such as cancer, arthritis, and cardiovascular diseases. This study aims to determine the effects of total

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flavonoid content and IC_{50} values in singkel leaves based on extraction methods, solvent concentration, and the ratio of extract to solvent. The methods used include maceration, soxhlet extraction, and Microwave-Assisted Extraction (MAE), with ethanol solvent concentrations of 60%, 70%, 80%, and 96%, and extract-to-solvent ratios of 1:10, 1:20, and 1:30. Antioxidant activity was measured using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method with UV-Vis spectrophotometry. The results showed that the soxhlet extraction method yielded the highest total flavonoid content of 24.56 ± 1.81 mgQE/g with an IC_{50} value of 20.31 ± 1.58 μ g/mL. The 96% solvent concentration provided the best results with a total flavonoid content of 26.12 ± 0.06 mgQE/g and an IC_{50} value of 15.51 ± 2.17 μ g/mL. In the solvent ratio testing, the 1:20 ratio showed the highest total flavonoid content of 19.37 ± 0.5 mgQE/g, while the 1:10 ratio provided the best IC_{50} value of 11.99 ± 1.02 μ g/mL. In conclusion, the extraction method, solvent concentration, and solvent ratio significantly affect the flavonoid content and antioxidant activity of singkel leaves.

Keywords: *Premna serratifolia* L.; total flavonoids; DPPH; IC_{50} .

INTRODUCTION

Secondary metabolites are specific to plant species or their botanical family. Flavonoids are secondary metabolites in various plant parts, including roots, leaves, flowers, fruits, and seeds. An extraction process is required to obtain these flavonoid compounds. Traditionally, extracting flavonoids from plant materials relies on methods that use large quantities of solvent, which involve lengthy separation periods and result in low recovery rates. The process of isolating flavonoid compounds can be improved by choosing appropriate solvents. The role of solvents is crucial in the extraction of chemical compounds, as their solubility characteristics significantly impact the efficiency of targeting specific compounds from raw materials.

Numerous studies have explored the extraction of flavonoid compounds using ethanol with varying concentrations and sample-to-solvent ratios. Optimal results with ethanol solvents depend on appropriate concentration levels, ratio factors, and extraction methods. Since different plants have unique properties, these factors cannot be

universally applied to all extraction processes. Therefore, this research was carried out to optimize the extraction conditions, including solvent ratio and concentration, for the total flavonoid content and antioxidant activity of singkel using the DPPH method.

DPPH (2,2-diphenyl-1-picrylhydrazyl) is a free radical widely used to test antioxidant activity in plant extracts. The DPPH molecule is a synthetic radical that dissolves in methanol and ethanol and remains stable with a wavelength range of 515-520 nm. The DPPH method offers some advantages. It only requires a small sample and is fast, simple, and accurate in determining antioxidant activity derived from natural materials.

Singkel is an herbal plant with potential as an exogenous antioxidant and is rich in flavonoids. According to Riduana *et al.*, (2021), phytochemical screening of singkel indicated the presence of flavonoids, phenols, tannins, saponins, and terpenoids. Additionally, Isnindar and Luliana (2020) found that the IC_{50} value of singkel was 22.1 ppm, with a total flavonoid content of 54.17% w/w EQ. Singkel exhibits anti-rheumatic, cardiogenic, antioxidant, and antidiabetic properties (Majumder, 2014).

Ethanol extract of singkel leaves contains polyphenols, alkaloids, flavonoids, and triterpenoids. *Premna latifolia* contained flavonoids, triterpenoids, and alkaloids in several preliminary studies, furthermore, the leaves of *Premna corymbosa* Rottl. Contains alkaloids, glycosides, flavonoids, steroids, and triterpenoids. Plants from the Verbenaceae family are known for their therapeutic properties, including treatments for cardiovascular issues, skin diseases, inflammatory conditions, arthritis, gonorrhea, rheumatism, anorexia, and jaundice (Firmansyah *et al.*, 2023).

This study aims to optimize the extraction of singkel leaves by evaluating the effect of extraction method, solvent concentration, and sample-to-solvent ratio on total flavonoid content and antioxidant activity of singkel (*Premna serratifolia* L.) leaves.

Method

The tools used in this study are UV-Vis spectrophotometry (*Shimadzu*[®]), a microwave-assisted extraction (MAE) unit (*Electrolux*[®]), a rotary evaporator (*Buchi*[®]), a food dehydrator (*Kriss*), soxhlet tools (*Pyrex*), a micropipette (*ThermoFisher*), glassware, an analytical balance (*Ohaus*), a stopwatch, a capillary tube, a Büchner funnel, and a TLC spray bottle (*Camag*).

The materials used in this study were singkel (*Premna serratifolia*) leaves, ethanol solvent (technical), ethanol p.a. (*SmartLab*), methanol p.a. (*SmartLab*), aluminum chloride (AlCl_3) (*Brand*), sodium acetate (CH_3COONa) 1M (*Brand*), quercetin (*Sigma Aldrich*), and distilled water.

Determination and Processing of Simplicia

Samples of fresh and healthy singkel leaves were obtained from Srikandi area, Sungai Raya Dalam, Pontianak City, West Kalimantan. Singkel leaves were determined at the Biology Laboratory of the FMIPA UNTAN using the common methods for determination and morphological examination. Collected singkel leaves were washed with running water to remove dirt. The leaves were then chopped to speed up the drying process. Chopping also influences the total flavonoid content, as smaller leaf pieces increase the surface area, leading to more efficient extraction. After chopping, the leaves were dried in an oven at 50°C for 3 days. This temperature was chosen to preserve the secondary metabolites in the leaves, remove moisture, and ensure the samples remain stable during long-term storage (Isnindar et al., 2016; Susilowati & Sari, 2020).

Extraction Method

Comparison of extraction methods used soxhletation, maceration, and MAE methods. The extraction process involved the use of 100 grams of singkel leaf powder. The MAE method used 800 mL of ethanol solvent, with a power setting of 400W, for 45 minutes. The soxhletation method, on the other hand, used

900 mL of ethanol solvent, at a temperature of 50°C. Meanwhile, the maceration required the use of 1000 mL of ethanol solvent, at room temperature.

Determination of Total Flavonoid Content

Preparation of Quercetin Stock Solution

To produce a 1000 ppm stock solution, 10 mg of quercetin was dissolved in 100 mL of anhydrous ethanol, ensuring the solution reached the calibration mark. This stock solution was then serially diluted to obtain final concentrations of 3, 5, 7, 9, and 11 ppm (Mag et al., 2015; Shirazi et al., 2014).

Determination of Maximum Wavelength

A specific concentration of the standard solution was transferred to a 5 mL measuring flask. This flask added 0.2 mL of 10% aluminum chloride (AlCl_3) and 0.2 mL of 1 molar sodium acetate. The solution was then diluted to the desired volume with distilled water, mixed thoroughly, and incubated for 15 minutes. Finally, the solution's absorbance was measured across a range of wavelengths from 200 to 500 nanometers. The wavelength exhibiting the highest absorbance was considered the maximum wavelength.

Determination of Operating Time

One concentration of the standard solution was put into a 5 mL measuring flask. Then, 0.2 mL of 10% AlCl_3 and 0.2 mL of 1M sodium acetate were added, diluted with distilled water to the limit and homogenized. The solution was incubated at room temperature, and the absorbance was measured at 0, 5, 10, 15, 20, 25, and 30 minutes with the maximum wavelength obtained.

Preparation of Quercetin Raw Curve

The quercetin standard series was made with concentration variations of 3, 5, 7, 9, and 11 ppm by pipetting 0.3, 0.5, 0.7, 0.9, and 1.1 mL of 100 ppm quercetin standard solution into individual 10 mL measuring flask. Three

mL of ethanol p.a, 0.2 mL AlCl_3 10%, and 0.2-mL sodium acetate 1M were added, diluted with distilled water to the limit, and then shaken homogeneously. The solution was incubated at room temperature according to the designated incubation time, and the absorbance was measured at the determined wavelength. The concentration of the standard solution and the absorbance obtained was plotted to make a curve, then a linear regression equation was produced.

Determination of Total Flavonoid Levels in Singkel Leaves Extracts from different Extraction Methods

A 5 mg of ethanol extract of singkel leaf was weighed and dissolved with ethanol p.a into a 10 mL volumetric flask. Two mL solutions were added to a 10 mL measuring flask. Subsequently, three mL of ethanol p.a, 0.2 mL of 10% AlCl_3 , and 0.2 mL of 1M sodium acetate were added, and then distilled water was added to the limit mark. The solution was left at room temperature by the designated incubation time, and the absorbance was measured at the maximum wavelength set. Tests on ethanol extracts were conducted in triplicate (Figure 1).

Determination of Flavonoid Content with Various Extraction Conditions

After obtaining the extraction method of singkel leaves, flavonoid levels from various extraction conditions were tested. The first condition was the solvent concentration using ethanol 60, 70, 80, and 96%; after obtaining the most optimal solvent concentration, the next stage involved looking at the sample: solvent ratio (1:10, 1:20, and 1:30 b/v). The total flavonoid content and antioxidant activity were then measured again, as shown in Figure 2 and Figure 3.

Determination of Antioxidant Activity with UV-Vis Spectrophotometer Preparation of DPPH Solution

The DPPH stock solution was prepared by weighing 10 mg of DPPH powder, which was then put into a 10 mL measuring flask

and diluted with methanol p.a up to the limit mark. A DPPH stock solution with a concentration of 1000 ppm was then obtained.

Determination of Maximum Wavelength

The maximum wavelength (λ_{max}) was measured in a 40 ppm DPPH solution and then scanned using wavelengths ranging from 450 to 550 nm.

Preparation of Sample Solution

Each extract of singkel leaves was made by weighing about 50 mg of the extract, dissolved with methanol p.a, and then homogenized to a volume of 50 mL, resulting in a sample stock solution with a concentration of 1000 ppm. This solution was then pipetted and added with methanol p.a until reaching the limit mark, thus achieving sample concentrations of 5, 10, 15, 20, 25, and 30 ppm depending on the IC_{50} of the plant against DPPH (Andriyani *et al.*, 2015; Hassan *et al.*, 2015).

Radical Absorbance Measurement

Each concentration of the sample solution was then added with 40 ppm of DPPH solution using the 1:3 ratio of the sample and DPPH. The solution was then stirred and incubated at room temperature for 30 minutes in a dark place. The absorbance was measured at the determined wavelength obtained, and each sample's percentage of antioxidant activity (%inhibition) was calculated. The blank concentration used was 40 ppm DPPH solution.

Data Analysis

Data analysis was carried out to see the effect of extraction methods and optimal conditions for the extraction of singkel leaves with solvent concentration factors and solid-to-solvent ratios to see the highest flavonoid compound content and antioxidant activity. The total flavonoid content and IC_{50} values of each sample were compared.

Research Scheme

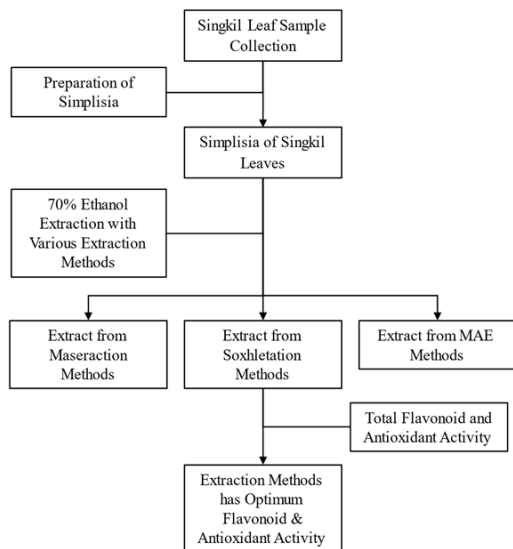


Figure 1.
Optimization of Ethanol Extract of Singkel Leaves Based on Extraction Method
Source: Author (2024)

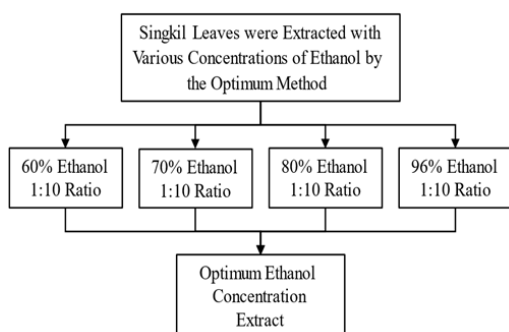


Figure 2.
Optimization of Ethanol Extract of Singkel Leaves Based on Solvent Concentration
Source: Author (2024)

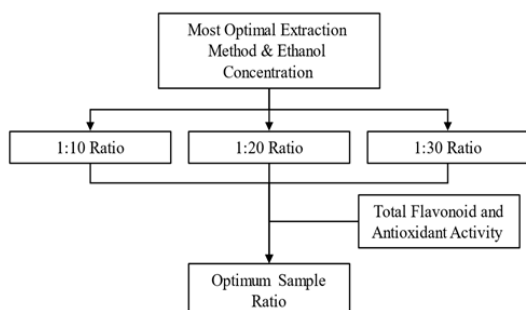


Figure 3.
Optimization of Ethanol Extract of Singkel Leaves Based on Solid-to-Solvent Ratio
Source: Author (2024)

RESULTS AND DISCUSSION

Comparison of Extraction Methods

MAE utilizes non-ionic electromagnetic waves ranging from 300 MHz to 300 GHz (Diantoro *et al.*, 2022). In the MAE process, extraction involves heating with a 400-watt microwave for 45 minutes. The yield from MAE is often less than that of maceration. The reduction in yield may be due to excessive MAE power, which can impede the interaction between the extract and the solvent, leading to a smaller yield. According to Putri *et al.* (2021), using excessive power in MAE can decrease extraction efficiency due to either sample degradation or the rapid boiling of the solvent, which disrupts the interaction between the extract and solvent. Additionally, Iriany *et al.* (2021) note that higher power levels during extraction can damage the bio-active compounds in plant extracts.

Antioxidant activity in Singkel leaves is due to the high content of phenolic compounds, especially the flavonoid group. This study found that the total flavonoid content for the soxhletation, maceration, and MAE methods were 24.56 ± 1.81 mgQE/g, 22.99 ± 0.08 mgQE/g, and 19.61 ± 0.37 mgQE/g, respectively. The soxhletation method yielded more flavonoids compared to both maceration and MAE. Based on the results of previous studies, it is known that the total flavonoid content identified in Singkel leaf extract reached 4.67 mg/g and 0.47% w/w, which indicates significant antioxidant potential (Puspita *et al.*, 2020). Puspitasari and Proyogo (2013) and Fadlilaturrahmah *et al.* (2020) showed similar results where soxhletation produced higher total flavonoid content than maceration and percolation.

Soxhletation involves continuous hot extraction with a single solvent, ensuring that the material remains in constant contact with the solvent, which leads to more efficient isolation of target compounds (Ridwan *et al.*, 2017). Wardhani *et al.* (2023) noted that increasing the number of extraction cycles enhances the total flavonoid content, as multiple cycles allow for better extraction due to higher temperatures. Higher temperatures

could extract more compounds because they assist in the breakdown of the plant cell walls (Missy *et al.*, 2024). However, optimizing the extraction temperature is crucial, as excessive heat can damage the desired compounds. The high flavonoid yield from soxhletation is attributed to its multiple cycles, which ensure thorough contact between the material and solvent, leading to the complete isolation of flavonoid compounds.

Antioxidant activity using the 50% inhibitory concentration (IC_{50}) parameter indicates the concentration needs to inhibit 50% of the oxidation process. A lower IC_{50} value suggests a more significant antioxidant potential of the compound. Generally, a compound is classified as having very high antioxidant activity if its IC_{50} is below 50 $\mu\text{g/mL}$. It is classified as having high activity when the IC_{50} is between 50-100 $\mu\text{g/mL}$, moderate activity when the IC_{50} is between 100-150 $\mu\text{g/mL}$, and low activity when the IC_{50} is above 150 $\mu\text{g/mL}$ (Kusumawati *et al.*, 2021).

The results of the determination of IC_{50} of ethanol extract of singkel leaves (*Premna serratifolia* L.) can be seen in Table 1. The ethanol extract of singkel leaves (*Premna serratifolia* L.) was found to have the highest IC_{50} value in the MAE method and the lowest in the soxhletation method. This result is inversely proportional to the results in the total flavonoid test, where the extract from soxhletation exhibited a higher total flavonoid. According to Jing *et al.* (2015), increasing the temperature can increase the movement of molecules, which accelerates the dissolution process and ultimately increases the levels of flavonoids. However, if the temperature increases excessively, this can cause damage to antioxidant compounds that are sensitive to heat because these compounds tend to be more stable at lower temperatures. Therefore, although the soxhletation method produces the highest levels of flavonoids, its activity in counteracting free radicals does not show significant effectiveness due to damage to antioxidant compounds.

Table 1.
Yield, Total Flavonoids, and Antioxidant Activity of Singkel Leaf Extracts

Extraction Methods	Yield (%)	Total Flavonoids (mgQE/g)	IC_{50} (ppm)
Soxhletation	29,3	24,56 $\pm$ 1,81	20,31 $\pm$ 1,58
Maceration	28,2	22,99 $\pm$ 0,08	15,75 $\pm$ 1,77
MAE	25,5	19,61 $\pm$ 0,37	13,21 $\pm$ 1,35

Source: Author's Analysis (2024)

Figure 5 shows the IC_{50} values of the three extraction methods, Microwave-Assisted Extraction (MAE), maceration, and soxhletation, with three replications for each method. The IC_{50} value of the MAE method was 13.21 $\pm$ 1.35, which was relatively low compared to other methods. This shows that microwave extraction effectively removes active compounds with high biological activity, which results in lower IC_{50} values. This method is known to be more efficient in extracting bioactive compounds than conventional methods due to shorter processing time and heat energy directly applied to the solvent (Alara *et al.*, 2020).

The IC_{50} value of the maceration was 15.75 $\pm$ 1.77. This method is simple and widely used but less efficient than MAE. This can be caused by a longer extraction time, causing some bioactive compounds to degrade. However, as this method does not involve intense heating, more heat-sensitive compounds tend to be preserved (Tambun *et al.*, 2021). The soxhletation method produced the most significant IC_{50} values at 20.31 $\pm$ 1.58 $\mu\text{g/mL}$. This suggests that soxhletation, which involves extraction with continuous heating for a longer time, is less effective in maintaining the integrity of bioactive compounds.

Continuous heating can lead to the degradation of thermolabile compounds, which causes the biological activity to decrease (Alara *et al.*, 2020). Among the three extraction methods, MAE showed the most effective results with the lowest value, indicating that this method can extract active compounds efficiently with little degradation.

Solvent Concentration Comparison

Based on Figure 4, the most suitable method for extracting compounds of singkel leaves was soxhletation, which yielded higher amounts and flavonoid values than other methods. Therefore, to determine flavonoid and antioxidant levels based on solvent concentration using the soxhletation method.

The ethanol extract of singkel leaves was prepared using the soxhletation extraction method. For this process, 15 grams of simplisia powder were used with 60%, 70%, 80%, and 96% ethanol. The resulting yields for the extracts with 60%, 70%, 80%, and 96% ethanol were 3.3 grams, 4.39 grams, 4.1 grams, and 4.3 grams, respectively. The extract yield percentages for these ethanol concentrations were 22%, 29.3%, 27.33%, and 28.66%. The use of 70% ethanol resulted in the highest yield percentage, which was 29.3%. This indicates that the ethanol concentration significantly influences extraction efficiency. With a more significant proportion of water, 70% ethanol is more polar and, thus, more effective at extracting polar metabolites such as flavonoids than 80% and 96% ethanol (Wahyudi & Minarsih, 2023).

The quantitative analysis of flavonoid compounds was conducted using UV-Vis spectrophotometry to assess the flavonoid content in the ethanol extract of singkel leaves, which were known to contain flavonoids. Quercetin was used as the standard solution for this analysis. It was selected due to its presence in the flavonoid group, its five hydroxyl groups, its ability to reduce free radicals, and its capacity to form complexes with $AlCl_3$. It is also a common flavonoid in plants. $AlCl_3$ is added to the sample solution to form some colored complexes, causing a shift in the wavelength to the visible spectrum and resulting in a yellow color. Sodium acetate is added to stabilize the wavelength in the visible range during the determination of flavonoid content (Khairunnisa *et al.*, 2022).

Absorbance measurements of quercetin standard solutions in ethanol at concentra-

tions of 9 ppm, 12 ppm, 15 ppm, 18 ppm, 21 ppm, and 24 ppm were conducted for each of the ethanol concentrations (60%, 70%, 80%, and 96%) after allowing the solutions to stand for 30 minutes. The resulting linear regression equation for the quercetin standard solution absorbance was $y = 0.03045x - 0.000912$, with a correlation coefficient (r) of 0.999. This high r -value, close to one, suggests a strong linear relationship between the quercetin concentration and the absorbance values

The absorbance of the singkel leaf ethanol extracts was measured in triplicate to ensure accuracy and to determine the standard deviation. The extracts, with ethanol concentrations of 60%, 70%, 80%, and 96%, were diluted to a concentration of 1000 ppm to reduce errors, as the Beer-Lambert law is applicable to dilute solutions and ensures light penetration. The samples were allowed to stand for 30 minutes to ensure complete reaction between the singkel leaf extract and the added reagent. Before the UV-Vis spectrophotometry measurement, a blank sample was used as a control. The blank is a calibration reference to exclude non-analyte compounds and other substances that might interfere with the absorbance measurements (Pujiastuti & El'Zeba, 2021).

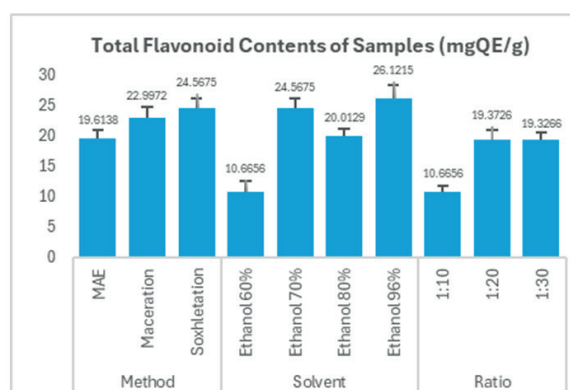


Figure 4.

Graph of Total Flavonoids Based on Extraction Method, Solvent Concentration, and Sample: Solvent Ratio

Source: Author's Analysis (2024)

According to Figure 4, the flavonoid content in the ethanol extracts of singkel leaves were 10.66 ± 0.13 mgQE/g for 60% ethanol, 24.56 ± 1.81 mgQE/g for 70% ethanol, 20.01 ± 0.14 mgQE/g for 80% ethanol, and 26.12 ± 0.96 mgQE/g for 96% ethanol. The highest flavonoid content was found in the 96% ethanol extract, indicating that 96% ethanol is the most effective for achieving optimal flavonoid levels in the extraction process. This finding aligns with research by Pujiastuti and El'Zeba (2021), which demonstrated that 96% ethanol is the most effective solvent for extracting flavonoids from red dragon fruit skin. Higher flavonoid content in 96% ethanol is likely due to its semi-polar nature, which better dissolves non-polar flavonoids. Hendryani *et al.* (2015) identified that non-polar flavonoids, which include isoflavones, flavones, and flavonols, dissolve more readily in 96% ethanol. The flavonoid content observed in this study was affected by the % yield of each solvent concentration. Generally, a higher yield corresponds to a higher total flavonoid content (Hidayah & Anggarani, 2022).). This finding aligns with the results, indicating that 96% ethanol, which achieved the second highest % yield, also showed high flavonoid content. Although the best yield was achieved with 70% ethanol, its flavonoid yield was lower compared to that of 96% ethanol. This discrepancy is likely due to the water content in the 70% ethanol extract, which contributes to a lower flavonoid yield.

Flavonoids are recognized for their potent antioxidant properties compared to the synthetic antioxidant (Hassanpour & Doroudi, 2023). Consequently, after determining the total flavonoid content in the ethanol extract of singkel leaves, the IC_{50} value was measured to assess the antioxidant activity of these leaves.

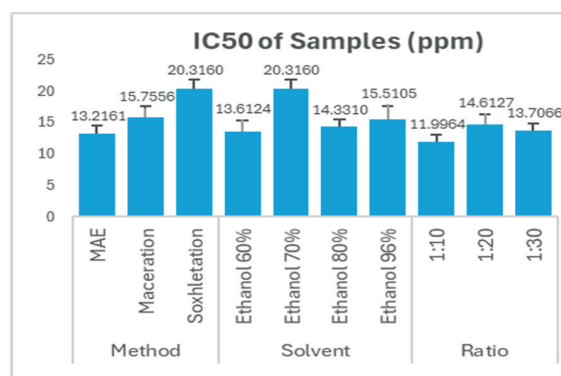


Figure 5.

IC_{50} of Samples Graph Based on Extraction Method, Solvent Concentration, and Sample: Solvent Ratio

Source: Author's Analysis (2024)

As shown in Figure 5, the antioxidant activity of singkel leaves extracts with 60%, 70%, 80%, and 96% ethanol was measured in triplicate, yielding IC_{50} values of 13.61 ± 1.78 , 20.31 ± 1.58 , 14.33 ± 1.16 , and 15.51 ± 2.17 ppm, respectively. The data indicates that 70% ethanol extract exhibited the lowest antioxidant activity; this suggests that a lower IC_{50} value of the 60% ethanol extract signifies a more potent antioxidant activity, as a lower IC_{50} is indicative of higher effectiveness. The variation in antioxidant activity among the different ethanol concentrations is likely due to differences in solvent polarity, with 60% ethanol being more polar than 70%, 80%, and 96% ethanol. This increased polarity enhances the extraction of secondary metabolites in singkel leaves (Wahyudi & Minarsih, 2023).

Table 2.

Yield, Total Flavonoids, and Antioxidant Activity of Singkel Leaf Extract Based on Solvent Concentration

Solvent Concentration	Yield (%)	Total Flavonoids (mgQE/g)	IC_{50} (ppm)
60% Ethanol	22,0	$10,66 \pm 0,13$	$13,61 \pm 1,78$
70% Ethanol	29,3	$24,56 \pm 1,81$	$20,31 \pm 1,58$
80% Ethanol	27,33	$20,01 \pm 0,14$	$14,33 \pm 1,16$
96% Ethanol	28,66	$26,12 \pm 0,06$	$15,51 \pm 2,17$

Source: Author's Analysis (2024)

Table 3.
Yield, Total Flavonoids, and Antioxidant Activity of Singkel Leaf Extracts

Ratio Sample: Solvent	Yield (%)	Total Flavonoids (mgQE/g)	IC ₅₀ (ppm)
1:10	33,33	10,66	11,99
1:20	28	19,37	14,61
1:30	26,67	19,32	13,70

Source: Author's Analysis (2024)

The result of the total flavonoid content and antioxidant activity of the ethanol extracts from singkel leaves indicated that the most effective solvent concentration was 96% ethanol among the four concentrations tested. This is due to the higher total flavonoid content observed with 96% ethanol compared to 60%, 70%, and 80% ethanol. Additionally, a higher yield achieved with 96% ethanol, compared to 60% and 80% ethanol, may contribute to its superior performance.

Sample and Solvent Ratio Comparison

The ratios used in the extractions were 1:10, 1:20, and 1:30. The yield gradually decreased with each solvent volume added. The highest yield was achieved with a ratio of 1:10, reaching 33.33% with 15 g of material and 150 mL of solvent. In contrast, the most negligible yield was observed at a ratio of 1:30, with a yield of 26.67%, while a ratio of 1:20 yielded 28%.

According to Table 2, the total flavonoid content for each ratio was 10.66±0.13 mQE/g, 19.37±0.5 mQE/g, and 19.32±0.8 mQE/g, respectively. The highest yield was observed at a 1:20 ratio, while the lowest was at a 1:10 ratio. The results indicated that increasing the solvent ratio up to 1:20 led to a corresponding rise in the total flavonoid content of the singkel leaf ethanol extract. This aligns with previous findings, which suggest that adding a certain amount of solvent optimizes compound release. However, beyond a certain point, further increases in solvent amount result in only minimal gains, as seen at the

1:30 ratio, where flavonoid content remained nearly constant compared to the 1:20 ratio.

As shown in Figure 5, the antioxidant activity of the three tested ratios yielded IC₅₀ values of 11.99±1.02, 14.61±1.65, and 13.7±1.13 ppm, respectively. The 1:10 ratio demonstrated the most vigorous activity with the lowest IC₅₀ of 11.99±1.02 ppm, likely due to the dissolution of bioactive compounds such as phenolics, which possess high antioxidant activity. In contrast, the 1:20 ratio showed the weakest activity, possibly because some bioactive compounds were not fully dissolved during extraction. As the sample-to-solvent ratio increases, antioxidant activity decreases, as a larger solvent volume dilutes the concentration of active compounds, resulting in reduced activity. When the ratio is smaller, the solution concentration nears equilibrium, leading to more efficient extraction of active compounds (Noviyanty *et al.*, 2019).). The 1:20 and 1:30 ratios showed minimal difference, suggesting that the sample may have reached its optimal extraction point at 1:20, and further solvent addition had little impact. The lower antioxidant activity could also be due to the extract containing flavonoids in a potentially impure form (Pratiwi *et al.*, 2023).

CONCLUSIONS

To conclude, regarding the extraction method optimization, this study that the soxhletation method yielded the best results with a total flavonoid content of 24.56±1.81 mgQE/g and an IC₅₀ of 20.31±1.58 µg/mL. Regarding the optimization of solvent concentration, the 96% ethanol concentration was proven most effective, producing a total flavonoid content of 26.12±0.06 mgQE/g and an IC₅₀ of 15.51±2.17 µg/mL. For solvent ratio optimization, the highest flavonoid content was achieved at a 1:20 ratio (19.37±0.5 mgQE/g), while the best IC₅₀ value was observed at a 1:10 ratio, with 11.99±1.02 µg/mL. Based on the findings of this research, future investigations should carry out in vivo studies using animal models to assess the pharmacological effects and potential toxicity of singkel leaf extract.

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