Pharmacoscript

Putri et al./Pharmacoscript, Volume 9, No, 2, Agustus 2026, 557-571

P-ISSN : 2622-4941 | E-ISSN : 2685-1121

COMPARISON OF MACERATION, INFUSION & SOXHLET EXTRACTION METHODS ON THE TOTAL FLAVONOID CONTENT OF *Syzygium polyanthum* LEAVES

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Received: 16/08/2026 Revised: 24/08/2026 Accepted: 07/09/2026 Published: 11/09/2026

ABSTRACT

Bay leaves (*Syzygium polyanthum*) contain flavonoid compounds that have the potential as anti-inflammatory, antimicrobial, anti-inflammatory, anti-allergic, antihyperuricemic, anticancer, and antioxidants. This study aims to determine the total flavonoid content and distribution pattern of compounds extracted from bay leaves using maceration, infusion, and soxhlet methods. Maceration and soxhlet used 70% ethanol as a solvent, while infusion used water as a solvent. Total flavonoid content was measured by UV-Vis spectrophotometry using quercetin as a standard, and the distribution pattern of compounds was analyzed by TLC. The results showed that the highest yield was obtained by the soxhlet method (28.08%), followed by maceration (23.08%), and infusion (18.52%), with all values meeting the yield requirement of ≥18.2%. The highest total flavonoid content was produced by soxhlet (82.2 mgQE/g extract), followed by maceration (59.2 mgQE/g extract), and infusion (1.6 mgQE/g extract). Under the conditions investigated, soxhlet extraction using 70% ethanol produced the highest measured extract yield and total flavonoid content.

Keywords: Syzygium Polyanthum, Total Flavonoids, Maceration, Soxhlet, Infusion

INTRODUCTION

Bay leaves (*Syzygium polyanthum*) are one of the plants commonly found in Indonesia today. They are a useful herb in traditional medicine, as well as a cooking spice. The use of bay leaves in traditional medicine is due to their content of secondary metabolites, which act as bioactive substances and can be used as treatments for various diseases. These secondary metabolites include flavonoids, tannins, and saponins (Alwie et al., 2021).

Flavonoids are the main compounds found in bay leaves. These compounds have been reported to have antioxidant, anti-inflammatory, antimicrobial, anti-allergic, antihyperuricemic, and anticancer activities. (Harismah & Chusniatun, 2016). The worsening of diseases is frequently caused by free radicals such as superoxide and hydroxyl. Flavonoid compounds found in

plants are highly effective at eliminating and scavenging damaging oxidizing species. As the primary polyphenolic compounds in bay leaves, flavonoids play a role in preventing degenerative diseases (Rheza et al., 2023).

Flavonoid compounds in bay leaves can be obtained through extraction. Extraction methods are generally classified into cold or hot techniques. Cold extraction methods include percolation and maceration, while hot extraction methods include reflux, digestion, Soxhlet, infusion, and decoction. The quality and content of the bioactive compounds obtained from plants are greatly influenced by the extraction method used. Selecting the appropriate extraction method can increase the concentration of secondary metabolites in the resulting extract (Sektiaji & Riyanta, 2023).

Several methods are used, including maceration, infusion, and Soxhlet extraction. The selection of this method is based on the advantages and disadvantages of each method. Maceration is the most commonly used method. This extraction method has the main advantages of simple equipment and procedures, and does not involve heating, which prevents the natural ingredients from decomposing or being damaged. However, this method has the disadvantage of requiring a long time and a large amount of solvent (Samudra et al., 2022). Research comparing maceration and percolation extraction methods for bay leaves indicates that maceration is more effective for isolating compounds, yielding a high extract recovery of 26.72% (Sektiaji & Riyanta, 2023).

Furthermore, another widely used type of extraction is infusion. This method has the advantage of requiring a relatively short time and simple equipment. However, this method has the disadvantage of only being able to handle compounds that are heat-stable (Pratiwi et al., 2023). Other extraction methods, such as soxhlet, have the advantage of using less solvent, yielding a larger extract, and being repeated throughout the process to ensure complete dissolution of the compound (Sulistiani & Isworo, 2022). However, this method has drawbacks, such as longer processing time and the risk of damage to compounds that are not heat-resistant (Khoiriyah, 2023). Research comparing percolation and soxhlet extraction methods for bay leaves demonstrated that the soxhlet method was more effective for compound isolation, yielding a high extraction yield of 23.62% (Hartanti et al., 2019). Different extraction methods affect the flavonoid content obtained, so an effective extraction method is needed to obtain the highest total flavonoid content from bay leaves.

In addition to the extraction method, the final outcome of the process is also influenced by the solvent; therefore, selecting the appropriate solvent is essential. This study utilized 70% ethanol due to its polar nature and because previous research indicated that 70% ethanol yields a higher total flavonoid content (Dewi Hastuti & Andini, 2021).

This study compared the total flavonoid content of bay leaf (*Syzygium polyanthum*) extracts obtained using maceration, infusion, and Soxhlet extraction methods through UV–Vis spectrophotometry. The compound distribution patterns in the extracts were further analyzed using thin-layer chromatography (TLC). The additional contribution of this study is the identification of the extraction method that provides the most favorable flavonoid yield under the conditions investigated.

METHODS

Tools and Materials

The tools used in this study were water bath (FAITHFUL), Erlenmeyer (IWAKI), oven (memmerth), soxhlet, infusion pan, rotary evaporator (IKA HB10), measuring flask (IWAKI), simplicia blender, analytical balance, stirring rod, dropper pipette, UV-Vis spectrophotometry (thermoscientific), porcelain cup (HERMA), micropipette (FAITHFUL). The materials used in this study were bay leaves, 70% p.a. ethanol solvent, filter paper, distilled water, p.a. quercetin comparator, $AlCl_3$ (aluminum chloride), magnesium, HCl and sodium acetate.

Research Path

1. Step I Plant Determination

Plant determination was carried out at the Batu Herbal Materia Medica Laboratory, East Java Provincial Health Office, and the results obtained were number 000.9.3/4901/102.20/2025.

2. Step II: Preparation of Plant Material

The plant material was prepared using 3,000 g of fresh bay leaves (*Syzygium polyanthum*), which underwent a dry sorting process to remove damaged parts and foreign materials. The leaves were then dried in an oven at 40°C and ground into powder. A total of 750 g of the resulting bay leaf powder was used for extraction. The powdered material was then stored in a clean, dry, and tightly closed container (Sinaga et al., 2021).

3. Step III Extract Preparation Using the Maceration Method

For the maceration procedure, 250 g of bay leaves (*Syzygium polyanthum*) powder were mixed with 2500 ml of 70% ethanol at a ratio of 1:10 (w/v), and macerated for 72

hours. The resulting macerate was filtered and evaporated at 50°C (Kiromah et al., 2021).

4. Step IV Extract Preparation Using the Infusion Method

For the infusion procedure, 250 g of bay leaves (*Syzygium polyanthum*) powder were mixed with 2500 ml of distilled water at a ratio of 1:10 (w/v). The mixture was heated in an infusion vessel at 90°C for 15 minutes, with the temperature maintained at 90°C throughout the heating period and the mixture stirred intermittently. The temperature and extraction time were selected based on the general infusion procedure and it can enhance the extraction process by increasing the molecular movement and diffusivity of the aqueous solvent (Hisbiah et al., 2025). The extract was then filtered through a flannel cloth while it cooled. The mixture was then concentrated using a water bath at 50°C until a thick infusion extract was obtained (Lestari et al., 2024).

5. Step V Extract Preparation Using the Soxhlet Method

For the soxhlet procedure, A total of 250 g of bay leaves (*Syzygium polyanthum*) powder was extracted using a soxhlet apparatus in five consecutive batches. Each batch consisted of 50 g of powder placed in a porous extraction thimble and extracted with 500 mL of 70% ethanol. The extraction process was carried out for 3 hours. Fresh solvent was used for each batch, resulting in a total sample to solvent ratio of 1:10 (w/v), corresponding to 250 g of powder and 2,500 mL of solvent. The extracts from all five batches were combined and concentrated at 50°C to obtain the soxhlet extract (Surya et al., 2021).

6. Step VI Qualitative Identification of Flavonoid Compounds

1 mg of solid ethanol extract was placed in a beaker, then 10 drops of methanol were added and stirred with a spatula until dissolved. Next, 10 mg of magnesium (Mg) and 4 drops of concentrated HCl were added to the mixture. The appearance of a yellow, blue, orange, or red color indicates a positive result for flavonoids (Ramadhan et al., 2026). In this study, TLC analyses were conducted using methanol: chloroform (1:9) and methanol: chloroform (5:5) as mobile phases.

7. Step VII Determination of Maximum Wavelength

The maximum wavelength of quercetin was determined by measuring a 6 ppm quercetin solution at a wavelength range of 400-500 nm using UV-Vis spectrophotometry. Next, the maximum wavelength with the highest absorbance value was determined (Dewi Hastuti & Andini, 2021).

8. Step VIII Determination of the Quercetin Standard Curve

In the first step, a quercetin stock solution was prepared by dissolving 10 mg of quercetin in ethanol p.a. to 100 ml (100 ppm concentration). Then, 0.2 ml, 0.4 ml, 0.6 ml, 0.8 ml, and 1 ml were pipetted, and 10 ml of ethanol p.a. were added to the standard curve. For concentrations of 2, 4, 6, 8, and 10 ppm, 0.2 ml of 10% $AlCl_3$ and 0.2 ml of sodium acetate were added to a 10 ml volumetric flask, and ethanol p.a. was added to the mark, then allowed to stand for 15 minutes. The absorbance was then read using UV-VIS spectrophotometry at a wavelength of 432 nm (the wavelength of highest absorption). Three replicates were performed to determine the standard curve (Dewi Hastuti & Andini, 2021).

9. Step IX Preparation of Test Sample Solution

The thick bay leaf (*Syzygium polyanthum*) extract was prepared by dissolving 1 mg of extract with 10 ml of ethanol p.a. was added in a 10 ml volumetric flask, resulting in a solution concentration of 100 ppm. This was replicated three times (Islamiyati & Noviana Saputri, 2018).

10. Step X Determination of Total Flavonoid Content

A 1 mL aliquot of the 100 ppm bay leaf (*Syzygium polyanthum*) extract solution was transferred into a 10 mL volumetric flask, followed by the addition of 0.2 mL of 10% $AlCl_3$ solution and 0.2 mL sodium acetate. Ethanol was then added to the mark, and the mixture was allowed to stand for 15 minutes. After incubation, the absorbance was measured using a UV-Vis spectrophotometer at the maximum wavelength of 432 nm, with three replicates. The total flavonoid content was determined using three analytical replicates, consisting of three repeated spectrophotometric measurements of the same extract solution (Dewi Hastuti & Andini, 2021).

Data Analysis

Total flavonoid content was calculated by substituting the sample's absorbance value into the quercetin standard curve equation, with the absorbance value serving as the y-variable and the resulting x-value representing the quercetin concentration in µg/mL. Subsequently, the total flavonoid content was calculated using the following formula:

$$F = \frac{V \times c \times Fp}{m} \times 100\%$$

Note:

F : Total flavonoid content
V : Volume of extract used (mL)
c : Quercetin concentration (mg/mL)
Fp : Dilution factor
m : Sample weight (g)

RESULTS AND DISCUSSION

The extraction yielded a thick extract with a yield of:

Table 1. Extract yield

Extraction Method	Powder Weight	Liquid Extract Weight	Yield	Requirements For *Syzygium polyanthum* Extract (Kemenkes RI, 2017)
Maceration	250 gr	57,7 gr	23,08 %	≥18,2 %
Soxhlet	250 gr	70,2 gr	28,08 %	
Infusion	250 gr	46,3 gr	18,52 %	

Differences in yield are derived from various extraction methods, solvents, and temperatures. In the maceration method, the extraction process is carried out under cold conditions. The process takes place without heating, at room temperature, and depends on the contact time during stirring, resulting in slower release of compounds from plant cells (Nur Fadillah et al., 2024). In the soxhlet method, the extraction process involves repeated heating until the solvent is clear, accelerating the transfer of compounds from the simplex to the solvent, thus yielding more extract (Agus et al., 2023). In the infusion method, the extraction process involves brief heating. The yield obtained by this method has the lowest value compared to the maceration and soxhlet methods. This occurs due to the difference in solvents used, with a temperature of 90°C during the infusion process. In the infusion method, the solvent used is water, which is effective in dissolving polar compounds. However, not all secondary metabolites are optimally soluble in water, and some compounds that are stable to heating temperatures will be extracted in greater quantities (Nur Fadillah et al., 2024).

The soxhlet method yields a higher percentage of extracts than maceration and infusion. This indicates that a higher yield indicates a greater number of compounds extracted or dissolved during the soxhlet process. Consequently, the amount of compounds extracted in the extract is also higher. Conversely, a lower yield indicates a smaller quantity of extracted compounds (Nur Fadillah et al., 2024).

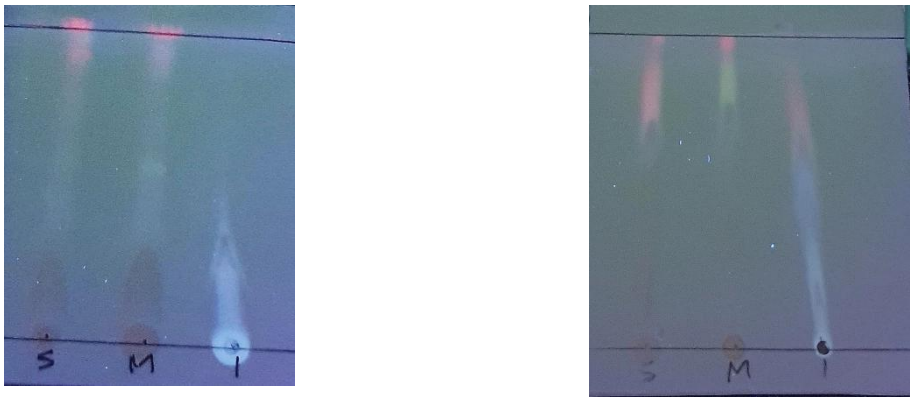
The results of the preliminary qualitative screening for flavonoids showed the following color changes in the tested extracts:

Table 2. Qualitative identification of flavonoid compounds

Extraction Method	Color Change	Identification Results
Maceration	Orange	+
Soxhlet	Orange	+
Infusion	Yellow	+

Based on the results of the identification test, all samples were positive for flavonoids. Although the test results produced different colors. The resulting color differences occurred due to differences in the methods used and the flavonoid content in the samples. This is in accordance with the research of Bahriul et al., (2017) that the more intense the resulting color approaching red or orange, the greater the flavonoid content in the sample (Bahriul et al., 2017). The infusion extraction method showed a yellow test result, which means the flavonoid content in the sample was less compared to the maceration and soxhlet method samples which produced an orange color.

In this study, TLC analysis was also conducted to observe the distribution patterns of compounds across the different extraction methods. The TLC results revealed that the soxhlet and maceration methods produced nearly identical spot patterns. Both methods successfully extracted non-polar compounds, evidenced by spots migrating significant distances in both the relatively non-polar mobile phase (methanol: chloroform 1:9) and the more polar mobile phase (methanol: chloroform 5:5). This indicates that both maceration and soxhlet extraction are capable of extracting the non-polar and polar compounds present in bay leaves, thereby facilitating more effective extraction of flavonoids. The spots migrated further when the more polar mobile phase methanol: chloroform (5:5) was used.



Methanol : Chloroform (1:9) Methanol : Chloroform (5:5)

Figure 1. TLC test results with annotations s = soxhlet m = maseration i = infusion

Results of determining maximum wavelength can be seen below:

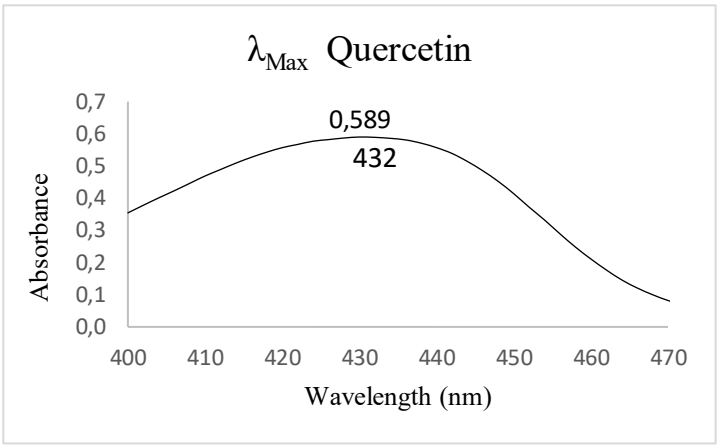


Figure 2. Maximum wavelength of quercetin

The maximum absorption wavelength was determined using a 6 ppm quercetin solution. A reaction mixture was prepared by adding 0.2 mL $AlCl_3$ and 0.2 mL sodium acetate, then adjusting to a final volume of 10 mL with ethanol p.a. in a volumetric flask. After incubation for 15 minutes. Measurement of the maximum wavelength of a 6 ppm quercetin solution series showed an absorbance value of 0.589 with a λ max of 432 nm. Absorbance measurements on quercetin can be performed at a λ max of 400-500 nm (Bahriul et al., 2017). The maximum wavelength measurement was carried out with the aim of determining the wavelength at which maximum absorption is achieved and having a relatively constant absorbance so that it can be optimal and accurate during sample testing (Mewar et al., 2023).

Determination of the quercetin standard curve can be seen below:

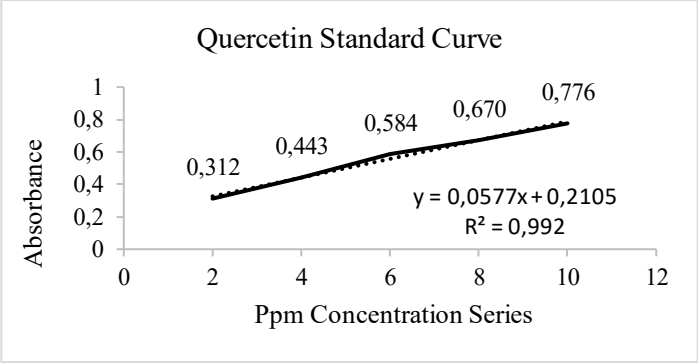


Figure 3. Quercetin Standard Curve

Based on Figure 3, it can be seen that the higher the quercetin content in ppm, the higher the absorbance produced. The absorbance produced at levels of 2 ppm, 4 ppm, 6 ppm, 8 ppm, and 10 ppm produced absorbance in the range of 0.2 – 0.8. The creation of this quercetin standard curve was carried out with the aim of helping determine the total flavonoid content in the sample through a linear regression equation. The standard curve produced a regression equation with a correlation coefficient (r) of 0.992. The value (r) obtained is close to 1 indicating that the regression equation is linear, so it can be said that absorbance and concentration have a very strong correlation, this indicates that the absorbance value is directly proportional to concentration, in accordance with the Lambert-Beer law. This means that the measurement of total flavonoid content with this method is accurate (Sadlia et al., 2024).

The results of the calculation of total flavonoid content are as follows:

Table 3. Total flavonoid content

Extraction Method	ppm	Absorbance (mean ± SD)	RSD (%)	Total Flavonoid Content (mgQE/g)
Maceration	100	0,553 ± 0,001	0,15	59,2
Infusion	100	0,221 ± 0,001	0,10	1,6
Soxhlet	100	0,685 ± 0,003	1,14	82,2

Note: values are expressed as mean ± standard deviation (SD) from three replicate, consisting of three repeated spectrophotometric measurements of the same extract solution. The relative standard deviation (RSD) values are expressed as percentages.

Based on the results, flavonoid concentrations exhibited a low % Relative Standard Deviation (%RSD) value, at ≤ 2%. This indicates good measurement precision and consistency across replicates (Sayuthi & Kurniawati, 2017).

The results of flavonoid concentrations obtained from bay leaf extracts by maceration, infusion, and soxhlet with a concentration of 100 ppm were 59.2 mgQE/g extract, 1.6 mgQE/g extract, and 82.2 mgQE/g extract, respectively. These levels are still within the range of bay leaf flavonoid levels that have been carried out based on previous research that the total flavonoid content obtained from maceration extraction results was 46.965 mgQE/g extract (Luh & Ari, 2025). Based on other studies that have been conducted, the total flavonoid content in bay leaves was 70.29 mgQE/g extract through the maceration process (Ramadhan et al., 2026). In other studies, the results of calculating the total flavonoid content in bay leaves showed a value of 165.658 mgQE/g extract by maceration (Aklimah & Ekayanti, 2022). In infusion extraction, the total flavonoid content produced a level of 0.6 mgQE/g, which generally produced a lower value than extraction using ethanol solvent (Lovena et al., 2025).

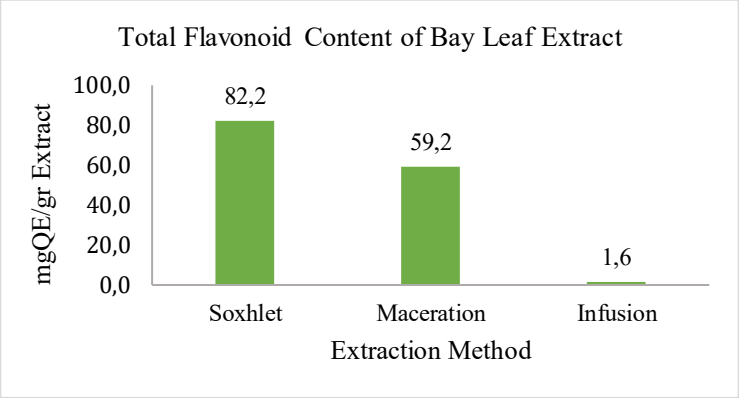


Figure 4. Total flavonoid content calculation graph

Figure 4 shows that soxhlet extraction yielded the highest total flavonoid content. Soxhlet extraction used 70% ethanol, which was repeatedly heated to near the ethanol's boiling point. This heating reduced the viscosity and surface tension of the solvent, making it thinner and smoother, facilitating deeper penetration into the plant matrix, which increased the number of compounds that could be extracted (Rosita et al., 2017).

Maceration extraction, however, resulted in a lower estimated total flavonoid content, as determined by the $AlCl_3$ colorimetric method than soxhlet extraction.

Maceration is performed at room temperature with periodic stirring to prevent the powdered material from settling or agglomerating, thereby facilitating solvent penetration and extraction of active compounds. However, the absence of heating may slow flavonoid diffusion and limit the extraction of certain compounds that are less soluble at room temperature, resulting in a lower estimated flavonoid content than that obtained by soxhlet extraction (Rosita et al., 2017). Nevertheless, the $AlCl_3$ colorimetric assay is not completely specific to all flavonoid structures because the response may vary depending on the chemical structure of the compounds and may be influenced by other constituents capable of reacting with $AlCl_3$. Therefore, the results should be interpreted as the total flavonoid content estimated using the $AlCl_3$ colorimetric method.

The infusion extraction method yielded a lower total flavonoid content than the maceration and soxhlet extraction methods. In this study, infusion was performed using distilled water at 90°C. Water is a highly polar solvent and may therefore favor the extraction of polar and water-soluble constituents. However, water alone may be less effective for extracting some moderately polar or less-polar flavonoids than aqueous-organic solvent systems. In addition, exposure to elevated temperature may affect thermolabile compounds through degradation or oxidation. Therefore, the lower total flavonoid content obtained by the infusion method may be associated with the solvent selectivity of water, the extraction conditions, and the possible thermal sensitivity of some flavonoid compounds (Hasanah et al., 2023).

CONCLUSIONS

The results of the flavonoid compound identification test in bay leaf extract *(Syzygium polyanthum)* through the maceration, infusion and soxhlet methods were positive for containing flavonoid compounds. Bay Leaf Extract (*Syzygium polyanthum*) had a total flavonoid content of 59.2 mgQE/g extract in the maceration method, 1.6 mgQE/g extract in the infusion method and 82.2 mgQE/g extract in the soxhlet method with the highest content obtained by the soxhlet method using 70% ethanol. The different extraction procedures produced different measured total flavonoid contents in bay leaf (*Syzygium polyanthum*) extracts. However, the observed differences may have been influenced not only by the extraction procedures but also by the solvents used, namely 70% ethanol for maceration and soxhlet extraction and distilled water for infusion.

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