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Comparative Analysis of Maceration and Soxhlation Extraction for The Total Flavonoid Content of Sungkai Leaves (*Peronema canescens* Jack.)

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Abstract

*The sungkai leaf (*P. canescens *Jack.) is an indigenous plant of Indonesia that has been utilized as a mouthwash and for treating minor wounds in traditional medicine. This study aimed to determine the total flavonoid content of* P. canescens *Jack leaf extract using maceration and soxhlation extraction methods. The total flavonoid content in the leaf extract of* P. canescens *Jack was determined using a UV-Vis spectrophotometer and a colorimetric technique ($AlCl_3$) at a wavelength of 412 nm. The results were reported as the total flavonoid content in quercetin equivalent (EQ). The maceration step yielded 7.30%, whereas the soxhlation process yielded 15.34%. The maceration method yielded a total flavonoid content of 81.19 mgEQ/gram extract, whereas the soxhlation process yielded a flavonoid content of 69.068 mgEQ/gram extract.*

Keywords: *Sungkai leaf, flavonoids, extraction, maceration, soxhlation*

Abstrak

Daun sungkai (P.canescens Jack.) merupakan tanaman asli Indoensia yang dikenal dengan nama daun sungkai telah lama digunakan dalam pengobatan tradisional sebagai obat kumur dan luka ringan. Penelitian ini bertujuan untuk mengetahui kadar flavonoid total dari ekstrak daun P. canescens Jack dengan menggunakan metode ekstraksi secara maserasi dan sokhletasi. Penentuan kadar flavonoid total pada ekstrak daun P. canescens Jack dilakukan secara spektrofotometer UV-Vis dengan metode kolorimetri ($AlCl_3$) pada λ 412 nm dan dinyatakan sebagai flavonoid total dalam ekuivalen kuersetin (EQ). Rendemen yang didapat dari proses maserasi sebesar 7,30% dan sokhletasi sebesar 15,34%. Kadar flavonoid total yang didapatkan dari proses maserasi sebesar 81,19 mgEQ/gram ekstrak dan sokhletasi sebesar 69,068 mgEQ/gram ekstrak..

Keywords: Daun sungkai (P.canescens Jack.), flavonoid, ekstraksi, maserasi, sokhletasi.

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1. INTRODUCTION

Sungkai (Peronema canescens Jack.) is a plant commonly found in Kalimantan. Sungkai has long been employed to treat minor wounds and as a mouthwash. The Dayak and Banjar tribes utilize the sungkai plant as a medicinal remedy to enhance the body's immune system, treat bruises, alleviate fever, and provide a bath for postpartum mothers (Sari et al., 2023). Multiple research findings also indicate that

sungkai plants have interesting activities. Yani et al. (2014) found that administering sungkai extract increased the number of leukocytes by 36% at a dose of 0.56 mg/kgBB in mice. Dillasamola et al. (2021) discovered that administering sungkai leaf extract enhanced the activity and capacity of macrophage cells and increased the number of leukocyte cells and overall leukocyte count in mice. These two studies demonstrate that the sungkai plant possesses immune-boosting properties. The antibacterial properties of Sungkai leaves have been scientifically studied and confirmed by Fransisca et al. in 2020. The preclinical evaluation of sungkai leaves has shown promise as an anti-hyperuricemic agent since it effectively lowers blood uric acid levels in rats (Latief et al., 2021).

Sungkai leaves have strong antioxidant activity with an IC50 value of 50,838 ppm for young leaves and 52,835 ppm for old leaves (Okfrianti et al., 2022). Kasumawati and Hasnah (2021) investigated the impact of the sungkai leaf simplicia drying method on the antioxidant activity of the ethanol extract. The results showed that the antioxidant activity of sungkai leaf ethanol extract was very strong in all samples with different drying methods. The antioxidant activity of samples with oven drying at 50°C had an IC50 value of 13,340 ppm, oven drying at 70°C was 17,034 ppm, wind drying at 14,610 ppm, and sun drying at 16,799 ppm. Sungkai leaves are rich in bioactive substances such as triterpenoids, alkaloids, flavonoids, phenolics, steroids, and saponins (Pindan et al., 2021). Ramadhani et al. (2022) stated that the total phenol content of sungkai bark extract was 14.97 ± 1.28 mgGAE/g extract and a total flavonoid content of 29.41 ± 0.64 mgEQ/gram extract. This study aimed to compare the overall flavonoid content of sungkai leaves using two distinct extraction techniques: maceration and soxhlation. These two extraction procedures are frequently employed in diverse research investigations. The total flavonoid content in sungkai leaf extract was quantified using a UV-Vis spectrophotometer using the colorimetric method. The results were reported as the total amount of flavonoids in quercetin equivalent (mgEQ/gram)..

2. MATERIALS AND METHODS

2.1. Materials and Tools

The materials used were sungkai leaf (Peronema canescens Jack.) obtained from the Banua Botanical Gardens in Banjarbaru, filter paper, ethanol p.a, methanol p.a, distilled water, acetic acid, sodium hydroxide, quercetin, and $AlCl_3$.

The tools used were analytical scales, ovens, porcelain dishes, glass beakers, test tubes, macerators, soxhlet extraction apparatus, water baths, Thermo Scientific Genesis 30 UV-VIS spectrophotometers, vortex mixer, and rotary evaporators.

2.2. Preparation of sungkai leaf simplicia powder

The preparation process commenced with wet sorting, separating dirt or foreign materials from sungkai leaves. The material was washed with clean water to remove soil and other impurities. The drying process was carried out in an oven at 50 ± 2°C. Dry sorting was then carried out to separate foreign organic materials and simplisia damaged by the previous process. The dry-sorted samples were pulverized into a coarse powder using a blender. Simplicia powder was stored in a clean and dry container (Praditya, 2016; Puspitasari & Proyogo, 2017).

2.3. Qualitative analysis of sungkai leaf flavonoid using chemical reaction methods

A total of 100 mg of sungkai leaf powder for the maceration and soxhlation methods was weighed and diluted with methanol p.a. Then, 2 mL of each was taken and put into a test tube. A negative control solution was also made as a

comparison. Then, a few drops of 10% sodium hydroxide were added to each test tube. If a yellow hue is produced, a positive test indicates the presence of flavonoids (Mailuhu et al., 2017).

2.4. Preparation of ethanol extract from sungkai leaves (maceration method)

A total of 25 g of sungkai leaf simplicia powder was put into a 1,000 mL beaker, added with 250 mL of ethanol until the entire sample was submerged, and stirred every 8 hours at room temperature. Extraction was carried out for three days (re-maceration) with solvent replacement every 1 x 24 hours. The liquid extract underwent filtration using filter paper to separate the sediment and extract. The liquid extract of sungkai leaves was evaporated using a rotary evaporator until a thick extract was obtained. The extract was concentrated using a water bath at 50°C until a constant weight was obtained (Leksono et al., 2018). The percent yield was calculated using Equation (1).

$$\%\ Yield = \frac{weight\ of\ extract\ obtained}{weight\ of\ simplicia}\ x\ 100\% \qquad (1)$$

2.5. Preparation of ethanol extract from sungkai leaves (Soxhlation Method)

A total of 25 grams of sungkai leaf simplicia powder was wrapped in filter paper, tied, and put into a sokhlet extractor. 250 mL of ethanol solvent was put into a round bottom flask. Soxhlation was carried out for approximately 18 hours at 64ºC until the solvent was clear. The extract was evaporated using a rotary evaporator at a 65ºC until a thick extract was obtained, then concentrated using a water bath until a concentrated extract was obtained. The extract replication process was repeated thrice (Mokoginta et al., 2013; Puspitasari & Proyogo, 2017; Riyani & Adawiah, 2015; Verawati et al., 2017).

2.6. Determination of total flavonoid levels using a UV-VIS spectrophotometer

2.6.1. Determination of maximum wavelength

A total of 1 mL of 40 ppm quercetin standard solution was put into a test tube and added with 1 mL of 2% $AlCl_3$ and 8 mL of 5% acetic acid. The solution was then homogenized with a vortex mixer and incubated for 30 minutes. Absorption was measured in the wavelength range of 400 – 500 nm (Asmorowati & Lindawati, 2019).

2.6.2. Determination of standard curve

A total of 0.4 mL, 0.6 mL, 0.8 mL, 1 mL, and 1.2 mL of 1000 ppm quercetin standard were put into a 10 mL volumetric flask and then diluted using methanol p.a. to have a final concentration of 40, 60, 80, 100, and 120 ppm. From each solution concentration, 1 mL was taken, put into a test tube, and added with 1 mL of 2% $AlCl_3$ and 8 mL of 5% acetic acid. The solution was homogenized with a vortex mixer and then incubated for 30 minutes at room temperature. The absorption at the maximum wavelength was then measured (Asmorowati & Lindawati, 2019).

2.6.3. Determination of total flavonoid levels

A total of 10 mg of sungkai leaf extract from the maceration method was diluted with methanol in a 10 mL volumetric flask. A total of 1 mL of the extract solution was put into a test tube, then added with 1 mL of 2% $AlCl_3$ reagent, and 8 mL of 5% acetic. The solution was homogenized with a vortex mixer then incubated for 30 minutes. After incubation, the sample absorption was measured using a spectrophotometer at the maximum wavelength previously obtained. Soxhlation results were extracted in the same way to obtain sample absorbance data.

2.7. Determination of Moisture Content

A total of 2 grams of sungkai leaf simplicia powder was put into a silica crucible that had been heated to a temperature of 105°C and measured. The simplicia was flattened by shaking the silica crucible. The silica crucible was placed in an open oven and then dried at 105°C until a constant weight was obtained. The silica crucible cup was cooled and weighed (MOH RI, 2008). Water content was calculated following Equation (2).

$$Moisture\ Content = \frac{initial\ weight\ -\ final\ weight}{initial\ weight\ of\ sample}\ x\ 100\% \quad (2)$$

3. RESULTS AND DISCUSSION

3.1. Plant Sampling

The samples consisted of leaves from the sungkai plant, which were collected in May 2023 at the Banua Botanical Gardens in Banjarbaru, South Kalimantan. The sungkai plant is a tall, woody plant that typically reaches a height of approximately 20-30 meters. Figure 1 displays the visual appearance of the plant. The leaves taken were mature leaves with green leaves and are still fresh because they contain more secondary metabolites than young leaves (Wijaya et al., 2013). Young leaves have a low photosynthetic capacity, while the photosynthesis process influences the content of secondary metabolites, such as flavonoids, in the leaves. Consequently, young leaves have a relatively low flavonoid content (Sjahid, 2008).

3.2. Preparation of Sungkai Leaf Simplicia Powder

The preparation of sungkai leaf simplicia powder started with wet sorting. Wet sorting aims to separate sungkai leaves that are dirty, damaged, and foreign objects. Afterward, it was washed with clean water to remove soil and other impurities attached to the leaves. Drying was carried out in an oven at 50 ± 2ºC. The drying aims to reduce the water content, thereby inhibiting microbial growth (Histifarina et al., 2004). This will prevent the growth of mold and mildew so that simplicia is produced, which is durable and can be stored for a long period (Djumaati et al., 2018). Manoi (2006) states that a water content above 10% will result in enzymatic reactions and microbial degradation.

Afterward, a dry sorting procedure was conducted to segregate samples that have incurred damage due to the preceding step. The sample grinding process was carried out using a blender so that a fine powder was obtained and the extraction process could run well (BPOM RI, 2013).

Figure 1. Plants (a) and sungkai leaves (b) (Personal Documentation)

Figure 2. Sungkai leaf powder

As stated by Kiswandono (2011), using a blender to refine simplicia enhances the interaction between the solvent and the sample during the extraction process.
This facilitates a more effective extraction of secondary metabolites from the sample. The organoleptic test results of sungkai leaf simplicia powder indicated that it was finely powdered, possessed a distinct odor, had a bitter taste, and exhibited a green hue (Figure 2).

3.3. Qualitative Analysis of the Active Flavonoid Content of Sungkai Leaves

Qualitative analysis of flavonoid testing using the chemical reaction method was a preliminary stage in a study that aims to provide an overview of the flavonoid compounds in sungkai leaves. The chemical reaction method was carried out by looking at the color testing reaction using a color reagent (Simaremare, 2014). The ethanol extract of sungkai leaves, obtained using maceration and soxhlation procedures, yielded identical findings in the flavonoid test when treated with NaOH reagent. The findings of this qualitative test indicate that the variation in extraction procedures has no impact on the flavonoid content. Both techniques are capable of extracting flavonoid chemicals. This is because flavonoids are phenolic compounds that can change color when alkali or ammonia are added, so they are easily detected in solution (Rais, 2015). Figure 3 presents the analysis of flavonoids in sungkai leaves using the chemical reaction method with sodium hydroxide reagent.

The presence of flavonoids in sungkai leaves was confirmed through sodium hydroxide tests; positive results were observed, as evidenced by the extract's transformation from dark green to yellow. The color shift is a result of the reaction between the sample and sodium hydroxide, which leads to the formation of a quinoid structure in the ring. This structure has longer and planar conjugated double bonds, allowing it to exhibit fluorescence (Mulyani & Laksana, 2011) (Figure 4).

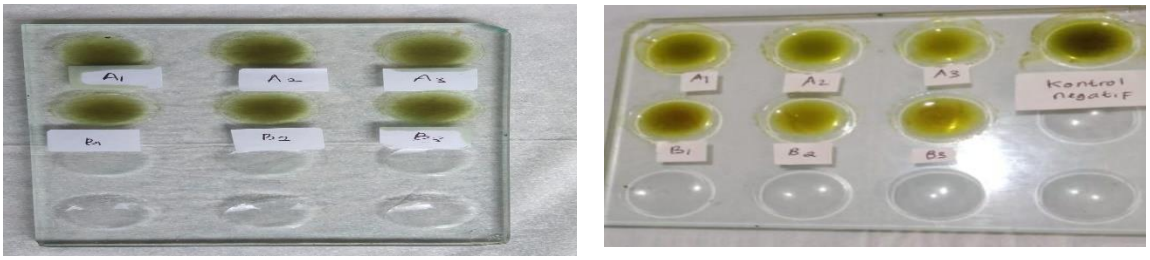

Figure 3. Qualitative analysis of the active flavonoid content of sungkai leaves before (left); and after right the addition of NaOH

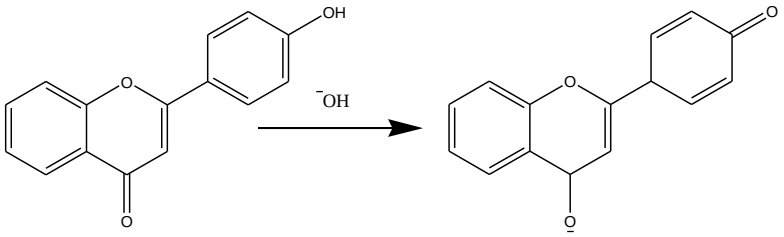

Figure 4. Reaction of flavonoids with NaOH (Mulyani & Laksana, 2011)

3.4. Sungkai Leaf Extraction

a. Maceration Method

The residue was re-macerated with ethanol solvent for two repetitions. The filtrate obtained from each repetition was collected and concentrated using a vacuum rotary evaporator and then heated using a water bath. The yield of ethanol extract of sungkai leaves by maceration method with three repetitions is presented in Table 1.

Table 1. The yield of sungkai leaf extract using the maceration method

Sungkai leaf extract sample	Yield (%)
Repetition-1 (A1)	6.08
Repetition-2 (A2)	7.60
Repetition-3 (A3)	8.24

b. Soxhlation Method

xtraction was carried out until an extract was obtained with a clear solvent. The extraction procedure was conducted from 8 am to 4 pm for three days. Soxhlet extraction was stopped when the solvent in the siphon tube containing the sample was visually clear. The extract solution obtained from the soxhlation process was then concentrated using a vacuum rotary evaporator and heated using a water bath. The yield of ethanol extract of sungkai leaves using the soxhlation method with three repetitions is presented in Table 2.

Table 2. Results of sungkai leaf extract yield using the soxhlation method

Sungkai leaf extract sample	Yield (%)
Repetition-1 (B1)	16.80
Repetition-2 (B2)	13.72
Repetition-3 (B3)	15.52

This study found that the yield of sungkai leaves was higher when using the soxhlation method (Table 2) than the maceration method (Table 1). These findings align with the studies conducted by Rahman (2017) and Firdaus (2019), which demonstrated that the soxhlation method yielded the highest yield of ethanol extract compared to the maceration method. The capabilities of the soxhlation method influence the difference in yield results. The heating process will bind more active compound components in sungkai leaves. Mukhriani (2014) states that the soxhlation extraction method offers the advantage of continuous extraction, where the material is extracted using pure solvent obtained from condensation. This leads to a higher yield compared to maceration extraction. Furthermore, applying heat in the soxhlation method enhances the solvent's capacity to extract insoluble compounds at ambient temperature, resulting in a more efficient chemical withdrawal process (Harborne, 1987). Rosidi et al. (2014) also affirmed that the large number of components extracted during the soxhlation process can cause the high yield value obtained.

3.5. Determination of Total Flavonoid Content Using UV-VIS Spectrophotometry

The colorimetric approach can be employed to quantify the total flavonoid content in the samples. The principle of determining total flavonoid levels is the reaction between flavonoids and $AlCl_3$, which forms a yellow complex whose absorbance is measured at the maximum wavelength. The reaction for determining flavonoid levels using the $AlCl_3$ colorimetric method is presented in Figure 5. The $AlCl_3$ method offers several advantages, including its simplicity, efficiency, and applicability for quantifying flavonoid levels by comparing them to quercetin (Lukman, 2015).

3.5.1. Determination of Maximum Wavelength

The objective of determining the maximum wavelength is to identify the specific wavelength at which the complex formed by AlCl3 and quercetin exhibits the most absorption, resulting in optimal absorbance (Suharyanto & Prima, 2020). The maximum wavelength was determined by measuring a standard solution of 40 ppm quercetin in the 400 – 500 nm range. The maximum wavelength obtained in this study was 412 nm. This maximum wavelength was used to measure the absorbance of the sungkai leaf extract sample.

3.5.2. Preparation of a Standard Curve

The quercetin standard curve was prepared by varying the standard series, which was read at an absorbance wavelength of 412 nm with an incubation time of 30 minutes. A calibration curve was constructed using different concentrations of quercetin, specifically 40 ppm, 60 ppm, 80 ppm, 100 ppm, 120 ppm, and 140 ppm, obtained from a 1000 ppm stock standard solution.

The regression equation was derived from the graph as follows: $y = 0.002x + 0.146$. The linearity value (r) obtained was 0.9995. The accuracy obtained was 99.95%. Linearity states a linear relationship between the concentration and absorbance of the concentration series solution obtained. The closer it is to 1, the more linear the results obtained. This statement is in accordance with the literature, which states that linearity is good if the correlation coefficient (r) value is close to 1 (Nafisa et al., 2015).

3.5.3. Determination of Total Flavonoid Levels

The total flavonoid content in sungkai leaf extract was determined using maceration and soxhlation extraction techniques. The absorbance value of each extract was entered into the quercetin standard curve equation obtained previously, namely $y = 0.002 x + 0.146$. The compound used as a standard in determining flavonoid levels was quercetin because quercetin is a flavonoid from the flavonol group, which has a keto group on the C-4 atom and also a hydroxyl group on the neighboring C-3 and C-5 atoms (Sari & Triyasmono, 2017). The total flavonoid content of sungkai leaves is presented in Table 3.

Table 3. Total flavonoid content of sungkai leaves

Extraction Method	Sample absorbance	Mean absorbance ± SD	Total flavonoid content (mgEQ/gr extract)	Average total flavonoid content (mg EQ/gr extract) ± SD
Maceration	0.312 0.305 0.313	0.31± 0.0044	80.582 80.303 82.673	81.19± 1.295
Soxhlation	0.280 0.290 0.292	0.29± 0.0064	65.048 70.588 71.568	69.068± 3.52

The Shapiro-Wilk test was conducted on the ethanol extract obtained using maceration and soxhlation procedures. The test yielded a significance value greater than 0.05, suggesting the data follows a normal distribution. The test of homogeneity of variances yielded a significance level greater than 0.05, indicating that the data was homogeneous. Subsequently, a One-way ANOVA analysis was carried out. A one-way ANOVA was conducted due to the normal distribution and homogeneity of the data. The One-way ANOVA analysis showed that the maceration and soxhlation methods differed significantly (sig < 0.05) on total flavonoid levels. Due to the notable disparities, the post hoc Test was conducted. The Post Hoc Test analysis findings indicated significant differences in total flavonoid levels between the maceration and soxhlation techniques, as evidenced by a value (sig < 0.05).

The maceration process resulted in a higher total flavonoid content of sungkai leaves compared to the soxhlation method, as shown in Table 3. These findings align with Rahman's (2017) research, which shows that the maceration approach yields a higher total flavonoid content compared to the soxhlation method. Based on the calculation results, the total flavonoid content of the maceration method averaged 81.19 ± 1.295 mg EQ/g extract, while the soxhlation method obtained an average of 69.068 ± 3.52 mg EQ/g extract. Therefore, it can be inferred that the sungkai leaves in this study are sensitive to heat, making them unstable when exposed to high temperatures. As a result, the maceration approach yields a higher flavonoid concentration compared to the soxhlation method.

Precision is expressed by the relative standard deviation (RSD) of a series of data (Alwi, 2017). The % RSD value in sungkai leaf extract using the maceration and soxhlation methods is in accordance with the percent RSD requirement, namely ≤ 4%. This demonstrates that the acquired results possess satisfactory repeatability, hence meeting the validation criteria (Gonzales et al., 2010).

3.6. Moisture Content

Determination of moisture content aims to provide a threshold or range for the amount of water and volatile compounds eliminated in the drying process. The remaining substance was measured by drying at a temperature of 105°C for 1 hour until the weight was constant. Table 4 presents the determination of the water content of sungkai leaves.

Table 4. Moisture content of sungkai leaves

Sungkai leaf extract sample	Moisture Content (%)
Repetition -1	6.71
Repetition -2	6.77
Repetition -3	6.72

According to the Indonesian Ministry of Health (1995), the drying shrinkage values range from no more than 10%. Therefore, the water content obtained from the sungkai leaves meets the specified requirements. A drying process is considered superior quality when the shrinkage value is lower. The water content in traditional medicine should not exceed 10% (Ministry of Health of the Republic of Indonesia, 1995). The material will easily grow mold if the water content exceeds 10%. Materials with low water content are less susceptible to mold contamination. This will affect the purity and contamination of a material (Ratnani et al., 2015).

4. CONCLUSION

The average yield of sungkai leaf extract using the maceration method was 7.30%, while the soxhlation method was 15.34%. The total flavonoid content of sungkai leaf extract from the maceration method was 81.19 mgEQ/gram of extract and the soxhlation method was 69.068 mgEQ/gram of extract.

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