

Isolation of Flavonoids from Walang Sangit Leaves (*Eryngium Foetidum*) Using Methanol and Acetone Maceration with UV–Visible Spectrophotometric Analysis

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ABSTRACT

Flavonoids are natural phytonutrient compounds in plants that exhibit antioxidant properties and play a role in scavenging free radicals. Walang sangit leaves have potential as a source of flavonoids, with samples consisting of young and mature leaves obtained from Pagebangan, Ciwandan District, Cilegon City. The sample preparation stage was carried out through extraction using the maceration method. The experimental procedure involved 10 g of dried walang sangit leaves ground to 60 mesh, which were macerated using 99.95% methanol and 80% acetone solutions. The volume of methanol and acetone used was 250 mL, with maceration times of 60 and 120 minutes in an Erlenmeyer flask. At 120 minutes, the methanolic extract yielded 2.81 mg QE/g (young leaves) and 4.12 mg QE/g (mature leaves), whereas the acetone extract only reached 1.03 mg QE/g and 2.88 mg QE/g, respectively. A similar pattern was also observed at 60 minutes, with methanol producing values of 1.39–2.32 mg QE/g, which were higher than those obtained with acetone (0.35–0.78 mg QE/g). The highest extraction rate constant was obtained for acetone–mature leaves (0.0124 min^{-1}) and for methanol–young leaves (0.0058 min^{-1}).



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1. INTRODUCTION

The extract of *walang sangit* leaves can be utilized as an active ingredient to support various industrial applications, particularly in the food industry [1]. This plant has potential as a herbal material [2], however, due to its strong and pungent aroma, it is often regarded as an abundant weed. The characteristic aroma of *walang sangit* leaves is attributed to the presence of a phenylalanine-derived compound, namely 2-acetyl-1-pyrroline [3].

Flavonoids are secondary metabolites belonging to the polyphenol group, commonly found in green plants, and play a significant role as bioactive compounds in medicinal materials [4]. Quantitatively, flavonoids are present at approximately 0.5–5% of the dry leaf weight, depending on the plant species and growth conditions. These compounds exhibit a wide range of biological activities, particularly as antioxidants that scavenge free radicals, as indicated by IC_{50} values ranging from 10 to 100 ppm across various plant extracts. In addition, flavonoids possess anti-inflammatory, anticancer, antidiabetic, and antibacterial activities [1], making them a primary target in phytochemical research.

The yield of flavonoid extraction is strongly influenced by the type of solvent, extraction time, and the condition of the raw material. Polar solvents such as methanol often produce higher total flavonoid yields, typically ranging from 40–70 mg QE/g extract, compared to less polar solvents such as acetone, which generally yield 25–55 mg QE/g, depending on the plant species. Extraction time also has a significant effect; increasing it from 30 minutes to 120 minutes can increase yield by approximately 15–30%. However, excessively long extraction times (>180 minutes) may reduce flavonoid content due to oxidative degradation [5]. In addition, leaf maturity influences flavonoid content. Several studies have reported that young leaves contain 10–25% higher flavonoid levels compared to mature leaves, although the opposite trend may occur in certain species [6].

Flavonoid compounds are generally isolated using extraction methods, most commonly by maceration with polar solvents [7]. As polar compounds, flavonoids readily dissolve in polar solvents such as ethanol, methanol, butanol, and dimethylformamide [8]. Flavonoid extraction from plant materials can be performed using several methods, including Soxhlet extraction, percolation, and maceration. Among these commonly used techniques, maceration is the most widely applied in various types of extraction. Maceration is a process of extracting *walang sangit* leaves with a suitable solvent to obtain the bioactive compounds present in the plant material. The advantages of the maceration method include its low cost, ease of operation, simple equipment requirements, and the absence of heating, which helps prevent the degradation of flavonoid compounds [9].

In previous studies, higher total flavonoid content was obtained using the maceration method. This is attributed to the heating process involved in Soxhlet extraction, which may reduce the total flavonoid content. In contrast, maceration is performed without heating, thereby minimizing the degradation of natural compounds and allowing more bioactive substances to be extracted. However, some compounds may exhibit limited solubility in the extraction solvent at room temperature (27–30 °C) [10]. Furthermore, a study by Hatam et

al. (2013) reported that the total flavonoid content of pineapple peel extract was higher with the Soxhlet method (5.115%) than with maceration (3.514%).

The novelty of this study lies in three main aspects. First, the utilization of walang sangit leaves as a raw material, which has been scarcely explored as a source of flavonoids. Second, the application of extraction methods using two different solvents, methanol and acetone, to evaluate their respective efficiencies in extracting flavonoids. Third, the analysis of differences in flavonoid content based on leaf maturity levels, namely young and mature leaves. Although it is well established that solvent type and leaf maturity can significantly influence flavonoid yield, studies directly comparing the efficiency of methanol and acetone, and specifically examining the effect of leaf maturity in walang sangit leaves, are still limited. This study is expected to provide systematic quantitative data and to determine the optimal extraction conditions for obtaining flavonoids from walang sangit leaves [11].

2. RESEARCH METHODS

2.1. Preparation of Raw Materials

The *walang sangit* leaves were washed using water at approximately 60°C, representing a moderate temperature condition. Subsequently, the leaves were oven-dried at 60 °C for 6 hours, as reported by Hendro et al. (2021) [12]. The dried leaves were then ground using a mixer until a fine powder was obtained and sieved through a 60-mesh sieve to achieve a uniform particle size suitable for the extraction process.

2.2. Raw Material Analysis

Moisture content was analyzed by weighing 2 g of the finely ground sample into a weighing bottle with a previously known initial mass. The sample was then dried in an oven at 100–105°C for 3–6 hours. Subsequent weighings were performed every 30 minutes after the sample had been cooled in a desiccator. This procedure was repeated until a constant weight was obtained, indicated by a difference between consecutive weighings of less than 0.2 mg [13].

2.3. Preparation of Walang Sangit Leaf Extract

This study employed the maceration method as the extraction technique. A total of 10 grams of walang sangit leaf powder was macerated with two solvents: 99.95% methanol and 80% acetone, each at 250 mL. The maceration process was carried out in an Erlenmeyer flask for 60 or 120 minutes, with stirring at 200 rpm using a magnetic stirrer. The resulting mixture was filtered using filter paper, and the filtrate was collected in a round-bottom flask. The filtrate was then concentrated using a rotary evaporator with the water bath temperature adjusted according to the boiling point of the solvent. The concentration process was continued until the extract volume reached approximately 20 mL. The concentrated extract was transferred into a porcelain dish with a known empty weight and subsequently dried in an oven at 50 °C until a dry extract was obtained. The maceration process was performed in duplicate.

2.4. Quantitative Analysis of Flavonoids in the Research Samples

The initial step was to prepare a quercetin standard solution to construct a calibration curve. A total of 15 mg of quercetin was dissolved and diluted to 100 ppm. This stock solution was further diluted to obtain a series of concentrations of 1, 2, 3, 4, 5, 10, 15, and 20 ppm, and their absorbance values were measured at the appropriate wavelength (λ_{max} , determined as the maximum wavelength) [13]. The absorbance data obtained were then used to construct the standard calibration curve for flavonoids. For sample analysis, 50 mg of the extract was weighed and dissolved in 50 mL of 99.9% methanol. A 10 mL aliquot of this solution was transferred into a 50 mL volumetric flask, followed by the addition of 1 mL of 10% AlCl_3 and 1 mL of 1 M potassium acetate, and then diluted to the mark. The absorbance was measured using a UV-Vis spectrophotometer at the appropriate wavelength. The same procedure was applied using 80% acetone as the solvent. The calibration curve results were further subjected to method validation of the spectrophotometric analysis, including the determination of the limit of detection (LOD) and limit of quantitation (LOQ).

2.5. Equipment Setup

The extraction apparatus for compounds from walang sangit leaves (*Eryngium foetidum*), with a particle size of 60 mesh, consists of a bubble-type condenser made of Pyrex borosilicate glass with an effective length of 300 mm; a glass thermometer with a measurement range of 0–300°C (Pyrex); a two-neck flask made of borosilicate glass with a capacity of 250–500 mL (IWAKI); and an electric heater in the form of a hot plate or heating mantle with temperature control up to approximately 350°C (IKA). This apparatus configuration complies with standard biomass extraction procedures [9].

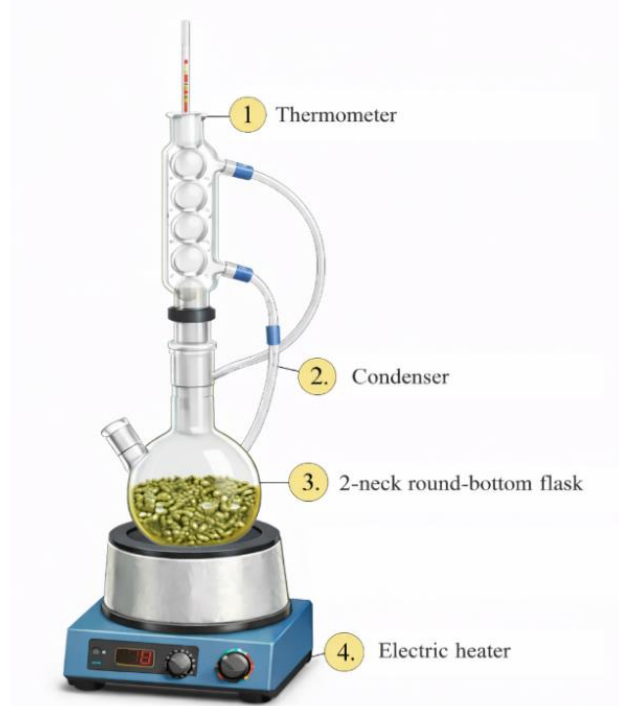


Figure 1. Extraction Apparatus Setup for Walang Sangit (*Eryngium Foetidum*) Leaves

The distillation apparatus for compounds from walang sangit (*Eryngium foetidum*) leaves consists of a double-pipe condenser made of Pyrex; a glass thermometer with a measurement range of 0–300°C (Pyrex); a two-neck flask made of borosilicate glass with a capacity of 250–500 mL (IWAKI); an electric heater in the form of a hot plate or heating mantle with temperature control up to approximately 350 °C (IKA); and a 100 mL graduated cylinder (Pyrex). This apparatus configuration complies with standard biomass distillation procedures [14].

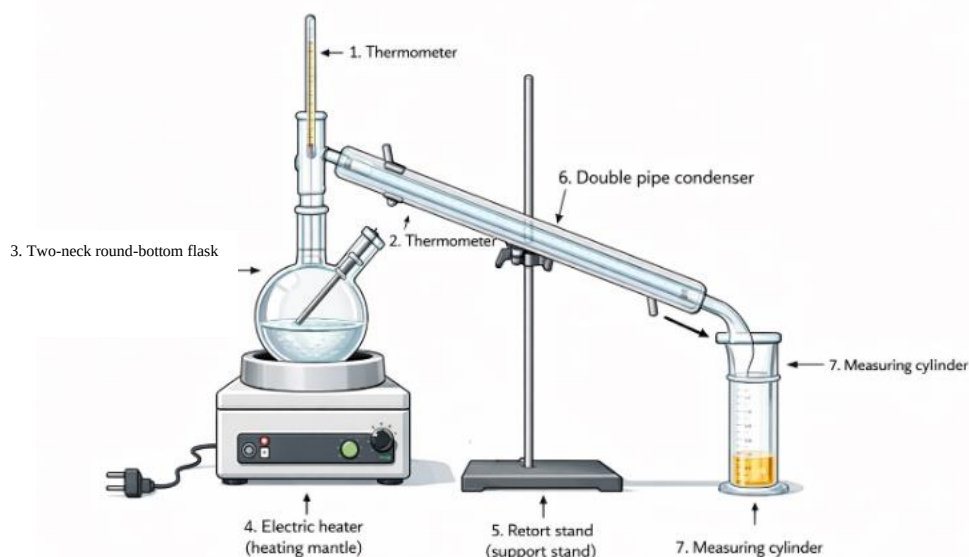


Figure 2. Distillation Apparatus Setup for Walang Sangit (*Eryngium Foetidum*) Leaves

3. RESULTS AND DISCUSSION

3.1. Walang Sangit Leaves Identification

In this study, mature and young walang sangit leaves (*Eryngium foetidum*) were used, obtained from the Pagebangan area, Ciwandan District, Cilegon City. Based on the observations, the mature leaves had an average length of 28.53 cm and a width of 3.22 cm, while the young leaves had an average length of approximately 26.43 cm and a width of 2.86 cm, as shown in Figure 3.1. Drying walang sangit leaves at 60°C for 6 hours was intended to reduce the moisture content without altering the chemical composition of secondary metabolites [15]. The mature leaves experienced a mass reduction from 24.24 g to 23.78 g with a moisture content of 0.02%, while the young leaves experienced a mass reduction from 22.68 g to 22.07 g with a moisture content of 0.03%.

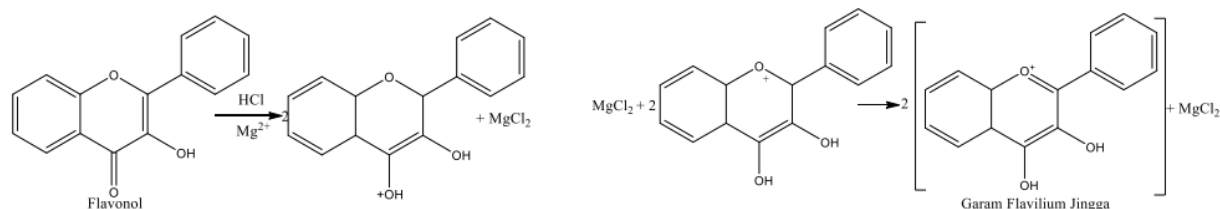


Figure 3. Flavonoid Reaction

Subsequently, grinding to 60 mesh was intended to increase the surface area and disrupt cell walls, thereby allowing the solvent to penetrate the tissue and dissolve the chemical compounds contained in the sample, as shown in Figure 3 [15]. The extraction process was carried out using the maceration method, which aims to extract active chemical components from walang sangit optimally into the solvent [16].

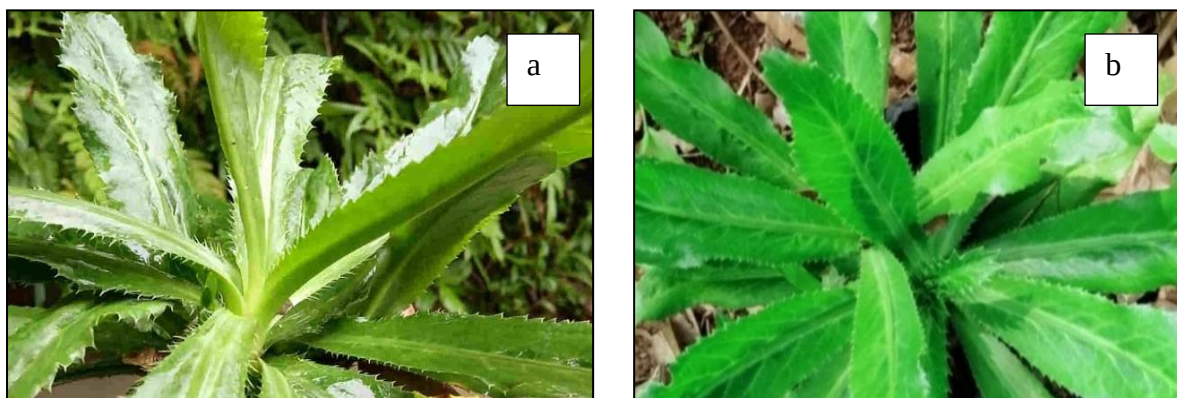


Figure 4. (a) Mature Walang Sangit Leaves (DMS Mature); (b) Young Walang Sangit Leaves (DMS Young)

3.2. Extraction of Walang Sangit Leaves

A total of 10 g of 60-mesh walang sangit leaves, both mature and young, were extracted using the maceration method with methanol and acetone as solvents. The maceration method was selected because it is simple to perform and does not require heating, thereby preventing the chemical compounds contained in the sample from being damaged or degraded. The soaking process applied to the plant samples disrupts cell walls and membranes due to pressure differences between the inside and outside of the cells, allowing the secondary metabolites present in the samples to dissolve into the organic solvent. Acetone is used as a solvent to extract semi-polar compounds, while methanol is used to extract polar compounds. The mechanism is illustrated in Figure 5.

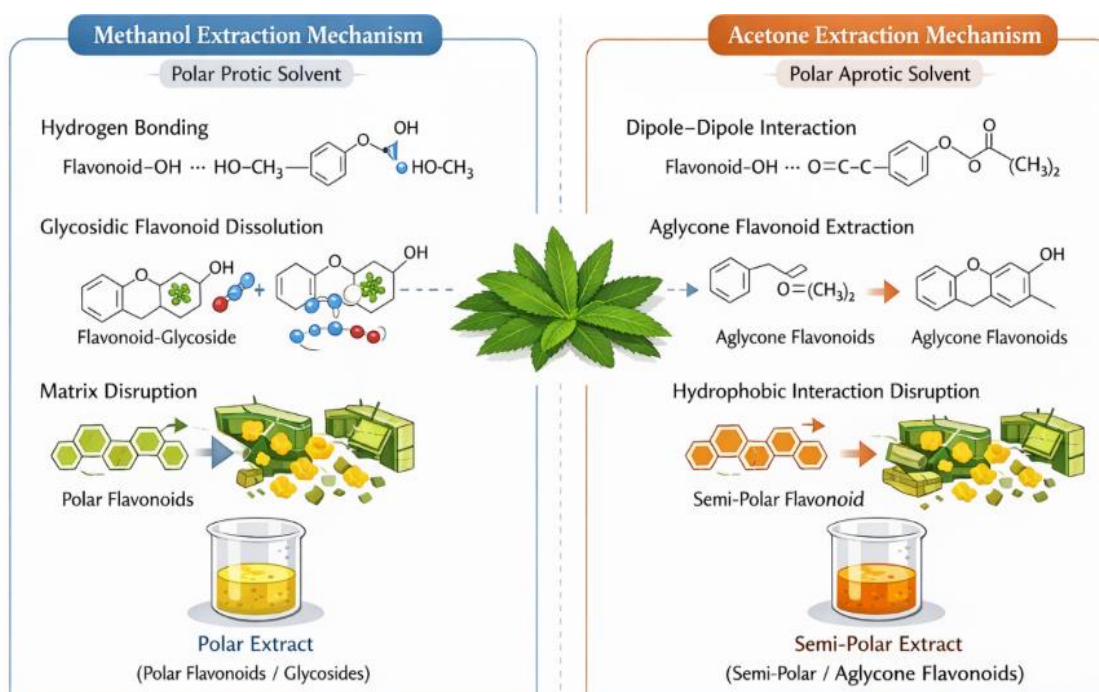


Figure 5. Flavonoid Isolation Mechanism

3.3. Total Flavonoid Content

The maximum wavelength was first determined using a UV–Vis spectrophotometer at 420 nm [2]. A quercetin standard solution was prepared at concentrations of 1, 2, 3, 4, 5, 10, 15, and 20 ppm. The relationship between absorbance and concentration was highly linear, with a correlation coefficient (R^2) of 0.9989. The calibration curve equation obtained was $y = 0.0005x + 0.004$. This equation was used to quantify flavonoid content (quercetin equivalents) in methanol and acetone extracts of mature and young walang sangit leaves [17]. The limit of detection (LOD) was 0.787 and the limit of quantitation (LOQ) was 2.385. The LOD value of 0.787 indicates that the spectrophotometric method used has good sensitivity in detecting low concentrations. Meanwhile, the LOQ value of 2.385 indicates that the quantification limit remains within the calibration curve range, indicating that this method is suitable for quantitative analysis of samples within the prepared concentration range.

Table 1. Quercetin Standard at a Wavelength of 420 nm.

Experiment	Concentration	Absorbance
A1	0	0.000
A2	1	0.006
A3	2	0.011
A4	3	0.017
A5	4	0.020
A6	5	0.027
A7	10	0.053
A8	15	0.084

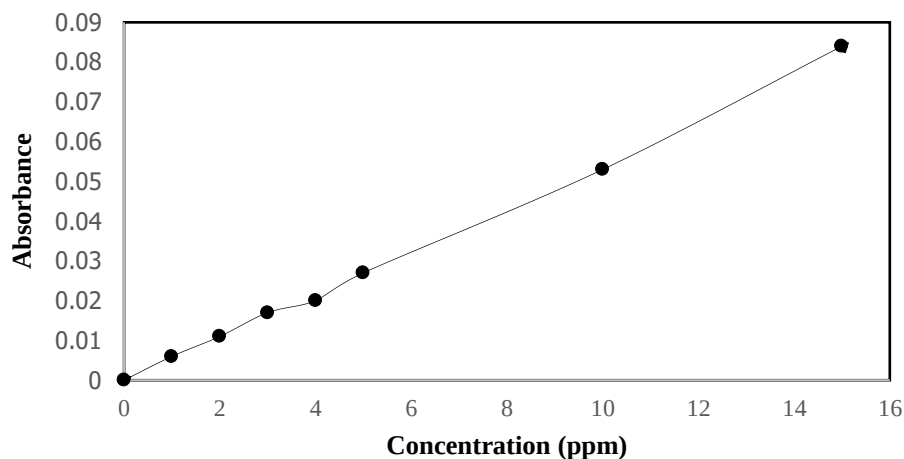


Figure 6. Quercetin Standard at a Wavelength of 420 nm

Table 1. indicates that extending the methanol extraction time from 60 to 120 minutes resulted in increased absorbance values and total flavonoid content in both young and mature walang sangit leaves (DWS). The absorbance values for DWS young and DWS mature rose from 0.143 and 0.236 to 0.285 and 0.416, respectively. Based on Equation 1, the total flavonoid content also increased significantly, from 278 to 562 ppm QE in DWS young and from 464 to 824 ppm QE in DWS mature.

$$\text{Total Flavonoid Content} = \frac{\text{Absorbansi} - 0.004}{0.005} \dots\dots\dots (1)$$

The determination of flavonoid content was performed using the Chang (2002) method, with quercetin as the standard [2]. The formula used is in accordance with Equation 2.

$$\text{Total Flavonoid Content} \left(\frac{\text{mgQE}}{\text{g}} \right) = \frac{C \times V}{m} \dots\dots\dots (2)$$

Description:

C = flavonoid concentration from the calibration curve (mg/L or ppm QE)

V = total extract volume (L)

m = mass of dry sample (g)

The total flavonoid content expressed in mg QE/g of dry sample also increased from 1.39 to 2.81 mg QE/g in DWS young and from 2.32 to 4.12 mg QE/g in DWS mature. Overall, DWS mature exhibited higher flavonoid content compared to DWS young, indicating that both leaf maturity and extraction time significantly influence the efficiency of flavonoid extraction using methanol as the solvent. The flavonoid content of walang sangit leaves is lower than that of mango leaves, where previous studies reported a flavonoid content of 12.280 mg QE/g and a moisture content of 7.66% [18].

Table 2. Total Flavonoid Content Using Methanol Extraction

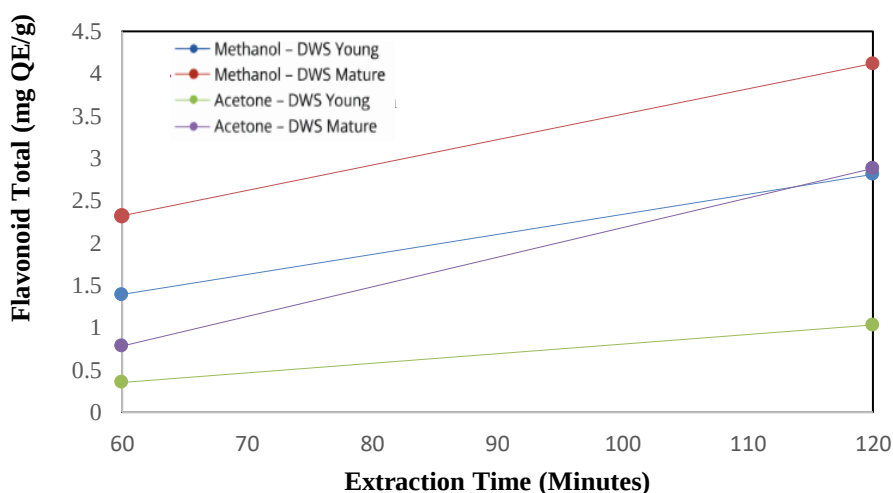
Time (Minutes)	Methanol Method (Absorbance)		Methanol Method (Total Flavonoid Content, ppm QE)		Methanol Method (Total Flavonoid Content, mg QE/g)	
	DWS Young	DWS Mature	DWS Young	DWS Mature	DWS Young	DWS Mature
60	0,143	0,236	278	464	1,39	2,32
120	0,285	0,416	562	824	2,81	4,12

Based on Table 2, the total flavonoid content of walang sangit leaves extracted using acetone increased with longer extraction time. At 60 minutes, the total flavonoid content obtained was 0.35 mg QE/g for DWS young and 0.78 mg QE/g for DWS mature, which then increased to 1.03 mg QE/g and 2.88 mg QE/g at 120 minutes.

DWS mature consistently produced higher flavonoid content compared to DWS young, indicating that leaf maturity influences extraction efficiency. The more fragile tissue structure of mature leaves allows better solvent penetration and more effective flavonoid diffusion. These results confirm that extraction time and raw material maturity are important factors in increasing flavonoid yield when acetone is used as the solvent.

Table 3. Total Flavonoids Using Acetone Extract

Time (Minutes)	Aceton Method (Absorbance)		Aceton Method (Total Flavonoid Content, ppm QE)		Aceton Method (Total Flavonoid Content, mg QE/g)	
	DWS Young	DWS Mature	DWS Young	DWS Mature	DWS Young	DWS Mature
60	0,039	0,107	70	206	0,35	0,78
120	0,082	0,292	156	576	1,03	2,88

**Figure 7. Effect of Extraction Time and Solvent Type on Total Flavonoid Content**

From these measurements, higher concentrations result in higher absorbance values. In quantitative analysis using UV-Vis spectrophotometry, a blank solution is used as a control to zero out or eliminate interference from compounds that are not being analyzed. In the

determination of total flavonoid content, the sample solution is treated with AlCl_3 , which forms a complex that shifts the absorbance toward the visible region. This is indicated by a more intense yellow coloration of the solution. The addition of potassium acetate aims to stabilize the wavelength within the visible region [12].

An incubation period of 1 hour prior to measurement is intended to ensure complete reaction and achieve maximum color intensity [18]. Based on the results of this study, the total flavonoid content of methanol extracts from mature walang sangit leaves is higher than that of young leaves. A similar trend is observed in acetone extracts, where mature leaves also show higher flavonoid content than young leaves, as presented in the table.

The highest flavonoid content was obtained using methanol as the solvent. This is because methanol has a higher polarity compared to acetone. Flavonoids are polar compounds containing hydroxyl groups, often in the form of glycosides with attached sugar moieties, making them more soluble in polar solvents. According to [19], factors influencing total flavonoid content in leaves include morphology and leaf age, which affect the production of secondary metabolites and bioactive compounds [12]. It is also reported that variations in flavonoid content are caused by differences in leaf developmental stages and their positions on the plant.

In this study, the spectrophotometric method was chosen to determine flavonoid content due to its feasibility and reliability. Additionally, this method offers simpler sample preparation and lower operational costs compared to HPLC (High Performance Liquid Chromatography).

3.4. Kinetics

The extraction kinetics of total flavonoids from walang sangit leaves (*Eryngium foetidum*) using methanol and acetone as solvents were analyzed using a first-order kinetic model [19], expressed by the following equation:

The extraction kinetics of total flavonoids from walang sangit leaves (*Eryngium foetidum*) using methanol and acetone solvents were analyzed using a first-order kinetic model [19], based on the following equation:

$$T \ln(C_{\max} - C_t) = -kt \dots\dots\dots (3)$$

$$k = \frac{T \ln(C_{\max} - C_t)}{t} \dots\dots\dots (4)$$

The extraction rate constant (k) values obtained ranged from 0.0058 to 0.0124 min^{-1} , indicating that the extraction process is controlled by the diffusion mechanism of flavonoid compounds from the plant cell matrix into the solvent phase.

The research results indicate that leaf maturity significantly affects the flavonoid extraction rate. For both solvents, mature walang-sangit leaves (DWS mature) exhibited higher k values than young leaves (DWS young). In methanol extraction, the k value increased from 0.0058 min^{-1} in DWS young to 0.0098 min^{-1} in DWS mature. A similar trend was observed in acetone extraction, where the k value increased from 0.0064 min^{-1} to 0.0124

min^{-1} . This increase in extraction rate suggests that the tissue structure of mature leaves is more easily penetrated by solvents due to increased porosity and reduced cell wall strength, thereby accelerating flavonoid release.

Comparison of solvent types shows that acetone provides a higher extraction rate than methanol, particularly in mature leaves. The highest k value was obtained in the acetone–DWS mature combination (0.0124 min^{-1}), indicating that the polarity compatibility of acetone with the dominant flavonoid compounds in mature leaves enhances mass transfer. In contrast, in DWS young, the difference in k values between methanol and acetone is relatively small, suggesting that diffusion limitations due to the still-compact tissue structure are more dominant than solvent type.

Overall, this kinetic analysis confirms that the combination of solvent type and raw material maturity plays a crucial role in determining the efficiency of flavonoid extraction. A first-order kinetic model has been shown to describe the extraction behavior of flavonoids adequately. It can serve as a basis for optimizing extraction conditions for pharmaceutical and functional food applications.

Table 4. Kinetics

Solvent	Sample	$k \text{ (minute}^{-1}\text{)}$
Methanol	DWS Young	0,0058
Methanol	DWS Mature	0,0098
Acetone	DWS Young	0,0064
Acetone	DWS Mature	0,0124

4. CONCLUSION

Extraction with methanol produced a higher total flavonoid content than acetone across all extraction times and leaf maturity levels. At 120 minutes, methanol yielded 2.81 mg QE/g (young leaves) and 4.12 mg QE/g (mature leaves), whereas acetone only produced 1.03 mg QE/g and 2.88 mg QE/g, respectively. A similar pattern was observed at 60 minutes, where methanol (1.39–2.32 mg QE/g) remained higher than acetone (0.35–0.78 mg QE/g). These results indicate that both solvent type and leaf maturity significantly influence extraction efficiency, and they hold potential for further development as natural antioxidant sources and for optimization of extraction methods in future studies.

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