Jurnal Akademika Kimia, 14(2): 112-117, May 2025
ISSN (online) 2477-5185 | ISSN (print) 2302-6030
http://jurnal.fkip.untad.ac.id/index.php/jak/

Comparison of Maceration and Soxhlet Extraction Methods on Flavonoid Contents from Papaya (*Carica papaya*)

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Received 14 April 2025, Revised 16 May 2025, Accepted 23 May 2025
doi: 10.22487/j24775185.2025.v14.i2.pp112-116

Abstract

The papaya plant offers numerous benefits. Each part of the papaya plant has its benefits, starting from the fruit, stem, roots, flowers, and leaves. Livestock Medicine confirmed the presence of at least 22 types of bioactive compounds. The papaya plant contains active compounds that act as antioxidants, including flavonoids. This research aims to compare maceration and Soxhlet extraction methods on the flavonoid content of papaya (Carica papaya) leaf extract as measured using a UV-Vis spectrophotometer. The methods used to separate flavonoid levels are maceration and Soxhlet extraction. Based on the research that has been carried out, it was found that two treatments were carried out in the maceration method: the first treatment was with 70 % ethanol solvent, namely 36.6 mg / 100g or 3.66 %, for 96 % ethanol solvent, namely 77.6 mg / 100g or 7.76 %. Two treatments are also carried out in the Soxhlet method: 70% ethanol (33.6 mg/100g, 3.36%) and 96% ethanol (92.6 mg/100g, 9.26%). These results indicate that Soxhlet extraction is the most effective method for producing flavonoids in papaya leaf extract, as the levels obtained are higher than those obtained by maceration.

Keywords: Carica papaya, flavonoid content, maceration, Soxhletation

Introduction

Papaya is a plant with numerous benefits and can be used as a medicinal plant. This plant offers numerous benefits, including the roots, stems, leaves, flowers, and fruit, as sources of vitamins, minerals, and other compounds for human health and fitness, and for medicinal properties in the health sector (Koul et al., 2022). Papaya contains several compounds beneficial to the body, including vitamins C and E and beta-carotene, which function as antioxidants that neutralize free radicals generated by neutrophil phagocytosis of debris and bacteria during the wound-healing process (Kong et al., 2021).

Papaya leaves contain saponins, which promote collagen formation and support wound healing (Marlinawati et al., 2023). Papain is beneficial as an anti-inflammatory and anti-edematous agent. It contains flavonoids and phenols, which exhibit antiseptic activity by preventing the formation of free radicals and minimizing oxidative damage to tissues. Papaya leaf extract is used to treat stomach aches. Young leaves are used to treat fever, increase appetite, vaginal discharge, and acne; increase milk supply; treat toothache; and, more recently, as a cancer treatment (Singh et al., 2020).

Papaya leaves contain active ingredients in the form of flavonoids, saponins, tannins, phenols, and papain, which act as anti-microbials that can kill and disrupt the growth of Aedes aegypti mosquito larvae (Fajriansyah & Sartika, 2022). Contains active ingredients that can be used as anti-microbials and prevent free radicals, called stomach poisoning or stomach toxins, which can disrupt the digestive system of Aedes aegypti larvae (Rahmaddiansyah et al., 2023). Hence, the larvae fail to develop and eventually die. The active ingredients in papaya leaves can be used as a natural, environmentally friendly larvicide without polluting the environment (Ilham et al., 2019).

The active substance found in papaya leaves can be extracted (Handayani & Azzahra, 2024). Extraction is the separation of materials from a mixture using a suitable solvent. The extraction process is stopped when equilibrium is reached between the concentrations of the compounds in the solvent and the concentrations in plant cells (Zhang et al., 2023). The extraction of bioactive compounds, including flavonoids, is influenced by the extraction method employed. Extraction is classified into two types based on the method used: traditional and modern (Bitwell et al., 2023).

Maceration is an extraction method in which vegetable simplicia are soaked in a specified solvent for a defined period, with occasional stirring or shaking (Abubakar & Haque, 2020). The working principle of maceration depends on the dissolution of the active substance, which occurs according to its solubility in the solvent used (like dissolves like).

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Soxhlet is the process of separating a natural material with an organic solvent using a Soxhlet tool. In general, the Soxhlet method is used to separate fats and vegetable oils (Ulfa et al., 2023).

Research shows that this method is highly effective for separating active substances from plant parts. This is demonstrated by Wijaya et al. (2022), who report that the use of the sokletation method yields a higher yield of turi stem extract than the maceration method. Extraction using the Soxhlet method also produced an 18.69% yield of coffee grounds extract, compared with only 5.62% using maceration (Berghuis & Maulana, 2023). In the past 9 years of research on flavonoid extraction from binjai leaves, the Soxhlet extraction method has consistently yielded the optimal extracts (Nurhasnawati et al., 2017). The next study showed that the total flavonoid content of papaya leaf ethanol extract was approximately 9.41%, with alkaloids more abundant than flavonoids (Alzanando et al., 2022). The advantages of each method must also be considered in terms of their economic viability. Therefore, an appropriate method is needed, regardless of the number of samples produced.

Therefore, this study extracted papaya leaves using ethanol solvent because polar flavonoid compounds are readily soluble in ethanol. Previous research shows that 70-96% ethanol effectively extracts flavonoids from papaya leaves. Flavonoid levels were measured using UV-Vis spectrophotometry, which offers speed, convenience, and specificity.

Methods

The samples used in this research were old papaya leaves in Sunju village, Marawola District, Sigi Regency, Palu City, Central Sulawesi. The following are the tools, materials, and work procedures.

Tool

In this research, the following tools were used: analytical scales, vacuum rotary evaporator, 100 ml measuring cup, dropper pipette, test tube, separating funnel, Soxhlet apparatus, filter paper, 500 ml Erlenmeyer flask, measuring flask, stirring rod, cuvette, and UV-Vis spectrophotometer.

Material

The main ingredients are old papaya leaves (Carica papaya), 70% and 96% ethanol, distilled water, aluminum foil, and tissue; concentrated 2 N HCl; 1 mL of 1.2% aluminum (III) chloride; and 1 mL of 120 M potassium acetate.

Sampling technique

Samples were taken in Sunju village, Marawola subdistrict, Sigi Regency. The old papaya leaves are cut into pieces, rinsed with running water, and air-dried at room temperature. without sunlight for 1 week. Once dry, grind the leaves in a blender to obtain papaya leaf powder.

Extraction process

Old papaya leaves (*Carica papaya L.*): select fresh ones, wash them thoroughly, cut them into pieces, and air-dry them; then blend them into a powder. A total of 50 grams of papaya leaf simplicia powder (*Carica papaya L.*) was weighed and then macerated with 70% and 96% ethanol.

Maceration process

Extraction of old papaya leaf samples was modified by preparing 2 empty Erlenmeyer flasks. Then put 50 grams of simplicia in each Erlenmeyer, then add 70 % ethanol solvent for Erlenmeyer 1 and 96% ethanol for Erlenmeyer 2 with a ratio of 1: 4. Cover the container with aluminum foil, then observe for 1 x 24 hours.

Soxhletation process

The Soxhlet apparatus is installed, and 50 grams of sample is wrapped in filter paper, tied with thread, and inserted into the Soxhlet apparatus. Subsequently, 70% ethanol is added to a 200 mL Soxhlet flask. Perform Soxhlet extraction until the cycle droplets are colorless, or for up to 7 cycles (Rahman et al., 2017). Repeat the steps above using 96% ethanol as the solvent.

Concentration

The extract solution is transferred into a round-bottom flask and connected to a rotary vacuum evaporator. Add distilled water to the water container until the water level is at the normal limit. Turn on the vacuum pump and set the rotary vacuum evaporator to 50 °C, 20 Psi, and 120 rpm. The concentration process was stopped when a thick line appeared at the bottom of the flask, and the solution turned thick orange-yellow. The extract solution was oven-dried at 50 °C to obtain the old papaya leaf extract. Then weigh the concentrated extract.

Determination of yield results

The yield of papaya leaf extract (*load papaya*) is determined used equetion 1.

$$Rendement = \frac{Extract\ weight}{Papaya\ leaf\ weight}\ x\ 100\% \qquad (1)$$

Qualitative test of flavonoid content

Papaya leaf extract was added to a separate test tube in a volume of up to 2 mL. Next, add a small amount of magnesium powder and 3-4 drops of concentrated HCl. The sample was shaken, then left to stand until a color change occurred. If the solution changes to red, yellow, or orange, it indicates the presence of flavonoids (Muthoharoh & Hikmah, 2019).

Quantitative test of flavonoid content

Determination of total flavonoid levels using the colorimetric method using a UV-Vis Spectrophotometer, which refers to a procedure with several modifications with quercetin (QE) as the standard (Wulan et al., 2019).

Determination of maximum wavelength (λ_{max}) to arsetin

Determination of the maximum wavelength of quercetin by running a quercetin solution in the wavelength range 400 - 500 nm (Pulukadang et al., 2024). The results show that the maximum absorbance of the standard quercetin is at 440 nm.

Preparation of quercetin standard solution

Making a standard quercetin solution by modifying several procedures carried out by (Pulukadang et al., 2024), namely weighing 25 mg of quercetin standard, then putting it into a 50 mL measuring flask, adding 96 % ethanol to the limit mark (mother solution 500 mg / L). Then, a series of standard solutions at 0.2, 0.4, 0.6, 0.8, and 1 mg was prepared. Pipette each standard solution into a test tube, then add 1.5 mL of 96% ethanol, 0.5 mL of 10% aluminum chloride ($AlCl_3$), 0.5 mL of 1 M potassium acetate, and 2.8 mL of distilled water. Subsequently, it was incubated at room temperature for 30 minutes. Absorbance was determined using the UV-Vis spectrophotometer method, and then a calibration curve was created.

Measurement of flavonoid levels

The concentrated papaya leaf extract was weighed out at 10 mg using a digital balance. Next, put 50 mL into each Erlenmeyer flask. Then add 10 ml of 70% ethanol to Erlenmeyer 1 and 10 ml of 96% ethanol to Erlenmeyer 2. Then pipette 1 mL of the solution. Then put them in each test tube. Next, 1.5 mL of 96% and 70% ethanol, 10% aluminum chloride ($AlCl_3$) (1 mL), 0.5 mL of 1 M potassium acetate, and 2.8 mL of distilled water were added to each tube. Subsequently, the solution mixture was shaken and then left to stand for 30 minutes. Filter each solution again to produce a filtrate. Next, the resulting filtrate is put into a cuvette. Then, measure the absorption value using a UV-Vis spectrophotometry tool at a wavelength of 440 nm to determine total flavonoids using the colorimetric method (Pulukadang et al., 2024).

Data analysis

Various methods can be used to determine flavonoid levels in herbal samples. The method recognized by the Indonesian Ministry of Health is UV spectrophotometry, which is based on colorimetric principles. The absorbance of the formed color was measured using a UV spectrometer. Total flavonoid levels were calculated as quercetin levels in the samples. This calculation is based on the Lambert-Beer law, which shows a straight relationship between absorbance and analyte content. To determine flavonoid levels in various food types from absorbance measurements, standard solution data are used. This standard solution data is used to create a regression equation, namely the equation used to calculate flavonoid levels in equation 2.

$$y = ax + b \quad (2)$$

Information:
y = absorbance value
x = amount of flavonoids
a,b = constant

Determination of total flavonoid levels using the colorimetric method, which refers to the Chang procedure (Chang et al., 2002; Ahmad et al., 2014), flavonoid content can be calculated using the formula:

$$F = \frac{c \; x \; V \; x \; 100}{m} \quad (3)$$

Information:
F = kadar flavonoid (mg / 100g)
c = equivalence of Quersetin (mg / L)
V = volume (L)
m = sample weight (g)

Results and Discussion

Papaya leaf extraction

Extraction of papaya leaves was carried out using maceration and Soxhlet extraction. 50 grams of sample were macerated with 300 mL of 70% and 96% ethanol. The macerated extract has the characteristics shown in **Table 1.**

Table 1. Yield results of papaya leaf extraction

Method	Solvent	Reagent	Color	information
Maceration	70 %		Orange	Contains Flavonoids
	96 %	Mg + metal	Dark red	Contains Flavonoids
Sokletasi	70 %	Concentrated HCl	Dark red	Contains Flavonoids
	96 %		Dark Red	Contains Flavonoids

Qualitative test of flavonoid compounds extracted using maceration and Soxhlet methods

Quantitative testing of flavonoid compounds was carried out using a UV-Vis Spectrophotometer, shown in **Table 2**, which aims to determine how much flavonoid content is contained in old papaya (Carica papaya L.) leaf extract. The working principle of the UV-Vis Spectrophotometer is that radiation in the 400-500 nm range interacts with the material, specifically with molecules (Pulukadang et al., 2024)

Quantitative test of flavonoid compounds

Measurement of flavonoid levels in old papaya leaves was at a maximum wavelength of 440 nm. Flavonoids were calculated using a linear regression equation from a previously measured quercetin calibration curve. The calibration curve is created based on the Lambert-Beer law, namely the linear equation y = ax + b. Where y is the absorbance value, x is the sample concentration, a is a constant, and b is the slope of the line. From the measurements, the straight-line equation is obtained: y = 0.1x – 0.0074, with a correlation coefficient (R^2) = 0.9937. Because the correlation coefficient (R^2) ≤ 1, the calibration curve obtained is linear. The results for the flavonoid content of papaya leaves are presented in **Table 3.**

Table 2. Qualitative test results for flavonoid compounds

Method	Solvent (%)	Sample Weight (g)	Concentrated Extract	% Rendeman	Color
Maceration	70	50	13.42	26.84	Orange
	96	50	15.82	31.64	Dark red
Sokletasi	70	50	23.50	47	Dark red
	96	50	33.06	66.12	Dark red

Table 3. Results of analysis of flavonoid levels of papaya leaf extract

Sample	Treatment	Absorbance	Flavonoid Concentration (mg/L)	Kadar Flavonoid (mg/100g)	How much Flavonoid (%)
Extract papaya leaves using a maceration process	1 (70% ethanol)	0.044	0.366	36.6	3.66
	2 (96% ethanol)	0.085	0.776	77.6	7.76
Extract papaya leaves by soxhletation process	1 (70% ethanol)	0.041	0.336	33.6	3.36
	2 (96% ethanol)	0.1	0.926	92.6	9.26

Quercetin is used as a standard because it is a flavonoid in the flavonol group, with a keto group at C-4 and hydroxyl groups at C-3 and C-5, which are adjacent to the C-4 keto group in neighboring flavones and flavonols (Frenț et al., 2024). Quercetin is also a flavonoid compound that can react with $AlCl_3$ form a complex. The addition of potassium acetate aims to maintain wavelengths in the visible region (Aminah et al., 2017).

The treatment was carried out twice: the first used papaya leaf extract prepared by maceration with 70% ethanol; the results were 36.6 mg/100g (3.66%). For 96% ethanol, the results were 77.6 mg/100g (7.76%). Two treatments are also carried out in the Soxhlet method: 70% ethanol (33.6 mg/100g, 3.36%) and 96% ethanol (92.6 mg/100g, 9.26%). These results indicate that Soxhlet extraction is a more effective method for determining flavonoid levels, as the levels obtained are higher than those obtained by maceration. This is consistent with the findings of research by Angraini et al. (2023). The analysis showed that the total flavonoid content obtained by Soxhlet extraction was higher than that obtained by maceration. Soxhlet extraction is a technique in which the sample is heated and soaked in a solvent. This process ruptures the cell wall and membrane due to the pressure difference between the inside and outside of the cell, thereby allowing secondary metabolites in the cytoplasm to dissolve in organic solvents. Additionally, repeated circulation during extraction yields a higher-quality extract. The Soxhlet method uses a higher temperature compared to maceration. Flavonoids are compounds that are stable at approximately 70 °C. Increasing temperature can increase flavonoid levels in the extract. Recent research indicates that flavonoids, particularly flavone derivatives, exhibit anti-inflammatory activity, including inhibition of arachidonic acid metabolism.

These results also show that higher ethanol concentrations increase the yield of papaya leaf extract. The high yield obtained from papaya fruit extract using 96 % ethanol as the extraction solvent indicates that this solvent effectively extracts compounds because its polarity is close to that of the compounds in papaya leaves. This is because the higher the solvent concentration, the greater the level that can be extracted (Puspitaningtyas et al., 2021)

Conclusions

Based on the research that has been carried out, it was found that the most effective method for producing flavonoid levels in papaya leaf extract is Soxhlet extraction, as the levels obtained are higher than those obtained by maceration. This is because the Soxhlet extraction method employs heating, thereby allowing the solvent to extract more flavonoid compounds from the simplicia.

Conflict of Interest

There is no conflict of interest disclosed by the writers.

Acknowledgment

The authors would like to sincerely thank everyone who helped to finish this study. We would especially want to thank the FKIP UNTAD Laboratory for support and help with sample preparation and analysis.

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