

TOTAL FLAVONOID CONTENT OF KAPUL FRUIT PEEL EXTRACT (*Baccaurea macrocarpa*) BASED ON SOLVENT TYPE VARIATION USING UV-VIS SPECTROPHOTOMETRY

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Abstract The solvent type plays a crucial role in influencing the extraction of active compounds from natural materials. The peel of kapul fruit (*Baccaurea macrocarpa*) is known to contain flavonoid compounds with notable antibacterial properties. This study aimed to quantify the total flavonoid content in kapul fruit peel extract by evaluating the effects of different solvent types using the UV-Vis spectrophotometric method. The analysis revealed the presence of secondary metabolites, including alkaloids, saponins, and flavonoids. The total flavonoid content of the kapul fruit peel extract varied significantly across different solvents: 4.7238 ± 0.5807 mgQE/g for ethanol, 2.9699 ± 0.1539 mgQE/g for ethyl acetate, and 2.0417 ± 0.4236 mgQE/g for n-hexane. Statistical analysis demonstrated a significant difference in flavonoid content among the three solvent extracts. These results highlight the importance of solvent selection in maximizing the extraction efficiency of flavonoids from kapul fruit peel, which may have implications for its potential therapeutic applications.

Keywords: *Baccaurea macrocarpa* • flavonoids • solvents • UV-Vis spectrophotometry



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Introduction

West Kutai is a district in East Kalimantan known for its diverse genetic resources (SDGs), particularly local fruits. One such fruit is the Kapul fruit, also known as tampoi (*Baccaurea macrocarpa*), which is widely consumed by the local community. It is commonly found in the inland and forest areas of West Kutai, its natural habitat (Hikmah and Gunawan, 2022).

The local population uses Kapul fruit in traditional medicine to address various health issues, such as aiding menstruation, alleviating abdominal pain, treating arthritis and constipation, and combating degenerative diseases like cancer, diabetes, and hypertension. It is also believed to boost immunity due to its antioxidant properties. The peel of the tampoi fruit contains flavonoid compounds, which have demonstrated antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* (Kimberi et al., 2022).

Flavonoids are found in plants in the form of glycosides that bind to a sugar, making them polar. Flavonoids are a group of secondary metabolites belonging to the polyphenol group, which are widely distributed in plant tissues. These compounds are commonly extracted using polar and semi-polar solvents such as water, methanol, ethanol, and ethyl acetate (Nyoman et al., 2019; Riyanto., 2022). The polarity of the solvent can affect the content of the compounds extracted, based on the principle of "like dissolves like." In this principle, polar compounds are soluble in polar solvents, while non-polar compounds are soluble in non-polar solvents (Arifianti et al., 2014). Ahdyani et al. (2022) reported the flavonoid content of *Baccaurea lanceolata* (limpasu) skin extract,

with n-hexane solvent yielding 44.1552 µg/ml, ethyl acetate 155.8506 µg/ml, and methanol 25.8506 µg/ml. This study used a different species than our research. Total flavonoid contents can be determined using the UV-Vis spectrophotometry method, as flavonoid compounds contain conjugated groups that enable them to absorb light in the UV-Vis region (Riwanti et al., 2020). The method for determining flavonoid contents is based on colorimetry using $AlCl_3$ reagents. The principle behind determining flavonoid contents with aluminum chloride involves the formation of aluminum chloride complexes with keto groups on C-4 atoms and hydroxy groups on C-3 or C-5 atoms, which are adjacent to the flavone and flavonol groups (Azizah et al., 2014).

Since *Baccaurea lanceolata* and *Baccaurea macrocarpa* belong to the same genus, it is highly likely that *Baccaurea macrocarpa* also contains significant amounts of flavonoids. To date, there is a lack of studies exploring the use of various solvent types in the extraction of flavonoids from this species. Therefore, this research aims to investigate the total flavonoid content in *Baccaurea macrocarpa* using different solvent types.

Materials and Methods

Glassware (Pyrex®), 40-mesh sifter, macerator, analytical balance (Ohaus®), oven (Mettler®), rotary evaporator, UV-Vis spectrophotometer (Shimadzu 1800®), water bath, Kapul fruit peel, distilled water, aluminum chloride, amyl alcohol, hydrochloric acid, ethanol, n-hexane, ethyl acetate, potassium acetate, quinine, sodium bicarbonate, and magnesium powder

Simplicia Processing

The processing of simplicia begins with the collection of Kapul fruits, followed by wet sorting, where the fruit peel is separated from the pulp. The peel is then weighed, typically at 1 kg. Subsequently, the peel is washed with clean water, drained, and allowed to dry. The fruit peel is then cut into small pieces and sun-dried under a black cloth. Once dried, the simplicia is finely ground and sieved through a 40-mesh sieve to obtain a moderately coarse powder (Ahdyani et al., 2022).

Determination of Drying Shrinkage

Weigh 1 g of simplicia into a porcelain crucible that has been preheated to a set temperature and previously measured. The material is flattened in the porcelain crucible by gently shaking it, then placed in an oven for 60 minutes at 105°C with the bottle cap left open. Afterward, the crucible is placed in a desiccator for 15 minutes (Kemenkes RI, 2017). Replication was carried out three times. The percentage of drying shrinkage is calculated using the following formula (Depkes RI, 2000).

$$\text{Drying shrinkage} = \frac{b - (c - a)}{b} \times 100\%$$

Extraction

Weigh 100 g of simplicia and place it into 3 separate glass vessels. Add 1000 ml of 95% ethanol, ethyl acetate, and n-hexane to each vessel. Stir the simplicia continuously for the first 2 hours, then allow it to stand for 22 hours before filtering. The fruit peel pulp is then re-extracted twice, each time with 500 ml of 95% ethanol, 500 ml of ethyl acetate, and 500 ml of n-hexane. The combined macerates are evaporated using a rotary evaporator, followed by concentration over a water bath until a thick extract is obtained (Supriningrum et al., 2019). The extract is weighed, and the yield is calculated using the formula provided by (Kemenkes RI, 2017).

$$\text{Rendemen}(\%) = \frac{\text{ekstrak weight}}{\text{simplicia weight}} \times 100\%$$

Identification of Flavonoid Compound

Each extract was accurately weighed to 0.5 g and dissolved in 20 ml of distilled water (aquades), then heated and filtered. A 1 ml aliquot of the filtrate was taken, followed by the addition of a small amount of magnesium powder, 2 ml of amyl alcohol, and 1 ml of concentrated hydrochloric acid (HCl). The mixture was then shaken. If a yellow, orange, or red coloration develops in the amyl alcohol layer, the extract is considered positive for the presence of flavonoid compounds (Supriningrum et al., 2019).

Determination of Flavonoid Content

The procedure for determining flavonoid content by UV-Vis spectrophotometry follows the method described by Syamsul et al. (2019) and includes the following stages:

- a. Preparation of 400 ppm quercetin stock solution. Weigh 10 mg of quercetin and dissolve it in 95% ethanol. Transfer the solution to a 100 mL volumetric flask and fill it to the mark with 95% ethanol. This yields a stock solution with a concentration of 400 ppm.
- b. Preparation of quercetin standard series solutions. Pipette 0.625, 1.5, 1.875, 2.5, and 3.125 mL of the stock solution into five 10 mL volumetric flasks. Dilute each solution with 95% ethanol to the mark, resulting in standard solutions with concentrations of 25, 50, 75, 100, and 125 ppm.
- c. Preparation of blank solution, the blank solution consists of 4 mL of 95% ethanol, 0.2 mL of potassium acetate, 0.2 mL of 10% aluminum chloride, and 5.6 mL of distilled water. Combine these in a 10 mL volumetric flask and dilute to the mark with distilled water. Shake until homogeneous.
- d. Measurement of maximum absorption wavelength. Pipette 1 mL of the 75-ppm standard solution into a 10 mL volumetric flask. Add 3 mL of 95% ethanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of potassium acetate, and 5.6 mL of distilled water. Shake until homogeneous. Measure the absorption of the solution using a UV-Vis spectrophotometer in the wavelength range of 350–550 nm.
- e. Calibration curve creation, using the maximum absorption wavelength obtained, prepare a calibration curve by pipetting 1 mL of each standard solution (25, 50, 75, 100, and 125 ppm) into separate 10 mL volumetric flasks. Add 3 mL of 95% ethanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 M

potassium acetate, and 5.6 mL of distilled water to each flask. Shake until homogeneous and incubate the solutions at room temperature for 30 minutes. Measure the absorption at the maximum wavelength using a UV-Vis spectrophotometer.

- f. Determination of flavonoid content in extracts. To prepare the sample solution, weigh 25 mg of kapul fruit peel extract and dissolve it in 5 mL of 95% ethanol in a chemical glass. Transfer the solution to a 25 mL volumetric flask and dilute to the mark with ethanol, resulting in a concentration of 1000 ppm. Pipette 1 mL of the sample solution into a 10 mL volumetric flask. Add 3 mL of 95% ethanol, 0.2 mL of 10% aluminum chloride, 0.2 mL of 1 M potassium acetate, and 5.6 mL of distilled water. Shake the mixture until homogeneous and incubate at room temperature for 30 minutes. Measure the absorption at the maximum wavelength using a UV-Vis spectrophotometer. Perform the procedure in triplicate.

Results and Discussion

Determination of Drying Shrinkage

The determination of drying shrinkage involves measuring the residual mass after drying at 105°C for 60 minutes or until a constant weight is achieved. The drying shrinkage is expressed as a percentage. The purpose of determining the drying shrinkage of simplicia is to establish the maximum allowable moisture content in the kapul fruit peel, which was found to contain 9.96% water. This moisture content complies with the required standard for simplicia, which should be $\leq 10\%$ (Kemenkes RI, 2017). The results of the drying shrinkage determination are shown in Table 1.

Table 1. Shrinkage of Kapul Fruit Peel Drying

No.	Drying Shrinkage (%)	Average (%)
1	10	9,66
2	8,91	
3	10,07	

Extraction

The viscous extract obtained from each solvent was used to calculate the yield value. The extract yield is determined by the ratio of the final weight (the weight of the extract produced) to the initial weight (the weight of the simplicia used), multiplied by 100% (Sani et al., 2014). The yield value is also indicative of the amount of bioactive content in the kapul fruit peel extract. Based on the yield values, the 95% ethanol extract demonstrated a higher yield compared to ethyl acetate and n-hexane. This suggests that the extract contains a higher proportion of polar compounds, as the highest yield was obtained with 95% ethanol solvent. The results of the yield calculation are presented in Table 2.

Table 2. Extract Yield

Extract	Weight of extract (g)	Yield (%)
Ethanol	7,93	7,93
Ethyl acetate	1,5	1,5
n-hexane	0,53	0,53

Flavonoid Extract Identification

The identification of flavonoid compounds in the extract is essential to verify the presence of flavonoids in the kapul fruit peel extract. The identification results confirmed that all three extracts, derived from different solvents,

contained flavonoid compounds. This finding is consistent with the study by Yunus et al. (2014), which reported that the methanol, ethyl acetate, and n-hexane fractions of *Baccaurea macrocarpa* fruit peel extract also tested positive for flavonoids.

Table 3. Results of Flavonoid Identification of Kapul Fruit Peel Extract

Extract	Compound	Result
Ethanol		Formed orange color (+)
Etil aseat	Flavonoid	Formed orange color (+)
n-Hexane		Formed orange color (+)

Determination of Flavonoid Content by Spectrophotometry

Flavonoid compounds are natural phenolic compounds known for their antioxidant potential (Selawa, 2013; Pardede et al., 2016). These compounds contain conjugated aromatic groups that exhibit strong absorption in spectrophotometry (Rais, 2015). To determine the flavonoid content, the kapul fruit peel extract, obtained using 95% ethanol, ethyl acetate, and n-hexane solvents, was reacted with aluminum chloride (AlCl_3), which functions as a complexing agent. Sodium acetate was added to stabilize the solution within the visible region (Kumalasari et al., 2023). A series of quercetin

solutions were prepared in concentrations of 25 ppm, 50 ppm, 75 ppm, 100 ppm, and 125 ppm, in accordance with (Kemenkes RI, 2017). A blank solution was prepared using 95% ethanol, 10% AlCl_3 , 1 M sodium acetate, and distilled water. The wavelength determination was performed in the range of 350–550 nm, and a maximum wavelength of 440 nm was obtained. The absorbance of each quercetin standard solution was measured, and a calibration curve was constructed. The linear equation obtained was $y = 0.00130x - 0.00271$, with a correlation coefficient (r^2) of 0.99762, indicating a very strong correlation between absorbance and concentration (Mewar et al., 2023). The standard curve is shown in Figure 1.

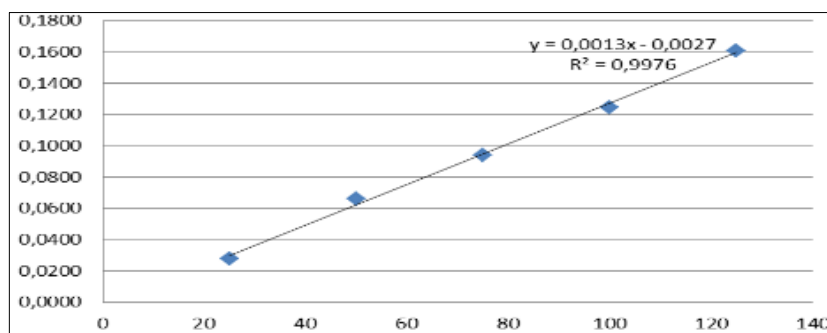


Figure 1. Quersetin standard curve (x: concentration of quercetin, y: absorbance)

The determination of total flavonoid contents was performed in triplicate for each extract sample to ensure data accuracy. The flavonoid content in the extract samples of 95% ethanol, ethyl acetate, and n-hexane was obtained by referencing the

absorbance values on the quercetin standard curve, using the equation $y = 0.0013x - 0.0027$. The results of the total flavonoid content for the 95% ethanol extract, ethyl acetate, and n-hexane extracts are presented in Table 4.

Table 4. Total Flavonoid content of Extract Kapul Fruit Peel

Extract	Up to flavonoids (mgQE/g)	Average contents of flavonoids (mgQE/g)
Ethanol	4,7853	4,7328 ± 0,5807
	4,6700	
	4,7161	
	2,9700	
Ethyl acetate	2,9546	2,9699 ± 0,1539
	2,9853	
	1,9930	
	2,0623	
n-Hexane	2,0700	2,0417 ± 0,4236

Table 4 shows that the extract with 95% ethanol solvent provides the highest average flavonoid content, which is 4.7238 ± 0.5807 mgQE/g, compared to the ethyl acetate extract (2.9699 ± 0.1539 mgQE/g) and n-hexane extract (2.0417 ± 0.4236 mgQE/g). A study by [Ahdyani et al. \(2022\)](#) on the peel of limpasu fruit (*Baccaurea lanceolata*) found that the flavonoid content of extracts with methanol, ethyl acetate, and n-hexane solvents were $44.1552 \mu\text{g/ml}$, $155.8506 \mu\text{g/ml}$, and $25.8506 \mu\text{g/ml}$, respectively. This study indicated that the ethyl acetate solvent yielded the highest flavonoid content. However, in this study, the kapul fruit peel extract (*Baccaurea macrocarpa*) with 95% ethanol solvent had the highest flavonoid content. This

suggests that the flavonoid compounds in kapul fruit peel are polar in nature. Differences in plant species and extraction solvents can significantly influence the type and content of bioactive compounds in natural materials.

The flavonoid content was statistically analyzed using the One-Way ANOVA test. The results showed significant differences among the three solvent concentrations, with a p-value of $0.000 < 0.05$. Subsequently, a Tukey test was conducted, confirming that the flavonoid content of the kapul fruit peel extract with the three solvent variations (95% ethanol, ethyl acetate, and n-hexane) also showed significant differences, all with p-values of $0.000 < 0.05$.

Conclusion

The total flavonoid content of kapul fruit peel extract (*Baccaurea macrocarpa*) was determined using three different solvents: ethanol, ethyl acetate, and n-hexane. The results showed that the ethanol extract exhibited the highest flavonoid content, with 4.7238 ± 0.5807 mgQE/g, followed by the ethyl acetate extract (2.9699 ± 0.1539 mgQE/g) and the n-hexane extract (2.0417 ± 0.4236 mgQE/g). Statistical analysis using One-

Way ANOVA revealed a significant difference in the flavonoid content among the three extracts ($p < 0.05$), indicating that the solvent type significantly influences the flavonoid yield. These findings suggest that ethanol is the most effective solvent for extracting flavonoids from kapul fruit peel.

Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

References

- Ahdyani. R. , Zamzani. I., Latifah .N. , Fatmasari.E. (2022). Determination of Total Flavonoid Contents of Laxative Fruit Peel Extract (*Baccaurea lanceolata*) by Uv-Vis Spectrophotometry Method. *JOPS (Journal Of Pharmacy and Science)* 6(1), 65-71
- Arifianti L, Oktarina RD, Kusumawati I. (2014). Effect of Extracting Solvent Type on Synensetin Contents in *Orthosiphon stamineus* Benth Leaf Extract. *J Planta Husada*. 2(1):3–6.
- Azizah DN, Kumolowati E, Faramayuda F. (2014) .Determination of Flavonoid Contents by $AlCl_3$ Method in Methanol Extract of Cocoa Pods (*Theobroma cacao* L.). *Kartika Jurnal Ilmu Farmasi*. 2(2):45–9.
- Depkes RI. (2000). *Parameter Standard Umum Ekstrak Tumbuhan Obat*. Jakarta : Depkes RI.
- Hikmah N, Gunawan G. (2022). Kapul Ethnobotany (*Baccaurea macrocarpa*) Limpasu (*Baccaurea lanceolata*) by the Dayak tribe and the Banjar tribe in South Hulu Sungai and Central Hulu Sungai Regencies. *Bioscientiae* 19(2).
- Kimberi, Regita E, Dianursanti, Arbianti, Rita, Apriliana CK. (2022). Optimization of the Process of Extraction of Flavonoid Compounds from Tampoi Fruit Peel (*Baccaurea macrocarpa*) as an Antibacterial Agent Using the Percolation Method. *Thesis*.
- Kemenkes RI. (2017). *Farmakope Herbal Jilid II*. Jakarta : Kemenkes RI.
- Mewar D, Sitti Hadijah, Nadila Bandian. (2023). Determination of Flavonoid Contents of Ethanol Extract of Itchy Leaves (*Laportea decumana* (Roxb.) Wedd) using the UV-Vis Spectrophotometry Method. *Indones J Pharm Nat Prod.*; 6(02):161–6.
- Nyoman C, M Permana, A Jambe. 2015. Effect of Solvent Type on Total Flavonoid Content and Antioxidant Activity of Matoa Leaf Extract (*Pometia pinnata*). *J Pharm Care Anwar Med*. 2(2):84–96.
- Pardede, A., Mashita, K., Ninomiya, M., Tanaka, K., & Koketsu, M. (2016). Flavonoid profile and antileukemic activity of *Coreopsis lanceolata* flowers. *Bioorganic & Medicinal Chemistry Letters*, 26(12), 2784-2787.
- Rais, I. R. (2015). Isolation and Determination of Flavonoid Contents of Sambiloto Herbal Ethanol Extract (*Andrographis paniculata* (Burm.F.) NESS). *Thesis*. Faculty of Pharmacy, Ahmad Dahlan University. Yogyakarta. 5(1): 101



- Riwanti P, Izazih F, Amaliyah A. (2020). Effect of differences in ethanol concentration on total flavonoid contents of 50, 70 and 96% ethanol extracts of *Sargassum polycystum* from Madura. *J Pharm Care Anwar Med.* 2(2):82–95.
- Riyanto A. 2022. Effect of Addition of Porang Tuber Flour and Variation in Pineapple Peel Flavonoid Concentration in the Making of Biofoam. *J Ilm Fis.* 19(2):101–11.
- Sani, R.N., Fithri C.N., Ria D.A., and Jaya M.M. (2014). Yield Analysis and Phytochemical Screening of Ethanol Extract of Marine Microalgae *Tetraselmis chuii*. *Journal of Food and Agroindustry.* 2(2):121-126.
- Selawa, W., Max, R.J.R., Gayatri, C. (2013). Flavonoid Content and Total Antioxidant Capacity of Binahong Leaf Ethanol Extract (*Annedera cordifolia* (Ten.) Steenis.). *Pharmacon* 2(1). Unsrat
- Supriningrum R, Fatimah N, Purwanti YE. Specific and non-specific characterization of Putat leaf ethanol extract (*Planchonia valida*). *Al Ulum J Science and Technology.* 2019; 5(1):6.
- Syamsul ES, Judge YY, Nurhasnawati H. (2019). Determination of Flavonoid Contents of Kelakai Leaf Extract (*Stenochlaena Palustris* (Burm. F.) Bedd.) By UV-VIS spectrophotometry method. *Indonesian Journal of Pharmaceutical Research.* 1(1):11-20.
- Yunus R, Hairil Alimuddin A, Ardiningsih P, Hadari Nawawi JH. (2014). Antibacterial Activity Test of Tampoi Fruit Peel Extract (*Baccaurea macrocarpa*) against *Escherichia coli* and *Staphylococcus aureus* bacteria. *Jurnal Kimia Khatulistiwa.*; 3(3):19–24.