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Validation of Vitamin C Analysis Method and Evaluation of Antioxidant Activity of Various Plant Extracts From South Kalimantan Using UV-Vis Spectrophotometer

Validasi Metode Analisis Vitamin C dan Evaluasi Aktivitas Antioksidan dari Berbagai Ekstrak Tanaman Asal Kalimantan Selatan Menggunakan Spektrofotometer UV-Vis

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Abstrak

Spektrofotometer UV-Vis adalah salah satu instrumen yang sering digunakan dalam analisis sampel untuk praktikum, penelitian maupun pengabdian pada masyarakat. Metode analisis sampel yang digunakan dalam kegiatan-kegiatan tersebut harus menggunakan metode analisis yang sudah divalidasi agar hasil analisis yang diperoleh bisa akurat, tepat, dan dapat dipertanggungjawabkan. Oleh karena itu, salah satu tujuan penelitian ini adalah melakukan validasi metode analisis vitamin C untuk linieritas, akurasi (% recovery), presisi (% RSD), batas deteksi (LOD) dan batas kuantitasi (LOQ), serta analisis kandungan vitamin C pada buah pampakin (Durio kutejensis Hassk (Becc.)) dan kalangkala (Litsea garciae Vidal) menggunakan metode analisis yang sudah divalidasi tersebut. Linieritas suatu metode diukur berdasarkan hubungan antara konsentrasi (sumbu x) dan respon (sumbu y). % recovery dilakukan dengan cara menentukan kadar vitamin C dalam sampel, selanjutnya dilakukan penentuan kadar vitamin C dalam sampel setelah penambahan larutan standar yang jumlahnya diketahui. Penentuan % RSD dilakukan dengan cara standar deviasi (SD) dibagi rata-rata kadar (µg/mL) dikalikan 100%. Penentuan LOD dilakukan dengan cara standar deviasi dibagi dengan nilai slope ($LOD = \frac{3\ x\ SD}{Slope}$). *Sedangkan, penentuan LOQ dilakukan dengan rumus* $LOQ = \frac{10\ x\ SD}{Slope}$. *Sementara itu, Kalimantan Selatan memiliki banyak tanaman yang berpotensi sebagai tanaman obat diantaranya pampakin dan kalangkala. Oleh karena itu, tujuan lain dari penelitian ini adalah mengevaluasi aktivitas antioksidan dengan metode DPPH dari ekstrak - ekstrak kedua tanaman ini yang dianalisis menggunakan Spektrofotometer UV-Vis. Hasil validasi metode analisis vitamin C yang diperoleh untuk linieritas, batas deteksi, batas kuantitasi, akurasi dan presisi berturut-turut adalah 0,999; 0,50 ppm; 5,02 ppm; 99,45-101,48%; dan 0,03-1,33%. Buah kalangkala dan pampakin mengandung vitamin C berturut-turut 0,24 dan 0,13 mg dalam 1,0 g sampel. Uji aktivitas antioksidan menunjukkan bahwa ekstrak daun kalangkala yang menghasilkan antioksidan yang baik adalah pelarut dengan urutan: metanol (IC50=23,03 µg/mL) > etilasetat (IC50= 27,16 µg/mL) > metilenklorida (IC50= 27,45 µg/mL) > n-heksana (IC50= 32,01 µg/mL). Daun pampakin, buah kalangkala, dan buah pampakin menunjukkan aktivitas antioksidan paling besar pada ekstrak metanol dengan IC50 berturut-turut adalah 50,29; 48,70; dan 45,30 µg/mL. Berdasarkan hasil penelitian ini, metode analisis vitamin C menggunakan spektrofotometer UV-Vis memenuhi syarat validasi dan adanya aktivitas antioksidan dari buah pampakin dan kalangkala pada radikal bebas DPPH kemungkinan karena vitamin C yang terkandung pada*

kedua buah tersebut. Sementara itu, ekstrak kedua tanaman yang diperoleh dari metanol menunjukkan aktivitas antioksidan paling tinggi dari pelarut lainnya seperti n-heksana, metilenklorida, dan etilasetat.

Kata Kunci: Antioksidan, *Durio kutejensis* Hassk (Becc), *Litsea garciae* Vidal, validasi metode.

Abstract

The UV-Vis spectrophotometer is commonly utilized for sample analysis in practicums, research, and community service. The analysis method employed in these activities must utilize a proven analytical technique to ensure the accuracy, precision, and accountability of the produced results. Hence, one of the aims of this study was to verify the vitamin C analysis technique for its linearity, accuracy (% recovery), precision (% RSD), limit of detection (LOD), and limit of quantitation (LOQ). Additionally, the validated analytical method will be employed to analyze the vitamin C content in pempaken fruit (Durio kutejensis Hassk (Becc.)) and kalangkala fruit (Litsea garciae Vidal). The linearity of a method was assessed by examining the correlation between the concentration (x-axis) and the response (y-axis). Percentage recovery was determined by quantifying the concentration of vitamin C in the sample and subsequently quantifying the concentration of vitamin C in the sample following the addition of a known quantity of a standard solution. The calculation of % RSD involves dividing the standard deviation (SD) by the average level (µg/mL) and then multiplying the result by 100%. LOD was determined by dividing the standard deviation by the slope value ($LOD = \frac{3\ x\ SD}{Slope}$). *Meanwhile, the determination of LOQ was accomplished by the use of a mathematical formula* $LOQ = \frac{10\ x\ SD}{Slope}$. *South Kalimantan is abundant in medicinal plants, such as pampakin and kalangkala. Hence, another objective of this study was to assess the antioxidant activity of the extracts from these two plants using the DPPH method, employing a UV-Vis Spectrophotometer for analysis. The linearity, detection limit, quantitation limit, accuracy, and precision of the validated vitamin C analysis method were as follows: 0.999, 0.50ppm, 5.02ppm, 99.45-101.48%, and 0.03-1.33%, respectively. A 1.0 g sample of kalangkala and pampakin fruit contained 0.24 mg and 0.13 mg of vitamin C, respectively. The antioxidant activity test revealed that the kalangkala leaf extract exhibited potent antioxidant properties, with the solvents ranked in the following order of efficacy: methanol (IC50=23.03 µg/mL) > ethyl acetate (IC50= 27.16 µg/mL) > methylenechloride (IC50= 27.45 µg /mL) > n-hexane (IC50= 32.01 µg/mL). Pampakin leaves, kalangkala fruit, and pampakin fruit showed the highest antioxidant activity in methanol extract with IC50, respectively 50.29, 48.70, and 45.30 µg/mL. Based on the findings of this study, the vitamin C analysis method utilizing a UV-Vis spectrophotometer passes the validation requirements, and the antioxidant activity of pampakin and kalangkala fruit on DPPH free radicals is most likely attributable to the vitamin C found in these fruits. Meanwhile, extracts of the two plants derived from methanol demonstrated the strongest antioxidant activity when compared to other solvents such as n-hexane, methylene chloride, and ethyl acetate.*

Keywords: Antioxidants, Durio kutejensis Hassk (Becc), Litsea garciae Vidal, method validation.

1. INTRODUCTION

The UV-Vis Spectrophotometry method is a commonly employed analytical technique in educational and research settings, including the Pharmacy Laboratory of FMIPA, Lambung Mangkurat University. Precision, comprehensiveness, and accuracy of the analytical data are essential criteria for determining the quality of an analysis. For the analytical data obtained from this spectrophotometer to be considered reliable, all aspects pertaining to this analytical method must have satisfied the validation criteria.

Validation of analytical methods is assessing certain parameters through laboratory experiments to prove that these parameters meet

the requirements for their use (Harmita, 2004). The analytical method can provide reliable data if it meets several required validation parameters, namely precision, accuracy, linearity, limit of detection (LOD), and limit of quantitation (LOQ) (Sayuthi & Kurniawan, 2017). A validation process was conducted to verify the accuracy of the vitamin C analysis method using a UV-Vis spectrophotometer. The purpose was to detect the presence of vitamin C in pampakin fruit (Durio kutejensis Hassk (Becc.)) and kalangkala (Litsea garciae Vidal). The second objective of this study was to verify the vitamin C analysis technique utilizing a UV-Vis spectrophotometer for the purpose of determining the vitamin C content in pampakin and kalangkala fruit.

In addition to verifying the vitamin C analysis technique using a UV-Vis spectrophotometer, this study aimed to gather data on plants with promising medicinal properties, such as pampakin and kalangkala. The bioactivity of the extracts from the two plants was assessed by conducting an antioxidant test on DPPH free radicals (1,1-diphenyl-2-picrylhydrazyl) using a UV-Vis spectrophotometer. Vitamin C in these two plants suggests antioxidant activity due to its strong antioxidant properties.

Pampakin and kalangkala are plants widespread across Indonesia, particularly in South Kalimantan. Pampakin is a variety of durian classified as D. kutejensis Hassk (Becc.) in scientific nomenclature. The local name for it in East Kalimantan is Lai. Pampakin comprises fat, protein, carbs, water, ash, and sugar (Belqis et al., 2016). The extract of Pampakin fruit possesses antioxidant characteristics that may be utilized for treating hyperpigmentation and as a skin-lightening agent (Arung et al., 2015). The kalangkala plant is a member of the Litsea genus, part of the Lauraceae family. Most of these plants thrive in natural forest habitats and are commonly farmed to harvest their durable timber for construction purposes. In South Kalimantan, the seeds are employed as a medicinal remedy for boils, while the young stems serve as an anti-irritant for insect bites.

Kalangkala is rich in secondary metabolites, including saponins, tannins, flavonoids, and alkaloids (Fitriyanti et al., 2020). Phenolic compounds such as N-trans-feruloyl-4-O-methyldopamine, N-cisferuloyltyramine, epicatechin-(4 beta->6)-epicathecin-(2 beta->7, 4 beta->8-epicathecin, 7-Hydroxy-3-(4-methoxyyphenyl)-4-prophyl-2H-1-benzopyran-2-one and 9-O-methylneodunol have been isolated from the methanol extract of the stem of this plant (Raduan et al., 2022). The leaves, stems, and roots of this plant contain compounds that have anticancer, anti-inflammatory, antibacterial, antioxidant, anti-diabetic, anti-HIV, and insecticidal properties (Amit & Zinyin, 2021). Few studies have been done on the pharmacology of this plant, making it intriguing to conduct deeper investigations into its bioactivity and constituent components. Based on this background, another aim of the research was to evaluate solvents that produce medicinal plant extracts with high antioxidant activity. The solvent study is expected to yield insights into suitable solvents for extracting medicinal plants with potent antioxidant properties.

2. MATERIALS AND METHODS

2.1. Materials

Pampakin was taken from the Mandiangin area, Martapura, South Kalimantan. Kalangkala was taken from the Barabai area, Hulu Sungai Tengah, South Kalimantan. The two plants were collected in April 2023 and were determined at the Basic Laboratory of FMIPA ULM with certificate numbers 174/LB.LABDASAR/V/2023 for pampakin and 148/LB.LABDASAR/V/2023 for kalangkala.

The ingredients used were leaf and fruit extracts from pampakin and kalangkala, Whatman filter paper, aluminum foil, plastic wrap, distilled water (H_2O), methanol (CH_3OH),

n-hexane (C_6H_{14}), dichloromethane (CH_2Cl_2), ethylacetate ($C_4H_8O_2$), DPPH (1,1-diphenyl-2-picrylhydrazyl/analytical reagent, Smart Lab), citric acid ($C_6H_8O_7.H_2O$/Merck), disodium hydrogen phosphate (Na_2HPO_4/Merck), sodium oxalate ($Na_2C_2O_4$/Merck), vitamin C ($C_6H_8O_6$/Merck), and Vitacimin.

The tools used were stirring rods, measuring cups, chemical glasses, watch glasses, test tubes, volume pipettes, dropper pipettes, vials, drying cabinets, hair dryers, analytical scales (Ohaus), vortex mixers (Jeio tech), micropipettes (Soccorex), maceration vessel, water bath (Memmert), centrifuge (Clement GS 150), and UV-Vis spectrophotometer (Genesys 10UV / Thermo Scientific).

2.2. Methods

2.2.1. Extraction

The extraction process of leaf and fruit of D. kutejensis Hassk. (Becc.) and L. garciae Vidal were carried out separately using four different solvents, namely n-hexane (HX), dichloromethane (MC), ethyl acetate (Ea), and methanol (MeOH). Both plants were subjected to separate extractions using 250 mL of n-hexane, dichloromethane, ethyl acetate, and methanol for 1x24 hours at room temperature. The extraction involved 20 g of leaves and dried fruit from each plant. The filtration process was employed to separate each extract using filter paper, followed by the concentration of the extracts in a water bath at 50°C.

2.2.2. Preparation of Standard Solutions

Vitamin C stock solution was prepared by dissolving 25 mg of vitamin C with distilled water in a 250 mL volumetric flask to obtain a concentration of 100 ppm. The concentration series for the vitamin C calibration curve was obtained by diluting the main standard solution made with various concentrations, namely 0.2 ppm, 0.4 ppm, 0.6 ppm, 0.8 ppm, 1.0 ppm, 1.2 ppm, and 1 .4 ppm (v/v).

2.2.3. Preparation of Vitamin C Test Samples

Samples of kalangkala and pampakin fruit were prepared by separating the skin from the flesh. The fruit's flesh was cut and mashed, then a portion representing the entire sample was taken and weighed at around 1 gram. About 4 mL of $Na_2C_2O_4$ 0.01 N was added to the sample and then centrifuged for 10 minutes at 2500 rpm. Next, the sample residue was separated by decantation. The centrifuge was repeated until the sample residue was colorless (Selimovic et al., 2011).

2.2.4. *Determination of Maximum Wavelength*

The maximum wavelength (λ) was determined using a UV-Vis Spectrophotometer. The maximum wavelength of the standard solution was determined by scanning. The scanning results determine the maximum wavelength at which the sample has the greatest absorbance value.

2.3. Validation of Analysis Methods

The validation of this analytical method referred to ICH (2005) for several parameters, such as linearity, accuracy, precision, detection limit, and quantitation limit.

2.3.1 *Linearity of the Calibration Curve*

A calibration curve was created by varying the standard solution 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 ppm using the specified maximum wavelength. The absorbance of sample solutions with varying concentrations was measured. A regression curve was made from the absorbance obtained and connected to the concentration to obtain a correlation coefficient value (r), which becomes more linear as it approaches 1. The calibration curve equation obtained was used to determine vitamin C levels. Linearity is considered favorable when the correlation coefficient value (r) equals or exceeds 0.999 (ICH, 2005).

2.3.2. Accuracy

Accuracy was assessed by conducting a recovery test. The procedure involved assessing the concentration of vitamin C in the sample,

followed by assessing the concentration of vitamin C in the sample after introducing a known quantity of standard solution. The standard concentration of vitamin C solution added was 50%, 100%, and 150%. Repetition was done thrice for each sample (ICH, 2005). The recovery percentage was calculated based on Equation 1.

$$\%\, recovery = \frac{A - B}{m}\, x\, 100\% \qquad (1)$$

Note:
A = Concentration of the substance after adding the standard solution
B = Concentration of the substance in the sample
m = Concentration of the standard solution added to the sample
Gonzales (2010) states that the recovery percentage requirement is 98% - 102%.

2.3.3. Precision

Precision is generally calculated using standard deviation (SD) to produce Relative Standard Deviation (RSD). The smaller the RSD percent, the higher the precision value. Precision can be expressed by the % relative standard deviation (% RSD) value, calculated based on Equation 2 (Harvey, 2000).

$$\%\, RSD = \frac{SD}{\ddot{X}}\, x\, 100\% \qquad (2)$$

Note:
RSD = Relative standard deviation, SD = Standard Deviation, $\ddot{X}$= Average level (µg/mL)
Where,

$$SD = \sqrt{\frac{\Sigma(Xi - \ddot{X})^2}{n - 1}} \qquad (3)$$

Note:
SD = Standard Deviation, Xi = i-th data, $\ddot{X}$ = average, n = number of data
The permitted RSD value is ≤ 2% (Gonzales et al., 2010).

2.3.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

The detection limit and quantitation limit can be calculated statistically via the linear line of the calibration curve. LOD was calculated based on Equation 4 and standard deviation (SD) based on Equation 3.

$$LOD = \frac{3\ x\ SD}{Slope} \qquad (4)$$

LOD= detection limit, SD= standard deviation
Meanwhile, the limit of quantitation (LOQ) was determined according to Equation 5.

$$LOQ = \frac{10\ x\ SD}{Slope} \qquad (5)$$

LOQ= the limit of quantitation, SD = standard deviation
The slope was obtained from the b value in the linear line equation y= a + bx (Harmita, 2004).

2.3.5. Determination of Antioxidant using the DPPH method

The antioxidant activity of the extract was tested using the DPPH (1,1-diphenyl-2-picrylhydrazyl) method, as carried out by Idris et al., (2022). A total of 33.3 µL of extract at different concentrations (159.73 - 4.99 µg/ mL) was added to 1 mL DPPH 6.0 × 10^{-5} M, mixed, and incubated for 20 minutes at room temperature in the dark. Absorbance was measured at 517 nm using a UV-visible spectrophotometer (Genesys 10Uv, Thermo Scientific) with three repetitions. Methanol and vitamin C were used as blanks and positive controls. The antioxidant activity percentage was determined using Equation 6.

$$\text{Inhibition } (\%) = \frac{(Blank\ absorbance - sample\ absorbance)}{\text{Blank absorbance}}\, x\, 100\% \qquad \ldots(6)$$

2.3.7. Statistic analysis

The data used were the average ± standard deviation. The linear regression equation was used to determine the concentration of vitamin C, and the nonlinear regression equation was used to determine the concentration of antioxidants. The t-test (Student's t-test) was employed as the statistical technique to assess differences between groups in antioxidant evaluation.

3. RESULTS AND DISCUSSION

3.1. Determination of Maximum Wavelength

Maximum wavelength measurements were carried out before analyzing the vitamin C content in the samples and validating the analytical method. The maximum wavelength measurement was carried out using a scanning method using a UV-Vis spectrophotometer. The wavelength was set from 200 to 300 nm. The concentration of vitamin C chosen in measuring the maximum wavelength was 0.4 ppm, with the maximum wavelength obtained being 257 nm with an absorbance of 0.921, as shown in **Figure 1**.

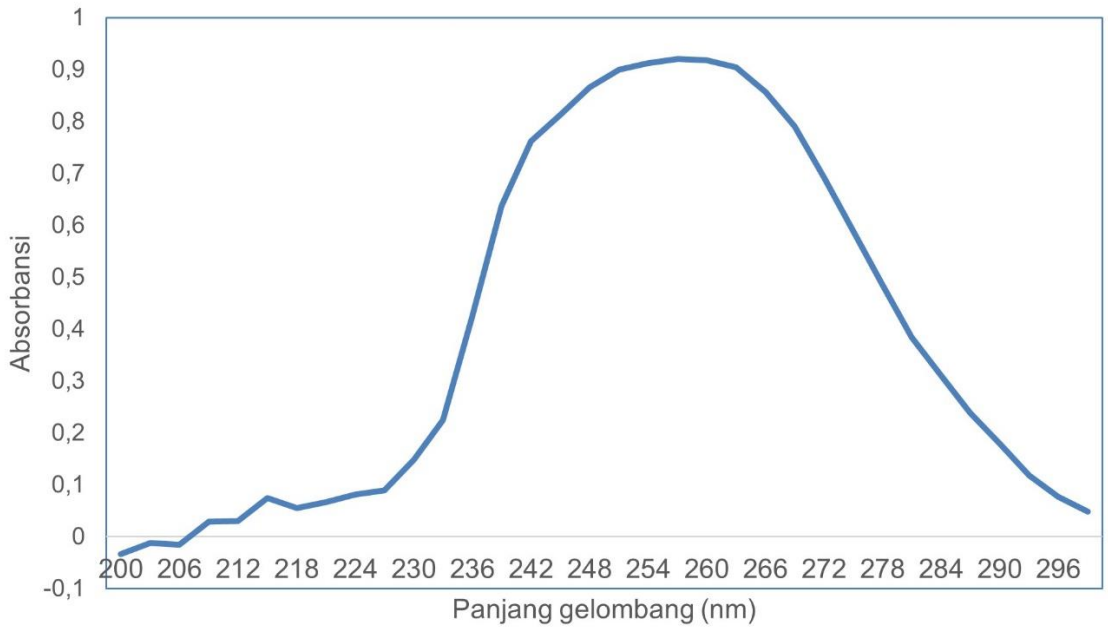

Figure 1. Maximum wavelength search for vitamin C analysis

3.2. Determination of Vitamin C Stability

Vitamin C stability measurements were carried out after determining the maximum wavelength. The standard concentration of vitamin C used in stability measurements was 0.2 ppm with a wavelength of 257 nm. Vitamin C stability measurements were carried out for 30 minutes, as presented in **Table 1**.

Table 1. Measurement of vitamin C stability

Time (minute)	1	5	10	15	20	25	30
Absorbance	0.021	0.019	0.019	0.019	0.020	0.019	0.019

Table 1 demonstrates that the vitamin C solution remains unchanged during storage for 30 minutes, as indicated by the absorbance value, which remains within the narrow range of 0.019 - 0.021 without any significant decline or increase. According to Selimovic (2011), vitamin C can be stable for 30 minutes at room temperature due to sodium oxalate (0.01 N) in the phosphate buffer solution. This stability time information is beneficial because researchers can estimate the safe storage time for standard solutions or vitamin C samples.

3.3. Linearity

Linearity is the ability of a method to obtain test results that are directly proportional to the analyte concentration in the range analyzed (Ganjar & Rohman, 2007). The linearity of a method is measured based on how well the curve connects the analyte concentration on the x-axis and the analyte response on the y-axis. The vitamin C standards analyzed were seven series, namely 0.2, 0.4, 0.6, 0.8, 1.0, 1.2, and 1.4 ppm, with three replications for each concentration series. Replication aims to ensure the consistency of the data obtained. The regression equation obtained was $y = 0.7639x + 0.0184$ with a correlation coefficient (r) = 0.999. This explains that 99.9% of absorbance changes are influenced by vitamin C concentration, while 0.1% is influenced by other factors, such as contamination during work (Snyder et al., 1997). This demonstrates that within the analysis range of 0.2 – 1.4 ppm, the method can deliver 99.9% accurate and precise results.

Linearity is considered favorable when the correlation coefficient (r) value equals or exceeds 0.999 (ICH, 2005). These findings indicate that the linearity parameters tested in this study have successfully fulfilled the specified criteria. There exists a direct correlation between the concentration and response (absorbance). As the concentration increases, the reaction (absorbance) increases, leading to a positive correlation coefficient value.

3.4. Limit of Detection (LOD) and Limit of Quantitation (LOQ)

LOD and LOQ aim to determine the lowest limit of analyte concentration that can be analyzed qualitatively (LOD) or quantitatively (LOQ). Statistically, LOD and LOQ can be calculated using the regression equation from the calibration curve. LOD and LOQ values can be obtained from the standard deviation and slope of the caffeine standard curve. Suppose the analyte concentration value is greater than the LOD. In that case, the analyte in the sample can be detected qualitatively. However, it cannot always be quantified. When the analyte concentration value is above the LOQ, the analyte in the sample can be determined qualitatively and quantitatively (Ganjar & Rohman, 2007). The LOD values were measured at 0.50 ppm and LOQ at 5.02 ppm. This LOD value indicates that samples can still be measured at this concentration, which provides accurate results for a tool based on the individual level of accuracy of the analysis results. Meanwhile, the LOQ value of 5.02 ppm indicates that if measurements are carried out at this concentration, it can still provide accurate analysis.

3.5. Accuracy

Accuracy in this study is expressed as a percentage of recovery. The acceptable range for

this percentage is between 98 and 102% (Gonzales et al., 2010). Recovery refers to the degree of precision of an analysis process or the proximity between the measured value and the received value, which can be a conversion, actual, or reference value.

Accuracy parameter testing was performed by adding several analytes to the sample at three concentrations, namely low, medium, and high, with three replications for each concentration. The addition of three different concentrations (low, medium, and high) of the analyte is intended to ascertain the proportion of the added analyte that can be detected.

The results of the % recovery calculation in Figure 2 indicate that the average % recovery value for each concentration falls within the acceptable range of 98 - 102%. This shows that the method used in this study is accurate in determining vitamin C levels.

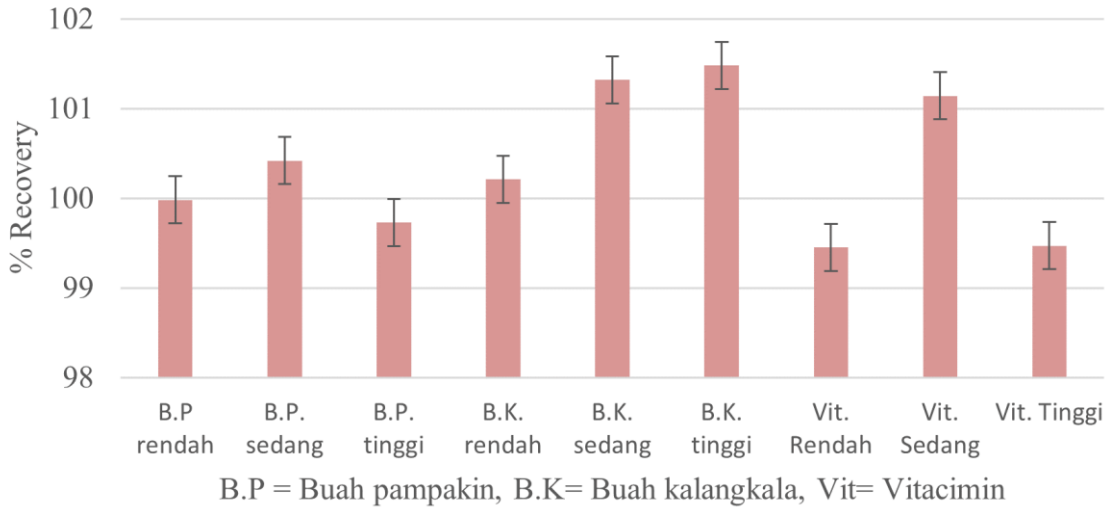

Figure 2. Percentage of recovery of pampakin fruit, kalangkala fruit, and Vitacimin.

3.6. Precision

Calculating precision is to ascertain the degree of accuracy exhibited by an analysis result when conducted under identical conditions. Conditions of analysis may consist of the location and time of the analysis, the apparatus utilized, and the analyst conducting the analysis. If there is a negligible discrepancy between one number and another in a sequence of measurement levels, the measurement findings can be regarded as accurate. The expression of precision is quantified by the percentage value of the relative standard deviation (RSD).

The % RSD number is significantly affected by the standard deviation value, with a higher standard deviation resulting in a higher % RSD value. The standard deviation value is affected by the variation in the vitamin C content among the samples within each replication and the average vitamin C content among the samples in the same treatment. The significance of the standard deviation value increases with a higher variance in content values.

Precision is considered to be good if the method provides a relative standard deviation (% RSD) or coefficient of variation ≤ 2% (Gonzales et al., 2010). The findings of the % RSD measurement in Table 2 indicate that the measurement exhibits excellent precision at each concentration level, as evidenced by a coefficient of variation value of < 2% (0.03-1.33%).

Table 2. Precision calculation (% RSD)

Sample	Concentration	SD	% RSD
Pampakin fruit	Low (±1.2 mg)	0.0100	0.67
	Medium (± 2.3 mg)	0.0300	1.33
	High (± 3.4 mg)	0.0300	0.89
Kalangkala fruit	Low (± 1.0 mg)	0.0100	0.76
	Medium (± 2.8 mg)	0.0200	0.71
	High (± 3.5 mg)	0.0180	0.47
Vitacimin	Low (± 1.7 mg)	0.0012	0.05
	Medium (± 2.7 mg)	0.0024	0.07
	High (± 3.1 mg)	0.0012	0.03

3.7. Analysis of vitamin C in samples

The sample was analyzed for its vitamin C content using the maximum wavelength obtained. Samples of pampakin and kalangkala fruit were weighed at 1 gram, while vitacimin was 2.5 mg. The samples were extracted using sodium oxalate solution (0.1 N) in phosphate buffer solution. Sodium oxalate in phosphate buffer functions as a solvent and stabilizer for ascorbic acid (vitamin C), as presented in Table 1. Vitamin C dissolved in sodium oxalate shows stability during 30 minutes of storage.

Table 3. Test results for vitamin C levels

Sample	Concentration (mg)			Average ± SD
	I	II	III	
Pampakin fruit*	0.127	0.128	0.125	0.127 ± 0.002
Kalangkala fruit*	0.233	0.234	0.252	0.240 ± 0.011
Vitacimin**	0.628	0.632	0.654	0.638 ± 0.014

* in 1 gram, ** in 2.5 mg

The Vitacimin utilized in this study was acquired from local convenience stores in the Banjarbaru region. The purpose of incorporating Vitacimin in this study was to evaluate the precision of the vitamin C test outcomes, as the vitamin C concentration was indicated on the package of Vitacimin. The test results obtained were 0.638 mg in 2.5 mg (0.0025 g) Vitacimin as shown in Table 3. The vitamin C content that should be obtained in 0.0025 g was 0.630 mg, which was calculated by 0.0025 g Vitacimin / 1.983 g tablet x 500 mg, while the vitamin C obtained in this analysis was 0.638. Therefore, 1.983 g of tablet contained 506.06 mg of vitamin C. The obtained result closely aligns with the vitamin C content indicated on the packaging of Vitacimin, which was 500.00 mg per 1.983 g tablet.

T vitamin C content of kalangkala and pampakin fruit in 1 g of the sample was 0.240

and 0.127 mg, respectively. Therefore, the vitamin C content of 1 g of kalangkala and pampakin fruit samples was 24.0% and 12.7%, respectively.

3.8. Extraction

Extraction of fruit and leaf from kalangkala and pampakin was carried out in a maceration vessel at room temperature for 1x24 hours. The liquid extract obtained was filtered using Whatman filter paper, collected, and then the solvent was evaporated using a water bath. **Table 4** displays the results of the extraction process conducted on the leaf and fruit of pampakin and kalangkala.

Table 4. Extraction of fruit and leaf from pampakin and kalangkala.

Sample	Solvent	Weight of dry powder (g)	Dry extract (g)
Kalangkala leaf	n-hexane	20.03	0.69
	Methylenechloride	20.04	0.75
	Ethyl acetate	20.07	0.71
	Methanol	20.05	5.28
Kalangkala fruits	n-hexane	20.05	1.56
	Methylenechloride	20.58	1.68
	Ethyl acetate	20.11	2.19
	Methanol	20.65	3.12
Pampakin leaf	n-hexane	20.00	0.17
	Methylenechloride	20.04	0.23
	Ethyl acetate	20.05	0.10
	Methanol	20.42	0.25
Pampakin fruits	n-hexane	20.01	2.60
	Methylenechloride	20.01	2.70
	Ethyl acetate	20.00	2.23
	Methanol	20.00	4.71

The DPPH method was employed to determine the antioxidant activity of the obtained dried extract. Table 1 shows that methanol solvent extracts more compounds compared to other solvents because methanol is the most polar solvent compared to ethyl acetate, dichloromethane, and n-hexane. Methanol has the ability to extract both nonpolar and polar chemicals, resulting in a higher yield of extract compared to other solvents employed in this study (Idris et al., 2022).

3.9. Antioxidant evaluation

Antioxidant screening was conducted on leaf and fruit extracts from pampakin and kalangkala using the DPPH method. Antioxidant chemicals will transfer their hydrogen atoms to DPPH free radicals, resulting in the reduction of DPPH and the formation of a non-radical state. The color change from purple to yellow indicates that the radicals are reduced by antioxidants through the contribution of hydrogen to stabilize the DPPH molecule (Idris et al., 2022). The extract concentration used in this antioxidant screening was 5000 ppm. Subsequently, the IC50 value was determined for extracts that had a good inhibition percentage (≥70%). Extracts that have an inhibition percentage of ≥70% have the potential to have antioxidant activity. The study included Vitamin C as a positive control (Molyneux, 2004).

Based on **Figure 3**, leaf extracts from kalangkala (n-hexane, dichloromethane, ethyl acetate, and methanol) and methanol extracts (kalangkala fruit and pampakin, as well as pampakin leaves) have the potential to be effective antioxidants due to their significant inhibition percentage (≥ 70%). Therefore, the test proceeds by finding the IC50 value. Vitamin C was selected as the control due to its strong antioxidant characteristics, which resulted in a significant inhibition percentage of 95.74% (IC50 of 0.80 µg/mL). In general, fruits and vegetables are known as healthy foods with high vitamin C content. Vitamin C functions as an antioxidant that removes toxic free radicals and other reactive oxygen species (ROS) (Arigoni et al., 2002). In addition, Vitacimin exhibits a significant level of inhibition (as an antioxidant), specifically 92.57%, with an IC50 value of 2.54 µg/mL due to its substantial content of vitamin C, which amounts to 500 mg each tablet (1 tablet = 1.983 g).

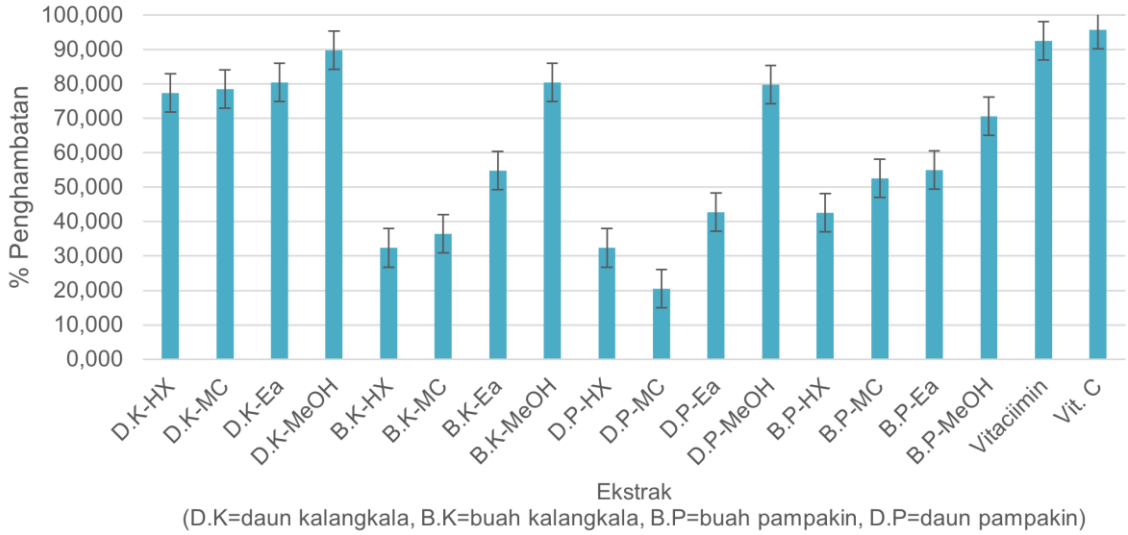

Figure 3. Antioxidant screening from pampakin and kalangkala plant extracts.

Comparatively, the antioxidant activity of the methanol extract of pampakin leaf is superior to that of the n-hexane, dichloromethane, and ethyl acetate extracts, as evidenced by the data presented in Figure 3 and Table 5. The antioxidant activity of the methanol extract of the leaf may be due to the presence of flavonoid and phenolic compounds. Previous studies have conducted phytochemical analyses on the methanol extract derived from pampakin leaf, wherein the findings have identified the presence of flavonoids, alkaloids, phenolics, saponins, and steroids (Manurung et al., 2022). According to Ahmed (2015), methanol is a good solvent

used to extract flavonoid and phenolic compounds. In his research, the total phenolic content of methanol, water, and n-hexane extracts from Adiantum caudatum leaf was 27.7, 21.1, and 16.7 µg gallic acid equivalents per mL. Meanwhile, the total flavonoid content in methanol, water, and hexane extracts was 13.2, 11.6, and 10.0 µg routine equivalents per mL. Therefore, it can be inferred that the majority of phenolic and flavonoid chemicals are extracted more effectively in methanol solvents than in water and n-hexane. Methanol is the most polar solvent used in this research. The order of polarity of these solvents is methanol (MeOH) > ethylacetate > dichloromethane > n-hexane. Methanol can extract polar molecules, such as phenolics and flavonoids, due to its polarity.

Table 5 presents the antioxidant activity (DPPH) of kalangkala leaf extract in descending order: methanol extract > ethylacetate > methylenechloride > n-hexane. Previous researchers have reported that n-hexane leaf extract contains phenolics of 30 ± 0.002 µg GAE/mg extract and flavonoids of 190 ± 0.004 µg GAE/mg extract (Wulandari et al., 2018). With an IC50 value of 32 ± 0.12 µg/mL, the n-hexane extract demonstrates excellent antioxidant activity due to its phenolic and flavonoid composition. More active than n-hexane extract, methylenechloride has an IC50 of 27.45 ± 0.14 µg/mL. The methanol extract of kalangkala leaf exhibited superior antioxidant activity against DPPH free radicals compared to other extracts, with an IC50 value of 23.03 ± 0.09 µg/mL. The IC50 value represents the concentration of the extract required to lower 50% of the total DPPH. In this case, the number 50 is used as a substitute for the y value. By inserting the value 50 for the y variable, the resulting x value will be the IC50 value. Wulandari (2018) reported that the ethanol extract of kalangkala leaves has a phenolic content of 100 ± 0.001 µg GAE/mg extract and a flavonoid content of 240 ± 0.001 µg GAE/mg extract. Since methanol belongs to the same class as ethanol, it is probable that the phenolic and flavonoid levels will likewise be elevated. Hence, the antioxidants of the methanol extract in this study are the highest among other extracts.

Table 5. Antioxidant activity of kalangkala and pampakin

Sample	Extract	IC50 (µg/mL) ± SD
Kalangkala leaf	n-hexane	32.01±0.12*
	Methylene chloride	27.45±0.14*
	Ethyl acetate	27.16±0.17*
	Methanol	23.03±0.09*
Kalangkala fruit	Methanol	48.70±1.10*
Pampakin leaf	Methanol	50.29±0.12*
Pampakin fruit	Methanol	45.30±0.06*
Vitacimin	-	2.54±0.13*
Vitamin C (positive control)	-	0.80±0.03

Data are expressed as mean ± SD with three replicates, *p<0.05 vs positive control

The IC50 value for the antioxidant activity of the methanol extract of kalangkala fruit was 48.70 µg/mL (Table 5). This result is also supported by other references that report that the antioxidant activity (DPPH) of the methanol extract of kalangkala fruit has an IC50 value of 60.0 ± 3.5 µg/mL (Hassan et al., 2013). The antioxidant activity results from this study were higher than those reported by Hassan (2013). This may occur due to the use of methanol solvents with different concentrations. This study used methanol with a purity percentage of 90%, while Hassan (2013) used 80% methanol. Another source reported that the methanol extract of kalangkala leaves showed antifungal activity against Colletotrichum gloeosporioides with growth inhibition of 13.31 ± 0.49 mm at a concentration of 0.01 µg/mL (Jhonny et al., 2010). The antioxidant activity of methanol extracts derived from pampakin fruit, kalangkala fruit, and pampakin leaf had IC50 values ranging from 45.30 – 50.29 µg/mL. The level of antioxidants in a sample is classified as "very good" if the IC50 value is less than 50 - 100 µg/mL, "medium" if the IC50 value is between 100 - 150 µg/mL, "weak" if the IC50 value is between 150 - 200 µg/mL, and "very weak" if the IC50 value is over 200 µg/mL (Kuspradini et al., 2018). The antioxidant activity of a substance is typically measured using the IC50 value, which represents the concentration of the substance needed to inhibit free radicals (DPPH) by 50%. A lower IC50 value indicates stronger antioxidant properties of the substance (Molyneux, 2004). The DPPH test results showed a significant difference between the extracts and the positive control in the t-test ($p<0.05$).

4. CONCLUSIONS

1. The vitamin C analysis method using UV-Vis spectrophotometry meets the validation requirements for linearity with a value of r=0.999, LOD= 0.50 ppm, LOQ= 5.02 ppm, accuracy (% recovery) = 99.45% - 101.48%, and precision (% RSD) = 0.03 % - 1.33%. The vitamin C levels of kalangkala and pampakin fruit using the validated analytical method were 0.24 and 0.13 mg, respectively, in 1g of sample.
2. The order of solvents for Kalangkala leaf extract, in terms of their antioxidant activity against DPPH free radicals, was as follows: methanol (IC50=23.03 µg/mL) > ethyl acetate (IC50= 27.16 µg/mL) > dichloromethane (IC50= 27.45 µg/ mL > n-hexane (IC50= 32.01 µg/mL). The antioxidant activity (DPPH) of pampakin leaves, kalangkala fruit, and pampakin fruit was greatest in methanol extract with IC50 of 50.29 and 45.30 µg/mL. The antioxidant activity of pampakin and kalangkala fruit on DPPH free radicals was most likely caused by the levels of vitamin C contained in these fruits.

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