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Validation of the Use of Anthocyanin as Indicators for Acid-Base Titration

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Abstract

Rambutan peel (*Nephelium lappaceum L.*) contains secondary metabolites, particularly flavonoids, including anthocyanins. Anthocyanins exhibit color changes in response to variations in pH, making them potential candidates for use as acid-base indicators. This study aims to validate using methanol fraction powder derived from rambutan peel as an acid-base titration indicator by evaluating method validation parameters such as precision, accuracy, and linearity. The fractionation process was conducted using liquid-liquid extraction with *n*-hexane, water, and methanol as solvents, followed by freeze-drying of the methanol fraction. The methanol fraction powder was analyzed and compared with the conventional phenolphthalein indicator to determine the color transition at the titration endpoint. The results indicated that the methanol fraction exhibited a color change to brown within a pH range of 8–11. Statistical analysis using an independent sample *t*-test showed no significant difference between the methanol fraction powder and phenolphthalein (significance value > 0.05). Therefore, based on the method validation results, including precision, accuracy, and linearity, the methanol fraction powder of rambutan peel demonstrates potential as a reliable acid-base indicator.

1. Introduction

The concentration of an acidic or basic solution can be determined through acid-base titration using a standardized acid or base solution. The equivalence point is reached when the number of moles of acid completely reacts with the number of moles of base. Since this point cannot be observed visually, an indicator is required to detect the color change in the sample solution due to a significant pH shift. Phenolphthalein is an example of a synthetic indicator used to determine the acidic or basic nature of a solution [1]. Alternatively, natural indicators can be derived from waste materials containing anthocyanin compounds.

Anthocyanins belong to the flavonoid group and are responsible for the blue, violet, red, orange, magenta, and purple pigments in plants, with maximum absorption in the 490–550 nm wavelength range [2, 3]. The intensity of a plant's color is directly related to its anthocyanin content [4]. However, anthocyanins are pH-sensitive and exhibit color changes accordingly: they appear red in acidic conditions and green in alkaline environments. This occurs due to structural transformations, where anthocyanins exist as flavylium cations in acidic conditions but convert into an unstable chalcone form in alkaline conditions [5].

Rambutan fruit (*Nephelium lappaceum* L.) is a tropical fruit that typically ripens during the rainy season. According to the Central Bureau of Statistics, Indonesia's national rambutan production reached 855,152 tons in 2022 [6]. Rambutan peels are often discarded as waste, contributing to environmental concerns. Phytochemical screening of rambutan peel ethanol extract has revealed the presence of polyphenols, tannins, saponins, steroids, flavonoids, monoterpenoids, sesquiterpenoids [7], and anthocyanins [8].

According to Tuslinah and Aprilia [8], the ethanol extract of rambutan peel contains 75.47 mg/L of anthocyanins. Previous research by Aprillia *et al.* [9] stated that the pH trajectory value of rambutan peel ethanol extract is 6–8, making it suitable as an indicator

in strong acid–strong base titrations. Based on this background, this study focuses on the preparation of a natural indicator powder from the methanol fraction of rambutan peel (*Nephelium lappaceum* L.) using the freeze-drying method.

Method validation is the process of ensuring that a method meets established requirements to obtain accurate and reliable results [10]. The validation of natural acid-base indicators was conducted by assessing the precision, accuracy, and linearity of the natural indicator derived from the methanol fraction of rambutan peel, using phenolphthalein as a reference. This validation aimed to confirm that the natural indicator from the rambutan peel methanol fraction does not exhibit significant differences compared to the phenolphthalein indicator.

2. Experimental

2.1. Materials

The plant material used in this study was rambutan peel. The chemicals and reagents included 96% ethanol (Hepta Indonesia), hydrochloric acid (Merck, Germany), sodium hydroxide (Merck, Germany), methanol (Hepta Indonesia), phenolphthalein indicator, citric acid (Merck, Germany), boric acid (Merck, Germany), potassium chloride (Merck, Germany), phosphoric acid (Merck, Germany), sodium citrate (Merck, Germany), acetic acid (Merck, Germany), potassium phosphate monobasic (Merck, Germany), and sodium hydrogen phosphate (Merck, Germany).

2.2. Instrumentation

The instruments used in this study were an analytical balance (Mettler Toledo ME204E), rotary evaporator (IKA RV 10 D S99), water bath (B-ONE DWBC-30L-6H), pH meter (Ohaus Starter 5000), UV-Vis spectrophotometer (Agilent Cary 60) and a freeze dryer (Biobase).

2.3. Simplisia Preparation

Six hundred grams of rambutan fruit, sourced from the Banjarsari area of Ciamis, were used in this study. The dark red skins were separated, thoroughly washed under running water, and sliced into thin strips [11].

2.4. Extract Preparation

Three hundred grams of rambutan peel simplicia were extracted using the maceration method with a solvent mixture of 96% ethanol and 1% HCl in a 9:1 ratio. The extraction was carried out for 72 hours (3×24 hours) in a location shielded from sunlight to produce a clear filtrate. The liquid extract was then concentrated using a rotary evaporator at 40°C to remove the solvent. The resulting concentrate was further evaporated using a water bath at 40°C until a thick extract was formed [9]. The rambutan peel ethanol extract yield was calculated using Equation (1).

$$\%\,yield = \frac{Extract\ weight}{Simplisia\ weight} \times 100\% \quad (1)$$

2.5. Extract Quality Testing

The quality of the extracts was assessed through various tests, including organoleptic examination, determination of total ash content, acid-insoluble ash content, water-soluble extractive content, and ethanol-soluble extractive content.

2.6. Fraction Preparation

Twenty-five grams of ethanol extract from rambutan peel were dissolved in water until completely dissolved. An equal volume of n-hexane (1:1 ratio) was added, and the mixture was homogenized thoroughly. The solution was then shaken using an orbital shaker for 1 hour. Afterward, the mixture was transferred to a separating funnel and left undisturbed for 24 hours to allow the formation of two distinct layers. The n-hexane fraction was collected, while the water fraction was transferred into a separate container and concentrated. The concentrated water fraction was mixed with 100 mL of methanol. The insoluble residue was separated, and the methanol-soluble portion was further concentrated. The thick methanol fraction was mixed with water and dried using the freeze-drying method with a freeze-dryer [12].

2.7. Flavonoid Qualitative Test

A 0.25-gram sample was dissolved in a 10 mL volumetric flask with water. Two milliliters of the solution were pipetted into a test tube and heated. Zinc or magnesium powder and 5 drops of 2 M HCl were added, followed by filtration of the mixture. Subsequently, 2 mL of amyl alcohol was added to the filtrate. The appearance of an orange-to-red color in the amyl alcohol layer indicated the presence of flavonoids in the sample [13].

2.8. Anthocyanin Qualitative Test

A 0.25-gram sample was dissolved in a 10 mL volumetric flask with water. Two milliliters of the sample solution were pipetted, followed by the addition of 3 mL of 2 M HCl. The mixture was heated at 100°C for 5 minutes. Subsequently, 2 M NaOH solution was added drop by drop until the color changed from red to blue-green and gradually faded [5].

2.9. Determination of Total Anthocyanin Content

A 0.25-gram sample of the methanol fraction of rambutan peel was dissolved in a 10 mL volumetric flask with water. Subsequently, 0.5 mL of this solution was transferred to a 5 mL volumetric flask, and buffer solutions with pH 1.0 and 4.5 were added to the mark. The solutions were incubated at room temperature for 15 minutes. The absorbance of the sample solutions at both pH levels was then measured at wavelengths of 516 nm and 700 nm [8]. Anthocyanin concentration was calculated using Equation (2).

$$Total\ anthocyanin = \frac{A \times DF \times 1000 \times MW}{\varepsilon \times b} \quad (2)$$

Where, A is (A_{max} - A_{700nm}) pH 1 - (A_{max} - A_{700nm}) pH 4.5, *DF* is the dilution factor, *MW* is the molecular weight (449.2 for Cyanidin 3-glucoside), ε is the polar absorbance of Cyanidin 3-glucoside (26900 L/mol.cm), *b* is the width of the cuvette (1 cm).

2.10. Color Testing on Buffer Solution pH 1-14

A 0.25-gram sample of the methanol fraction of rambutan peel was dissolved in a 10 mL volumetric flask with water. Then, 0.5 mL of this solution was transferred to a 5 mL volumetric flask, and buffer solutions with pH ranging from 1 to 14 were added to the mark. The resulting color changes were observed and recorded. The absorbance of each solution was then measured across the wavelength range of 400–800 nm using UV-Vis spectrophotometry [9].

2.11. Application of Natural Indicator Powder

2.11.1. Strong Acid-Strong Base Titration

Ten milliliters of 0.1 N HCl solution were placed in an Erlenmeyer flask, and 200 mg of the methanol fraction powder from rambutan peel was added. The mixture was then titrated with a 0.1 N NaOH solution until a color change occurred. Phenolphthalein was used as an indicator for visual comparison during the titration process [14].

2.11.2. Weak Acid-Strong Base Titration

Ten mL of 0.1 N CH_3COOH solution was placed in an Erlenmeyer flask, and 200 mg powdered methanol fraction of rambutan peel was added. The mixture was titrated with 0.1 N NaOH solution until a color change occurred. The phenolphthalein indicator was used as a comparison throughout the titration process [14].

2.12. Method Validation

2.12.1. Precision

The strong acid-strong base titration and weak acid-strong base titration were performed six times for each titration. The relative standard deviation value was calculated using Equation (3) [10].

$$\% \, RSD = \frac{SD}{\bar{X}} \times 100\% \quad (3)$$

Where, *SD* is the standard deviation, $\bar{X}$ is the average value, and *RSD* is the relative standard deviation.

2.12.2. Accuracy

Strong acid-strong base titration and weak acid-strong base titration were carried out using three sample concentrations in the range of 80–120%, with three replications at each concentration. The recovery of the two titration processes was calculated using the Equation (4) [10].

$$\% \, Recovery = \frac{Level \ obtained}{Actual \ level} \times 100\% \quad (4)$$

2.12.3. Linearity

Strong acid-strong base and weak acid–strong base titrations were performed using eight sample concentrations ranging from 60% to 130%, with three replications at each concentration. The obtained data were then analyzed to determine the correlation coefficient (R^2) of the linear regression for both titration processes [10].

2.12.4. Data Analysis

The data obtained from the method validation parameters were tested for significance using SPSS. If the data were normally distributed ($p > 0.05$), an independent sample *t*-test was conducted. Otherwise ($p < 0.05$), the Mann-Whitney test was used [15].

3. Results and Discussion

Plant determination was conducted to identify and confirm the plants to be used. This plant identification was carried out at the Herbarium Jatinangor, Plant Taxonomy Laboratory, Department of Biology, FMIPA UNPAD. Based on the plant identification sheet No. 30/HB/01/2023 dated January 7, 2023, the plant used is rambutan, with the Latin *Nephelium lappaceum* L.

The simplisia used in this study was fresh simplisia, which refers to natural materials utilized in their fresh or undried state [16]. The purpose of processing fresh simplisia was to preserve the targeted compounds, particularly anthocyanins, which are thermolabile [8]. The processing of simplisia yielded 300 grams of fresh simplisia from 600 grams of rambutan fruit. To ensure purity, the rambutan fruit skin was separated from impurities and foreign substances, such as the white inner layer, which can affect the simplisia. If extracted, this white layer may cause the extract to become sticky due to its pectin content [17]. The fresh simplisia was cut into small pieces to reduce the size of the rambutan fruit skin and increase its surface area, facilitating the maximal extraction of its compounds [18].

The extraction of rambutan peel was performed using the maceration method, which was chosen to prevent anthocyanin oxidation. Since anthocyanins contain hydroxyl groups that are highly susceptible to oxidation when exposed to heat, a cold extraction process—maceration—was used [19]. The extraction solvents comprised 96% ethanol and 1% HCl in a 9:1 ratio. The selection of 96% ethanol was intended to optimize the extraction of secondary metabolites, as ethanol can break down plant tissues, allowing for the extraction of both polar and non-polar compounds.

Table 1. Extract quality testing result

Test type	Test result
Organoleptic	Odor: Characteristic Odor Color: Red Shape: Thick Taste: Bitter
Water-soluble juice content	18.16 ± 0.03%
Ethanol-soluble juice content	17.02 ± 0.04%
Total ash content	2.73 ± 0.17%
Acid-insoluble ash content	0.23 ± 0.02%

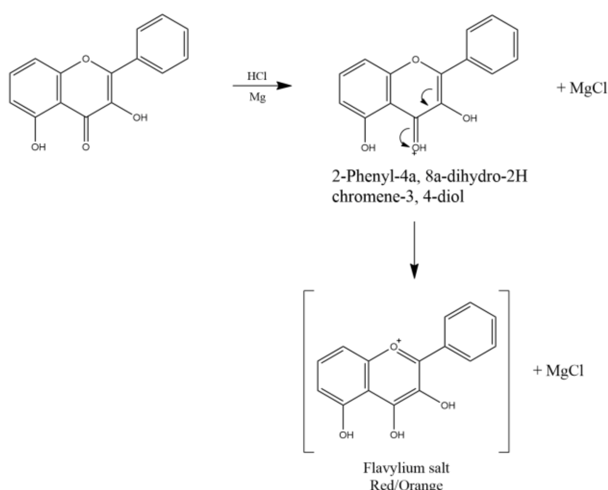

Figure 1. Flavonoid reaction

Additionally, ethanol evaporates easily, reducing the required evaporation time [20]. Meanwhile, 1% HCl was used to break glycosidic bonds, converting glycosides into glycones and aglycones [21]. During maceration, solvent replacement—commonly known as remaceration—was necessary to prevent solvent saturation. Without replacement, equilibrium between the simplisia and the solvent could hinder optimal compound extraction [22].

In the maceration process, a duration of 24 hours is required to allow sufficient time for compound migration into the solvent, maximizing the contact time between active compounds and the solvent [23]. Additionally, stirring during maceration enhances this contact by increasing the frequency of interactions between the simplisia and the solvent [24]. Maceration is considered complete when the filtrate becomes clear, indicating that the compounds in the simplisia have been fully extracted [25]. The extraction of rambutan peel yielded 18.11%.

Extract quality testing was conducted to ensure that the produced extract meets the quality standards outlined in the Indonesian Herbal Pharmacopoeia (FHI) or Materia Medika Indonesia (MMI) [26]. The quality test results of rambutan peel ethanol extract can be seen in Table 1.

The physical characteristics of the extract were evaluated organoleptically, assessing its odor, taste, shape, and color using the five senses. The organoleptic examination revealed that the extract had a distinctive odor, a dark red color, a thick consistency, and a bitter taste. The observed color aligns with the characteristic hue of anthocyanins, as reported in the literature; according to Andriyani *et al.* [27], anthocyanins have a red-violet color.

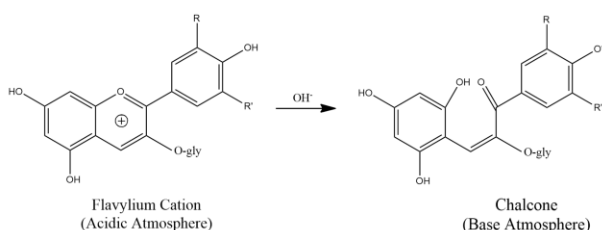

Figure 2. Anthocyanin reaction in acidic and base atmospheres

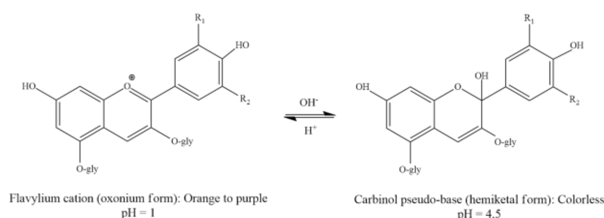

Figure 3. Structure of anthocyanin at pH 1 and 4.5

The percentage of internal and external mineral content in the extract was determined by measuring the total ash content [28]. The average total ash content of the rambutan peel ethanol extract was 2.73 ± 0.17%, which is consistent with the findings of Faramayuda *et al.* [29], who reported a value of 2.74 ± 0.04%. The ash content represents the mineral components present in the extract, including oxidizing minerals such as Mn, Cr, and Cd, as well as non-oxidizing minerals like Ca, Na, and K. A low total ash content in the ethanol extract of rambutan peel is essential to minimize anthocyanin oxidation [28].

The percentage of external factor contamination was determined by measuring the acid-insoluble ash content [30]. The average acid-insoluble ash content of the rambutan peel ethanol extract was 0.23 ± 0.02%, which is lower than the value reported by Faramayuda *et al.* [29] (0.65 ± 0.13%). Acid-insoluble ash represents contamination from external sources, such as silica found in sand and soil [30].

The determination of ethanol-soluble extract content was based on measuring the weight of the precipitate obtained from the extract. This test aimed to assess the amount of active compounds dissolved in ethanol [26]. The average ethanol-soluble extract content of the rambutan peel ethanol extract was 17.02 ± 0.04%.

Table 2. Results of color testing on buffer solution

Buffer solution	Color solution of methanol fraction of rambutan peel	Maximum wavelength (nm)
pH 1	Red +++	517
pH 2	Red +++	515
pH 3	Red +++	513
pH 4	Red ++	513
pH 5	Red +	513
pH 6	Brown +	508
pH 7	Brown ++	500
pH 8	Brown +	540
pH 9	Brown ++	546
pH 10	Brown +++	541
pH 11	Brown +++	550
pH 12	Green ++	583
pH 13	Green +++	585
pH 14	Green +++	607

Table 3. Calculation results of method validation parameters for anthocyanin content analysis

Parameter	Strong acid-strong base titration		Weak acid-strong base titration		Condition [10]
	Phenolphthalein indicator	Methanol fraction powder of rambutan peel	Phenolphthalein indicator	Methanol fraction powder of rambutan peel	
Presicion (%)	0.77	0.39	0.44	0.57	< 2
Accuracy (%)	101.98	101.40	100	100.86	98-102
Linearity	0.9986	0.9985	0.9981	0.9981	> 0.997

Figure 4. The color variation of anthocyanins in buffer solutions at different pH levels: (a) pH 1, (b) pH 2, (c) pH 3, (d) pH 4, (e) pH 5, and (f) pH 6

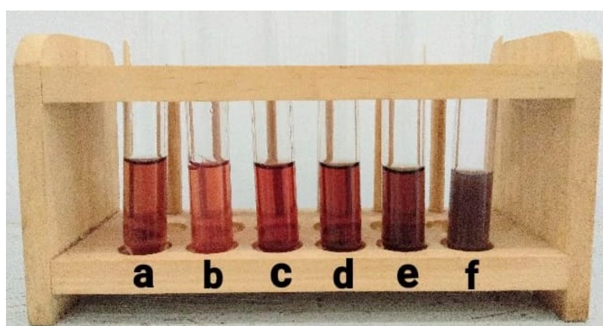

Figure 5. The color variation of anthocyanins in buffer solutions at different pH levels: (a) 7, (b) 8, (c) 9, (d) 10, (e) 11, and (f) 12

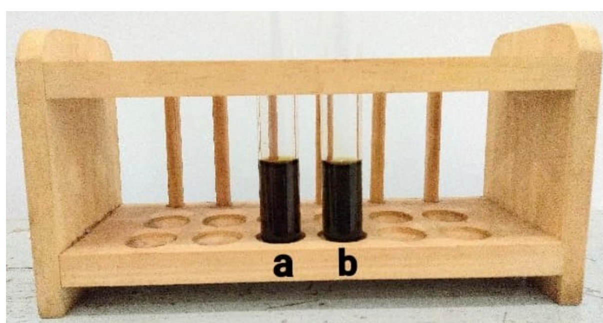

Figure 6. Anthocyanin color in buffer solutions at pH (a) 13 and (b) 14

The determination of water-soluble extract content was based on measuring the weight of the precipitate obtained from the extract. This test aimed to assess the amount of active compounds dissolved in water [26]. The average water-soluble extract content of the rambutan peel ethanol extract was 18.16 ± 0.03%. The water-soluble extract content was higher than the ethanol-soluble extract content. This difference is attributed to the variation in polarity between water and ethanol, as water has a higher polarity than ethanol. As a result, polar active compounds, particularly anthocyanins, dissolve more readily in water [31].

The fractionation of rambutan peel was performed to separate secondary metabolites based on their polarity levels selectively [32]. In this study, fractionation was conducted using the liquid-liquid extraction method. The solvents used for fractionation were n-hexane, water, and methanol, selected based on their varying polarity levels. This selection ensured that the fractionated metabolites were optimally distributed according to their polarity, allowing for more specific separation.

n-Hexane was used to extract resins or sap, alkaloids in their base form, and steroids, while water was used to extract flavonoids (especially anthocyanins), tannins, and phenols [33]. After extraction with water, methanol was employed to further extract anthocyanins due to its similar polarity to water [34]. From the fractionation of 25 grams of rambutan peel, the process yielded a water fraction of 47.11% and a methanol fraction of 45.48%.

Freeze-drying was performed to dry the methanol fraction obtained, enhancing its stability. From 25 grams of the viscous methanol fraction of rambutan peel, 7 grams of dried fraction was obtained. This process operates under high pressure and low temperatures, allowing water to evaporate rapidly and facilitating faster drying. The working principle of freeze-drying involves the direct transition of a substance from solid to gas through sublimation without passing through the liquid phase. The drying process consists of three stages: pre-freezing, primary, and secondary. Pre-freezing involves cooling the sample below its eutectic temperature, the lowest melting point of a substance. Primary drying removes water through sublimation at a temperature and pressure below the triple point of 0.0099°C and 4.579 mmHg. Secondary drying eliminates any remaining water by maintaining low pressure while gradually increasing the temperature of the freeze dryer [35].

Qualitative testing of flavonoids and anthocyanins in the extracts and fractions of rambutan peel was conducted to identify their presence. The qualitative test for flavonoids involved the addition of Mg and HCl powder, which protonates flavonoids in the benzopyrone core, leading to the formation of flavonoid salts [36]. The test confirmed the presence of flavonoids in the ethanol extract and methanol fraction of rambutan peel, as indicated by the formation of a red color in the amyl alcohol layer. The reaction is illustrated in Figure 1.

Table 4. Results of strong acid–strong base titration

Indicator	Concentration (N)	Average volume of NaOH (mL)	Real level (N)
Phenolphtalein indicator	0.0567	5.95	0.0596
	0.0652	6.76	0.0678
	0.0761	7.75	0.0777
	0.0845	8.65	0.0867
	0.0929	9.53	0.0956
	0.1052	10.65	0.1068
	0.1162	11.7	0.1173
	0.1249	12.6	0.1263
Methanol fraction powder of rambutan peel	0.0567	5.68	0.0570
	0.0652	6.53	0.0655
	0.0761	7.6	0.0762
	0.0845	8.53	0.0855
	0.0929	9.52	0.0954
	0.1052	10.58	0.1061
	0.1162	11.75	0.1178
	0.1249	12.46	0.1250

Table 5. Results of weak acid–strong base titration

Indicator	Concentration (N)	Average volume of NaOH (mL)	Real level (N)
Phenolphtalein indicator	0.0633	6.2	0.0633
	0.0730	7.15	0.0730
	0.0814	7.96	0.0814
	0.0909	8.9	0.0909
	0.1037	10.15	0.1037
	0.1139	11.15	0.1139
	0.1231	12.05	0.1231
	0.1343	13.15	0.1343
Methanol fraction powder of rambutan peel	0.0633	6.31	0.0645
	0.0730	7.21	0.0737
	0.0814	8.03	0.0821
	0.0909	9.03	0.0923
	0.1037	10.21	0.1043
	0.1139	11.28	0.1152
	0.1231	12.18	0.1245
	0.1343	13.28	0.1357

Qualitative tests for anthocyanins in the extracts and fractions of rambutan peel revealed a red color in an acidic environment after adding HCl. This occurs because anthocyanins exist in the form of flavylium cations, which impart a red hue to the sample solution [37]. Conversely, the addition of NaOH results in a green color due to the transformation of anthocyanins into chalcone forms, causing the red color to disappear [8]. The structure of anthocyanins in an acidic and base atmosphere is shown in Figure 2.

The total anthocyanin content in the methanol fraction of rambutan peel was 44.252 ± 0.289 mg/L, measured using the pH differential method with buffer solutions at pH 1.0 and 4.5. This method was selected due to structural variations of anthocyanins at different pH levels. At pH 1.0, anthocyanins appear red due to stable oxonium compounds, while at pH 4.5, they become colorless as they transform into hemiketal carbinol compounds [37]. The structural forms of anthocyanins at pH 1 and 4.5 are illustrated in Figure 3.

The analysis of total anthocyanin content was conducted at various wavelengths, including the maximum absorption wavelength of anthocyanins 516 nm and 700 nm, the latter being used to assess sample clarity and contamination. A sample is considered clear if the absorbance value at 700 nm is zero [38]. The absorbance measurements at 700 nm were 0.015 in the pH 1.0 buffer solution and 0.016 in the pH 4.5 buffer solution, indicating the presence of impurities or contamination in the sample.

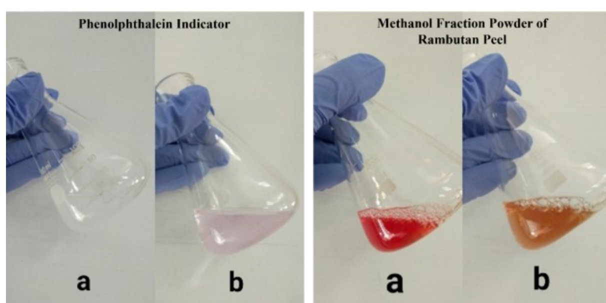

Figure 7. Color change in strong acid–strong base titration: (a) before titration, (b) after titration

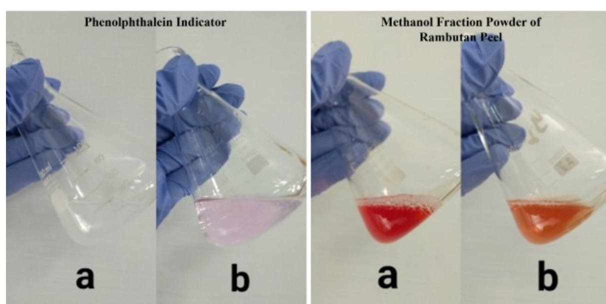

Figure 8. Color change in weak acid–strong base titration: (a) before titration, (b) after titration

The results of the color testing on the buffer solution are presented in Table 2, showing significant changes in color and wavelength in the pH 6–14 range. The color variation of anthocyanins in buffer solutions at pH 1-14 is presented in Figures 4-6. The observed wavelength shift follows a bathochromic trend, driven by structural changes in anthocyanins from the flavylium cation form in an acidic environment to the chalcone form in an alkaline environment. This shift occurs as the number of π bonds and auxochrome groups increases, causing the absorption wavelength to move further into the bathochromic region [5].

The method validation parameters, including precision, accuracy, and linearity, were determined using acid-base titration by observing the titration endpoint. The color changes in strong and weak acid-base titration are presented in Figures 7 and 8. The titration results of strong and weak acid-base titration are presented in Tables 4 and 5. The results of the method validation parameter calculations are shown in Table 3. The precision parameters in method validation were aimed at determining the correlation of homogeneous samples through repeated measurements [10]. The precision results for strong acid-base and weak acid-base titrations, using the methanol fraction powder of rambutan peel as an indicator and phenolphthalein as a comparison, yielded %RSD values of 0.77, 0.39, 0.44, and 0.57%, respectively. All %RSD values indicate that the methanol fraction powder of rambutan peel has good precision as an acid-base indicator, as they meet the precision requirement of <2% [10].

The accuracy parameters in method validation were assessed to determine the recovery of analyte levels in the sample by comparing the test results to the actual analyte levels [10]. The accuracy results for strong base-strong acid and weak base-strong acid titrations, using the methanol fraction powder of rambutan peel as an indicator and phenolphthalein as a comparison, yielded % recovery values of 101.98, 101.40, 100, and 100.86%, respectively. All % recovery values indicate that the methanol fraction powder of rambutan peel has good accuracy as an acid-base indicator, as they meet the accuracy requirement of 98–102% [10].

The linearity parameters in method validation were assessed to determine the relationship between concentration and response [10]. The linearity results for strong base-strong acid and weak base-strong acid titrations, using the methanol fraction powder of rambutan peel as an indicator and phenolphthalein as a comparison, yielded R^2 values of 0.9986, 0.9985, 0.9981, and 0.9981, respectively. All R^2 values indicate that the methanol fraction powder of rambutan peel exhibits good linearity as an acid-base indicator, as they meet the linearity requirement of > 0.997 [10].

The method validation parameters were statistically analyzed using SPSS with the Mann-Whitney test to determine whether there were significant differences between the validation parameters of rambutan peel methanol fraction powder and the phenolphthalein indicator. Since the data groups in this study were independent, they were categorized as an independent group. The normality test results indicated that the data were not normally distributed ($p < 0.05$), necessitating the use of the Mann-Whitney test. The significance values obtained for precision, accuracy, and linearity were 0.439, 1.000, and 0.683, respectively. All significance values indicated no significant differences between the two indicators ($p > 0.05$) [15].

4. Conclusion

The research findings indicate that the methanol fraction powder of rambutan peel (*Nephelium lappaceum* L.) functions effectively as an acid-base titration indicator, showing no significant difference from the phenolphthalein indicator, with a significance value greater than 0.05. The method validation results, including precision, accuracy, and linearity, support its applicability as an acid-base indicator.

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