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Evaluation of anthocyanin extracted from Lempeni (*Ardisia Elliptica* l.) fruit as a new natural acid-base indicator

Abstract: The *Ardisia elliptica* species is usually known as Lempeni fruit has a purplish red color which contains the color pigment of anthocyanins. This work aimed to obtain anthocyanins from Lempeni fruit and applied them as new acid-based indicators. The extraction method was carried out using a combination solvent containing ethanol and HCl with appropriate ratio, followed by the addition of anthocyanin-containing extract with acid and base solutions. The results showed that anthocyanin from Lempeni fruit induced a signicant color response depending on the pH values of acid-base solutions. The color was changed to red in acidic environments and brownish green in alkaline environments. Besides, the filter paper indicator and acid-base titrations were performed with good performances. The utilization of a natural acid-base indicators from anthocyanins offers potential applications in qualitative analysis and scientific education, by exploring new sources of anthocyanins. This research contributes to the understanding of the potential use of anthocyanins from Lempeni fruit in environmentally friendly and economical analytical chemistry applications.
Key words: Extraction, Anthocyanin; Lempeni fruit; Natural indicator; Acid base.

Introduction

Anthocyanins are a group of water-soluble pigments widely distributed in the plant kingdom, particularly in fruits, vegetables, and flowers [1]. These natural compounds are responsible for a wide range of colors such as red, purple, and blue, which vary depending on their molecular structure and the pH of their environment [2]. In acidic conditions, anthocyanins typically appear red, while in neutral conditions they may turn purple, and in alkaline environments they can shift to bluish-green [3]. This reversible color change makes anthocyanins not only visually striking but also functionally useful as natural pH indicators [4]. Their ability to respond sensitively and rapidly to pH variations has attracted significant attention for applications in analytical chemistry, environmental monitoring, food packaging, and even cosmetics.

Indicators are substances that exhibit a specific color at a particular pH level and undergo a noticeable color change when the pH shifts [5]. This transformation provides a visual signal to determine whether a solution in acidic or basic. While synthetic indicators such as phenolphthalein, methyl orange, bromothymol blue, and methyl red are commonly used in laboratories, they often raise concerns due to toxicity, high production costs, and environmental persistence [6]. In light of these concerns, researchers have increasingly turned to plant-based alternative that are biodegradable, less toxic, and often more accessible [7]. Natural indicators derived from plant pigments offer a safer and more sustainable option for educational, laboratory, and field-based pH testing [8].

Several studies have demonstrated the extracts of anthocyanins from various plant sources for use as natural indicators such as purple cabbage, magenta, amaranth, red beet, flamingo lily, passion flower, garden dahlia, mulberry, jungle flame flower, *Butea monosperma*, etc. [9, 10]. Besides, Ernawati and Rahayu investigated the extraction of anthocyanins from mangosteen peel using ethanol: HCl solvents [11]. Their results indicated that the ethanol-HCl solvent combination produced the most concentrated extract with the clearest and most intense color transitions across acidic and basic conditions when applied to filter paper. This study highlighted the importance of solvent selection of optimizing anthocyanin yield and indicator effectiveness, setting a precedent for further research into other anthocyanin-rich plant materials.

One such promising yet underutilized source is Lempeni (*Ardisia elliptica* L.) fruit, which is known

for its deep coloration and significant anthocyanin content [12]. Locally known in Indonesia as "lempeni," this fruit refers to *Ardisia elliptica*, a tropical shrub or small tree native to Southeast Asia, including Indonesia, Malaysia, and India. Figure 1 shows the Lempeni fruit with black peel color. Despite its abundance in certain tropical regions, Lempeni fruit has not been extensively studied for its chemical properties or potential applications. Utilizing this fruit as a raw material for natural pH indicators not only adds value to a locally available botanical resource but also supports the development of green analytical techniques. The exploration of such lesserkwon species contributes to biodiversity utilization and promotes innovation in sustainable chemistry practices.

In this work, anthocyanins were extracted from Lempeni fruit using the maceration methods, with a solvent mixture of 96% ethanol and 1% HCl (1:1), over a 24-h period. The resulted extract was evaluated across a full pH spectrum (pH 1-14), both through spot tests and application on filter paper, to observe the color changes at various pH levels. Furthermore, the usability of the extract in acid-base titrations will be tested to assess its functionality compared to conventional synthetic indicators. By examining both the qualitative and quantitative performance of Lempeni's anthocyanins, this study aimed to validate their effectiveness in real-world analytical scenarios. The broader goal of this work was to advance the development of natural, biodegradable, and cost-effective pH indicators derived from local plant sources.

Figure 1 – The Lempeni fruit appearance

Materials and methods

Materials and Equipment. Lempeni fruit samples were collected directly from the coastal area of Kuala Baru Laut, Aceh Singkil, Indonesia. The chemical reagents involved included distilled water (H_2O), 96% ethanol (C_2H_5OH), hydrochloric acid (HCl), sodium hydroxide (NaOH), acetic acid (CH_3COOH), and ammonium hydroxide (NH_4OH). The equipment employed in this study consisted of a stir bar, grinder, filter paper, burette, funnel, Erlenmeyer flask, beaker, volumetric flask, analytical balance, dropper pipette, measuring pipette, drop plate, test tubes, rotary evaporator, and a UV-Vis spectrophotometer.

Lempeni fruit extraction. 200 grams fruit were washed, drained, and dried until the fruit was completely dried. Then, the samples were ground into a fine powder using a grinder. 50 grams of the powder were placed into 500 mL beaker containing a solvent (96% ethanol + 1% HCl, 1:1). The mixture was stirred using a glass spatula to ensure the complete mixing of them. The mixture was subsequently subjected to maceration at room temperature and kept for 48 hours, with occasional stirring every 6 hours. After that, the mixture was filtered using a Whatman filter paper and concentrated using a rotary evaporator to obtain the fruit extract without solvent.

Determination of anthocyanins. To determine the maximum wavelength, Lempeni fruit extract was placed into a cuvette and analyzed using a UV-Vis spectrophotometer within the wavelength range of 400-800 nm. The maximum absorbance value was then recorded. Following this, a qualitative test for anthocyanin content was carried out using two methods. In the first method, 2 M HCl was added to the extract in a test tube, which was then heated at 100°C for 2 minutes. The sample was observed for any color change, if the red color remained stable, it indicated the presence of anthocyanins. In the second method, 2 M NaOH was added dropwise to a separate sample. A color change from red to blue-green that gradually faded confirmed the presence of anthocyanins [13].

Testing Lempeni Fruit Extract as an Indicator. Five drops each of HCl, NaOH, CH_3COOH, and NH_4OH solutions were placed into separate wells of a drop plate. The pH of each solution was measured using a pH meter. Subsequently, 5-8 drops of Lempeni fruit extract were added to each well, and the resulting color changes were observed to assess the extract's response at known pH levels.

Preparation of Acid-Base Indicator Paper. Whatman filter paper was used as the base for the indicator. Lempeni fruit extract was poured into a basin, and the filter paper was fully immersed in the extract for approximately 2 hours. After soaking, the paper was removed and placed on a tray or baking sheet to air dry. Once completely dry, the paper was observed for any color development, then cut into strips measuring 1 x 4.5 cm. These strips, referred to as Lempeni fruit indicator paper, were then tested in various solutions including HCl, NaOH, CH_3COOH, and NH_4OH. The indicator paper was considered stable if it turns dark red or pink in acidic solutions and changes to green, yellow, or purple in alkaline solutions.

Titration Using Lempeni Fruit Extract as an Indicator. A total of 10 mL of a strong acid solution (0.1 N HCl) was pipetted into a 250 mL Erlenmeyer flask, followed by the addition of 3 drops of Lempeni fruit extract as an indicator. The solution was then titrated with a strong base (0.1 N NaOH) until a visible color change occurred. This procedure was repeated three times, and the volume of NaOH used in each titration was recorded. The same method was applied to a strong base (0.1 N NaOH), which was titrated with a strong acid (0.1 N HCl). Additionally, titrations were performed for a weak acid (0.1 N CH_3COOH) using a strong base (0.1 N NaOH), and for a weak base (0.1 N NH_4OH) using a strong acid (0.1 N HCl), with the volume of titrant used in each case being recorded. For comparison, each titration was also repeated using standard indicators (phenolphthalein and methyl orange) in place of the Lempeni fruit extract.

Results and discussion

Lempeni fruit extraction process. The extraction of Lempeni fruit was conducted using the maceration technique, a method that involves soaking plant material in a solvent to draw out the desired compounds [14]. Initially, the fruit is ground with a grinder, then immersed in an organic solvent for a specified duration. Maceration is widely favored for isolating natural substances due to its simplicity and low cost [15]. During soaking, pressure differences inside and outside the cell walls cause them to break down, allowing secondary metabolites in the cytoplasm to dissolve into the solvent, resulting in effective extraction. The soaking time can be adjusted as needed. In this process, maceration was performed over 48 hours (2×24 hours), with stirring every 6 hours. After maceration, the extract was filtered to separate the solid residue from the liquid extract. The ideal extraction time was found to be two days, as longer contact between the solvent and the plant material allows for more thorough extraction. However, if this contact exceeds the optimal duration, compound degradation can occur [16]. A limitation of the maceration method is its relatively low efficiency, as only about 40% of the active compounds are extracted, and some may become less soluble. Therefore, temperature evaporation is used to concentrate the extract. This is done with a rotary evaporator, which effectively removes the solvent without damaging the compounds. However, excessive pressure during evaporation can degrade sensitive compounds like anthocyanins. In this process, the extract was concentrated at 40°C for 90 minutes, resulting in a thick red Lempeni fruit extract.

Determining the maximum wavelength of Lempeni fruit extract. To identify the maximum wavelength of Lempeni fruit extract, a UV-Vis spectrophotometer was used. This instrument measures absorbance within the visible light spectrum, which ranges from 400 to 800 nm. This range is suitable because the compounds responsible for anthocyanins which typically absorb light within this region. Based on Figure 2, the Lempeni fruit extract exhibited its peak absorbance within the 400-800 nm range and the broad absorption peak ranged from 450 to 550 nm, which is consistent with the absorption spectrum of the anthocyanin color groups [17]. This peak absorbance was essential in identifying the most effective wavelength for analyzing or utilizing the extract, such as in dye applications or compound quantification. The wavelength at which this maximum occurs represents the optimal point for future spectrophotometric measurements of the extract.

Anthocyanin verification test. To confirm the presence of anthocyanins in Lempeni fruit extract, a qualitative test was conducted using acidic and basic solutions. Anthocyanins are pH-sensitive pigments, so their color tends to change depending on the pH level of the solution [18]. In this test, 2 M HCl, a strong acid, was added to the Lempeni fruit extract to observe any visible color change. The mixture was then heated for 5 minutes to ensure proper reaction. After heating, it was observed that the color of the Lempeni fruit extract remained unchanged compared to its initial appearance. Figures 3a-b show the original extract as a control sample and the extract after the addition of 2 M HCl after heating, respectively. Un acidic condition, the color of solution was consistent, suggesting that the extract contains anthocyanins which tend to maintain their red to purple in acidic environments. This finding confirms that anthocyanins are presenting in Lempeni fruit extract, indicating the stability of this pigment under acidic conditions.

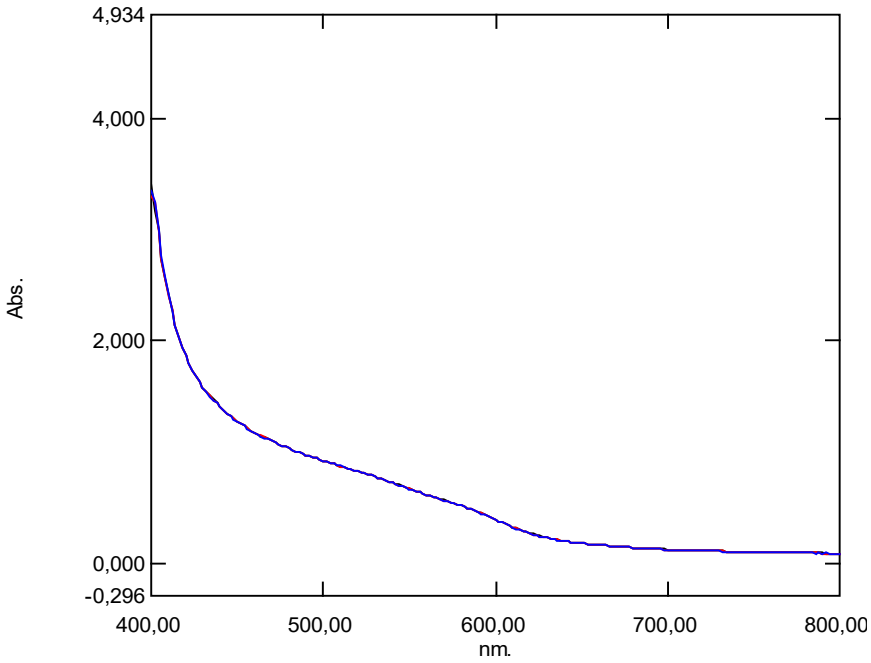

Figure 2 – The UV-Vis absorbance of anthocyanins from Lempeni fruit extract

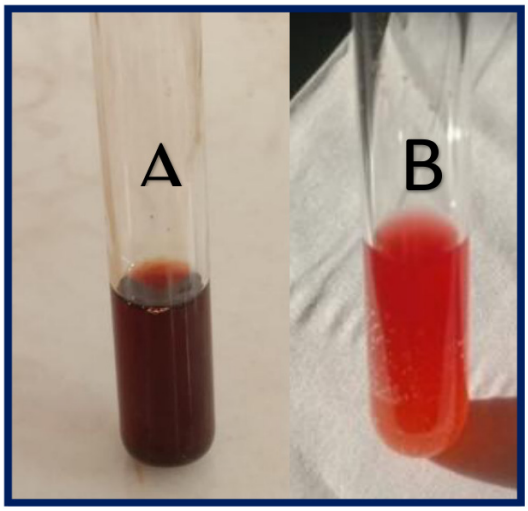

Figure 3 – Color change of extract with HCl solution

In the next phase of the anthocyanin verification test, the Lempeni fruit extract was treated with a strong base to observe its response to alkaline conditions. A few drops of 2 M NaOH solution were added to the extract. As a result, a visible color change occurred from the original hue to a brownish green, as shown in Figure 4. Figure 4 visually compares the untreated extract (A) with the extract after the addition of 2 M NaOH (B). The transition to a brownish green color upon exposure to an alkaline environment is a clear indicator of the presence of anthocyanins. The solution appeared in red to pink color in the acidic conditions and changed to green or yellow-brown color under alkaline conditions.

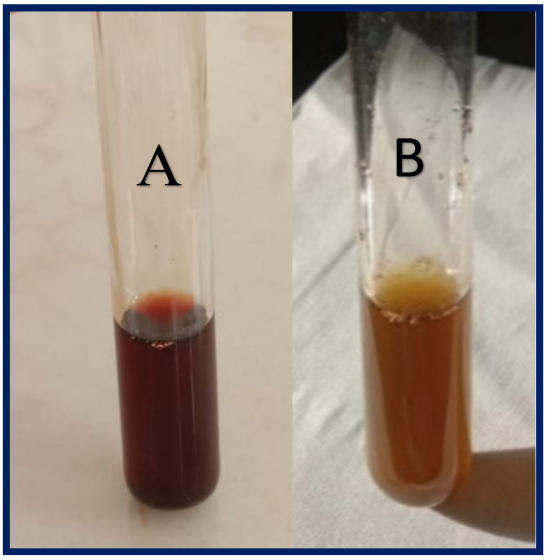

Figure 4 – The appearance of extract' color change after adding NaOH solution

This color change further illustrates a typical trait of anthocyanins, as their molecular structure changes with pH variations, influencing their light absorption and reflection properties. The observation showed that the color of the extract changed to brownish green after adding NaOH as a basic solution. But it remained pink color in acidic solutions even after heating (Figure 3), indicating the presence of anthocyanin compounds. In addition, the anthocyanin induces greater stability in acidic conditions, whereas they commonly produce color's change in basic conditions owing to their reactivity and cationic characters. This phenomenon not only demonstrates the anthocyanin existence but also has potential as a new natural indicator.

Performance of natural indicator from extract. Anthocyanins exhibit several colors based on the pH values of environments. These typical characteristics offer them suitable as the pH indicators. Before the use of Lempeni fruit extract as a natural acid-base indicator in titration, it is important to identify its certain pH range for color changes. Subsequently, the extract was introduced into two acidic solutions (HCl and CH_3COOH) and two basic solutions (NaOH and NH_4OH) in order to test their color changes. Figure 5 and Table 1 exhibit a visual observation of the color changes when the extract interacted with the solutions of HCl (1), NaOH (2), CH_3COOH (3), and NH_4OH (4). The extract color appeared from purplish red to vivid red in acidic solutions. Meanwhile, the extract color changed from purplish red to brownish green in basic solutions. These naked eye color changes are specific character of anthocyanins, which react susceptibly to pH changes.

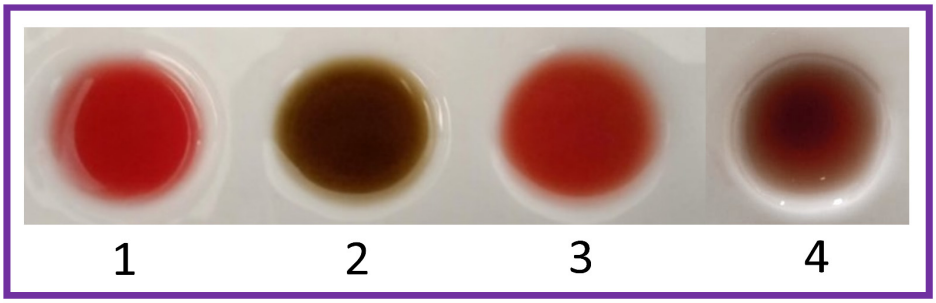

Figure 5 – The change in color of acid-base solutions after mixing with the extract

Table 1 depicts the results of the discoloration of the extract mixing with several solutions. The fruit extract was still in red in acid solutions (HCl and CH_3COOH), indicating the stability of anthocyanins. In NaOH, which is a strong base, the extract changed to dark green, while in NH_4OH, a milder base, it transformed into a brownish red. These unique changes further validate the existence of anthocyanins and illustrate their effectiveness as a natural pH indicator. Color variation happens as anthocyanins experience structural modifications at various pH levels, resulting in different molecular forms [19]. At low pH, they primarily appear as flavylium cations, which are red in color. As the pH rises, the structure changes, resulting in greenish or brownish shades caused by the degradation or creation of quinonoidal and chalcone forms [20]. Understanding this behavior is crucial for employing Lempeni fruit extract successfully as a natural and eco-friendly indicator in acid-base titrations.

Table 1 – The color change of extract after adding into acidic and basic solutions

Extract	Solution	Type	Discoloration
Lempeni Fruit	HCl	Strong Acid	Red
	NaOH	Strong Base	Brownish green
	CH_3COOH	Weak Acid	Red
	NH_4OH	Weak Base	Brownish red

Acid-base indicator paper from Lempeni fruit extract. Lempeni fruit extract served as an acid-base indicator due to its ability to change color when exposed to various solutions such as HCl, NaOH, CH_3COOH, and NH_4OH. According to Ernawati and Rahayu [21], filter paper is commonly used for this purpose. Filter paper, made of pure cellulose, has excellent absorption properties. As shown in Figure 6, when dipped in HCl and CH_3COOH, the indicator paper made with Lempeni extract did not show any color change. However, it turned dark green in NaOH and brownish in NH_4OH. The observed color changes are due to the purplish-red pigment found in anthocyanins, which can function as chemosensory compounds in acid-base indicators. Anthocyanins are sensitive to pH changes; in acidic environments (pH < 7), the paper remains red, while in basic environments (pH > 7), it shifts to dark green. Therefore, based on the above data, Lempeni fruit extract can be effectively used as a natural pH indicator.

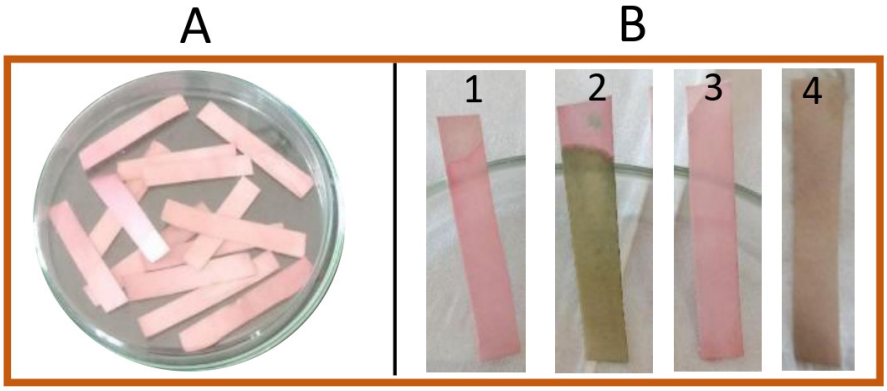

Figure 6 – (a) Filter paper and (b) the results of color changes after being dipped in acidic and basic solutions

Lempeni fruit extract indicator for acid-base titration. Acid-base indicators can exist in neutral, positively charged, or negatively charged forms [22]. Some indicators display a single color, with that appearance affected not only by pH but also by the indicator's concentration. For instance, phenolphthalein (PP) is a single-color indicator that works within a pH range of 8.0-9.6 where colorless in its acidic form and pink in its basic form [23]. Lempeni extract shows promise as a natural acid-base indicator. To evaluate its effectiveness, acid-base titration experiments were performed using this extract alongside standard indicators like PP and methyl orange (MO). Both acid and base concentrations were set at 0.1 N. The titrations conducted included four combinations: strong acid with strong base, strong base with strong acid, strong acid with weak base, and strong base with weak acid. Specifically, the titration pairs were NaOH vs HCl, HCl vs NaOH, NaOH vs CH_3COOH, and HCl vs NH_4OH. In each experiment, three drops of the Lempeni extract, PP, and MO were added to the titrand, with 10 mL of titrant used. Initial colors were recorded immediately after adding the indicators. The findings exhibited that the fruit extract could identify titration endpoints accurately, nearly matching those observed with the standard and commercial indicators. The change of pink color to green color happened at almost same volume addition, indicating that the anthocyanin derived from the fruit extract responded similarly to the commercial indicators. The pink color in acidic solutions changed to brownish in basic ones, informing that the extract functions as a pH indicator for basic solutions. The changes in color dan pH values across different titration combinations are summarized in Table 2. Hence, the results suggest that the Lempeni fruit extract is an alternative indicator toward commercial acid-base indicators.

Table 2 – The changes in colors and pH values at the end point of the titration

Titrand	Titrant	Indicator	Color Changes	pH
	NaOH			
HCl	9.05 mL	Phenolphthalein	Colorless to pink	8.52
10.00 mL	9.10 mL	Methyl Orange	Orange to yellow	6.23
	9.95 mL	Fruit Extract	Pink to brownish green	8.55
	HCl			
NaOH	12.70 mL	Phenolphthalein	Pink to colorless	6.83
10.00 mL	12.25 mL	Methyl Orange	Yellow to orange	3.86
	12.90 mL	Fruit Extract	Brownish green to colorless	6.88
	NaOH			
CH_3COOH	7.20 mL	Phenolphthalein	Colorless to pink	8.40
10.00 mL	7.50 mL	Methyl Orange	Yellow to orange	4.23
	7.35 mL	Fruit Extract	Pink to brownish green	8.38
	HCl			
NH_4OH	8.10 mL	Phenolphthalein	Pink to colorless	5.02
10.00 mL	8.50 mL	Methyl Orange	Yellow to pink	3.65
	8.97 mL	Fruit Extract	Brownish red to colorless	5.30

Conclusions

In this study, the anthocyanin compound from Lempeni fruit was extracted and used as a natural acid-base indicator. Using the maceration method with a mixture of 96% ethanol and 1% HCl, the anthocyanin-rich extract was obtained and tested across a broad pH spectrum. Results showed consistent and distinct color changes, shifting from red in acidic environments to brownish green and brownish red under alkaline conditions. These observations, supported by UV-Vis spectrophotometric analysis, confirmed the presence and stability of anthocyanins in the extract, particularly within the 450-550 nm absorbance range. The extract's color sensitivity to pH made it a valuable candidate for various applications. When tested on indicator paper, Lempeni extract produced clear and predictable color changes in response to strong and weak acids and bases. More importantly, in titration experiments, the extract reliably indicated endpoint detection, performing comparably to standard synthetic indicators such as phenolphthalein and methyl orange. These results illustrate the accuracy and real-world utility of Lempeni fruit extract in analytical chemistry. As a plant-derived, biodegradable substitute for synthetic indicators, it provides both ecological and economic benefits. Its ease of use and efficiency also render it a useful educational resource for instructing on acid-base reactions in an eco-friendly manner. In general, Lempeni anthocyanins demonstrate essential attributes of a natural pH indicator, underscoring the fruit's viability a local resource for sustainable analytical practices in both educational and laboratory environments.

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Conflict of interest

All authors are aware of the article's content and declare no conflict of interest.

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