

## Research Article

# Blooming chemistry: Vinca as your pocket-friendly pH indicator

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## ABSTRACT

This study investigates the extraction of anthocyanins from Vinca flower by cold extraction method. The pH sensitivity of the extracts was evaluated across a broad range (pH 1-14), with distinct color changes observed: red/pink in acidic conditions (pH 1-3), colorless at pH 5, green in the neutral to slightly alkaline range (pH 7-9), and yellow in strongly alkaline solutions (pH 11-14). Ultraviolet-visible spectroscopy confirms anthocyanins as a primary pigment with additional sugars. In acid-base titration experiments the Vinca flower provide a clear, concise and reliable pH endpoints, comparable to synthetic indicators involving sodium hydroxide versus hydrochloric acid (Methyl Red (10.339), Methyl Orange (10.350), Phenolphthalein (10.348); Vinca: Purple (10.351), Red (10.348), Violet (10.346), Pink (10.346), Light Pink (10.346), White (10.351), hydrochloric acid versus ammonium hydroxide (Methyl Red (10.380), Methyl Orange (10.368), Phenolphthalein (10.381); Vinca: Purple (10.383), Red (10.382), Violet (10.38), Pink (10.385), Light Pink (10.386), White (10.387), and sodium hydroxide versus acetic acid (Methyl Red (10.339), Methyl Orange (10.343), Phenolphthalein (10.36); Vinca: Purple (10.347), Red (10.346), Violet (10.363), Pink (10.366), Light Pink (10.363), White (10.368). The pH endpoints observed with the Vinca extracts closely matched those obtained with the synthetic indicators, demonstrating their accuracy and reliability. This study highlights the unique application of Vinca flowers as a natural, eco-friendly, and cost-effective substitute for synthetic pH indicators in both educational and practical settings.

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## INTRODUCTION

In chemistry, indicators play an essential role in determining chemical conditions, such as pH levels, end points, or equivalence points, through visible color changes [1]. Traditional pH indicators, such as litmus paper, undergo color transitions [2] in response to varying acidity, neutrality, or alkalinity [3]. For instance, litmus paper is red in acidic conditions (low pH), blue in basic conditions (high pH), and remains purple or unchanged in neutral conditions [4].

Most commonly used indicators today are synthetic, organic compounds, [5] including thymol blue, phenolphthalein, methyl orange, methyl red, and others [6, 7]. These indicators are reli-

able and produce distinct color changes, [8] but they often come with significant drawbacks: toxicity, environmental pollution, high cost, and reactivity with the substances being tested [9]. Given these limitations, the scientific community is increasingly turning to natural indicators—compounds derived from plant sources—as more sustainable alternatives [10].

Natural indicators, such as those derived from anthocyanins in plants, [11] have been used for centuries due to their eco-friendly nature, ease of use, and simplicity in chemical analysis [12, 13]. Examples include litmus from lichens, which turns red in acidic environments and blue in alkaline media with pH range 4.5-8.3, [14] and anthocyanins from plants like red cabbage, which transition from red to blue depending on the pH [15].

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These indicators are particularly popular in educational settings due to their cost-effectiveness and straightforward application [16, 17]. However, they tend to have a narrower pH range and can be less precise than synthetic indicators, with their accuracy sometimes affected by environmental factors like light and temperature [18].

Natural indicators are environmentally friendly indicators that have a variable pH range, exhibit dynamic color change similar to synthetic ones [19], some examples are given in Table 1. Among the variety of natural pH indicators, Vinca, commonly known as periwinkle or Madagascar periwinkle used for the treatment of cancer, tumor [20], Lymphoblastic leukemia and Hodgkin's disease [21], has garnered attention for its distinct pH-responsive color changes [22]. The plant contains anthocyanin pigments that exhibit a dramatic shift in color based on the surrounding pH. Such types of natural pH indicators not only effective for lab experiments but also useful for food safety [23], food monitoring [24], food spoilage detector [25] and as coloring agents [26].

This study focuses on exploring the pH indicator properties of six distinct-colored Vinca flowers (purple, magenta, pink, white, red, and violet). We compare their pH sensitivity and color transitions with those of synthetic indicators such as phenolphthalein, methyl orange, and methyl yellow during acid-base titration processes. The novelty of this research lies in the systematic analysis of Vinca's potential as a natural, non-toxic pH indicator, offering a more sustainable and accessible alternative to traditional synthetic dyes.

Beyond its educational value, the practical application of Vinca as a pH indicator in industries such as food science holds significant promise. Given its natural origin and non-toxic nature, Vinca could be utilized in food testing to monitor pH levels, offering a safer and more sustainable solution than synthetic alternatives. However, it's important to note that the reliability and effectiveness of Vinca as a pH indicator may be limited by factors such as the variability in pigment concentration among different flower colors and its sensitivity to light and temperature.

## EXPERIMENTAL

### Study Material

Six different color varieties of Vinca (periwinkle) flowers were collected for this study to evaluate their potential as acid-base indicators.

### Extraction of Natural Indicator

Fresh Vinca petals were separated from the flowers, washed thoroughly with distilled water to remove dust, and dried gently using tissue paper. A total of 20 g of the petals was ground using a mortar and pestle in the presence of 150 mL of 70% ethanol. The mixture was left to rest for 30 minutes to allow the extraction of anthocyanins. The resulting solution was filtered twice using Whatman filter paper to remove any flower material. The clear extract was stored for further use, in a tightly sealed brown container to protect it from light and preserve its stability.

### Analytical Chemistry Profiling of Vinca Extract

To identify the key compounds responsible for the acid-base indicator properties of Vinca, a thorough analytical profiling was performed using UV-Vis spectroscopy and TLC. The extract was examined for the presence of anthocyanins. By using Rf values and specific wavelengths, we confirmed the identity and purity of these compounds that is necessary to establish a correlation between color change and pH.

### Preliminary Activity of Vinca Extract

The preliminary effectiveness of Vinca extract as a natural acid-base indicator was explored by observing its vibrant color transformations across a wide pH spectrum. Using a pH meter, the extract was tested from highly acidic (pH 1) to strongly alkaline (pH 14) conditions. The resulting color shifts were carefully documented, revealing the extract's dynamic response to pH changes and highlighting its potential as a natural, visually striking pH indicator.

### Acid-Base titrations

0.5M standard solutions of acids ( $\text{HCl}/\text{CH}_3\text{COOH}$ ) and bases ( $\text{NaOH}/\text{NH}_4\text{OH}$ ) were titrated using few drops of synthetic (phenolphthalein, methyl orange, methyl yellow) or natural (Vinca) indicators. All the experiments were performed in triplicate to enhance the accuracy.

## RESULTS AND DISCUSSION

Vinca flowers, known for their vibrant and diverse colors, including shades of pink, purple, magenta, white, violet, and red, have demonstrated potential as natural pH indicators. Their visually striking hues not only enhance the aesthetic value of gardens but also possess inherent chemical properties that make them suitable for use in acid-base titrations. The results of this study reveal that the color changes of Vinca extract in response to varying pH conditions are comparable to those observed with conventional synthetic indicators like methyl yellow, methyl orange, and phenolphthalein. This finding highlights the potential of Vinca extract as a natural alternative for pH detection in acid-base titrations, including titrations of strong acids with strong bases, strong acids with weak bases, and weak acids with strong bases.

### Extraction Method and Solvent Choice

Cold method was used for extracting color from these flowers in 70% ethanol. The choice of solvent was supported by the literature, maximum extraction can be done in alcoholic solution and responsible for extracting both polar and non-polar components [38, 39].

**Table 1.** Some natural indicators and their comparison with Vinca

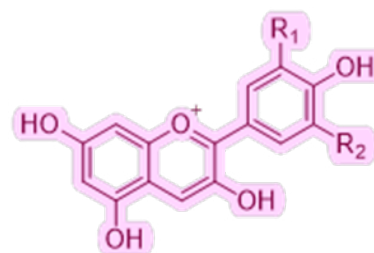
Natural Indicator	Color Change (acid-neutral-base)	pH Range	Advantages	Limitations
Turmeric [18]	yellow-yellow-red	7-9	Simple to use	Narrow pH range; less accurate
Red cabbage [27]	Red/pink-violet-blue/green	1-12	Wide pH range, inexpensive, clear color transitions	Preparation needed heating; can fade over time
Rose [28]	Purple/pink-baby pink-blue/green	4-8	Simple to use, widely available	Narrow pH range
Flame Lily [29]	Pink-pink-green	2-7	Unique color transitions	Narrow pH range; less effective
Pomegranate [30]		3-10	Wide pH range	Require extraction process
Bougainvillea [31]	Red-yellow	7-10	Visible color change	Limited pH range
Mulberry [32, 33]	Blue-pink	5.5-8.5	Simple to use, visible color change	Limited pH range; less effective
Marigold [34, 35]	Yellow-green-orange	-	Easy to use, visible color change	Sensitive to environment
Vinca [36, 37]	Red/pink-green/yellow	1-14	Non-toxic, eco-friendly, Wide range of colors, wide pH range	Sensitive to light, temperature

### Color Change Across the pH Range

The Vinca extract was tested across a full pH range (1–14), from strongly acidic to highly alkaline conditions. As shown in Figures 1-6, the extract exhibited distinct and striking color changes. In acidic conditions, the color deepened to red or pink as the acidity increased (lower pH). Around pH 5, the extract became colorless, and as the pH rose, a green hue emerged, intensifying as the pH approached 9. In highly alkaline conditions (above pH 10), a yellow color gradually appeared, growing more vibrant and darker with increasing alkalinity. These dynamic shifts in color highlight the Vinca extract's clear pH-dependent behavior.

### Connecting Experimental Data

Anthocyanins (Figure 7), a vibrant group of flavonoid phenolic compounds [26] are the key colorants in Vinca flowers. These pigments remain stable in acidic environments, maintaining their rich hues, but their color fades as the pH rises [40]. Six distinct anthocyanin structures have been identified (Table 2), each differing in the arrangement of hydroxyl, hydrogen, and methyl ester groups [41]. The aglycosylated forms of these compounds, known as anthocyanidins [26] are especially interesting, as increasing methyl ester concentrations lead to a captivating shift toward a striking blue color [42].

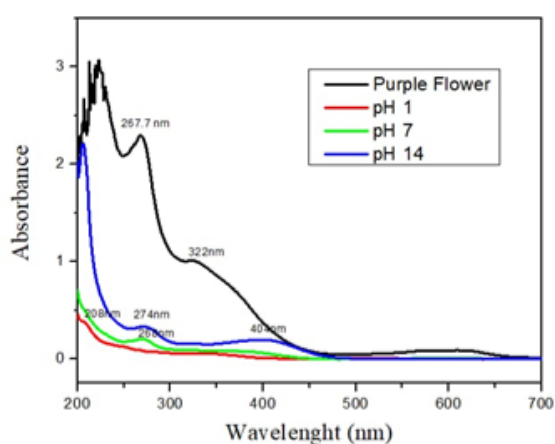
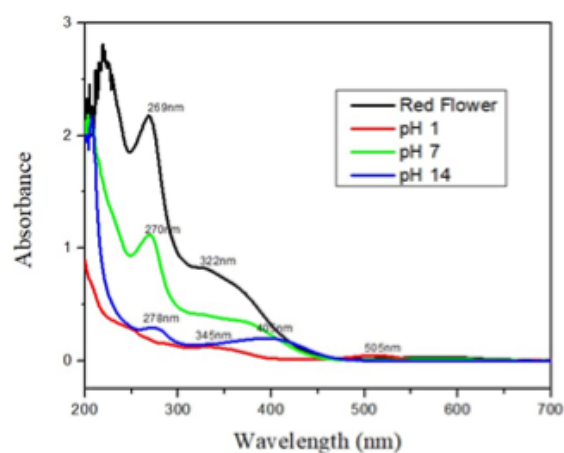
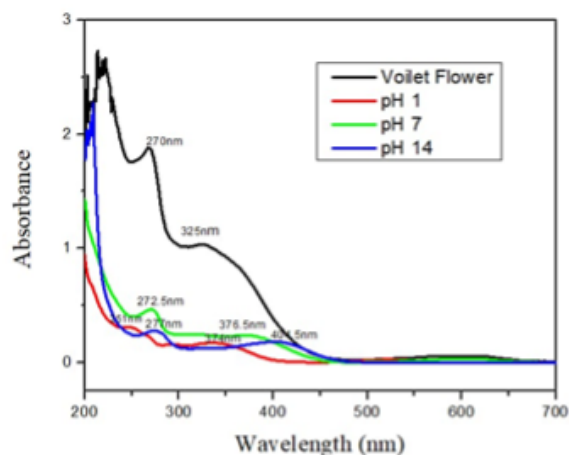
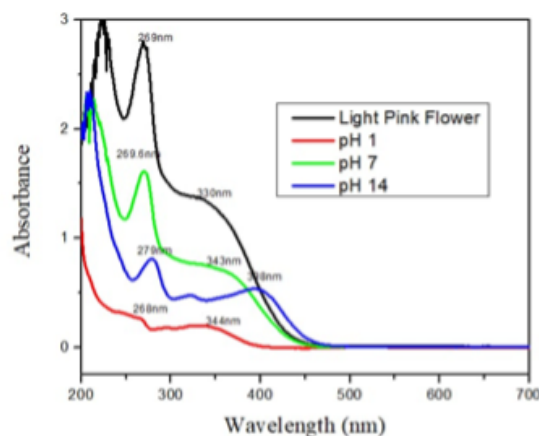
**Figure 1.** Purple flower extract at pH (1-14)**Figure 2.** Red flower extract at pH (1-14)**Figure 3.** Violet flower extract at pH (1-14)**Figure 4.** Pink flower extract at pH (1-14)**Figure 5.** Light pink flower extract at pH (1-14)**Figure 6.** White flower extract at pH (1-14)**Figure 7.** Basic structure of Anthocyanin

**Table 2.** Common Anthocyanidins that stably exist in nature [43]

Anthocyanidins	R <sub>1</sub>	R <sub>2</sub>
Pelargonidin	H	H
Cyanidin	OH	H
Delphinidin	OH	OH
Peonidin	OCH <sub>3</sub>	H
Petunidin	OH	OCH <sub>3</sub>
Malvidin	OCH <sub>3</sub>	OCH <sub>3</sub>

Anthocyanin, the key compound responsible for the color change in Vinca flowers, was confirmed through UV-Vis spectroscopy (Figures 8-13). This finding is consistent with existing literature, which indicates that anthocyanins exhibit a distinct absorption band in the UV region (260-280 nm) due to their delocalized  $\pi$ -conjugated system. When anthocyanin is bound to a sugar molecule, an additional absorption peak appears around 310-340 nm, with the peak size varying based on the number of sugar molecules attached [44]. Moreover, an increase in the number of hydroxyl groups leads to a bathochromic shift in the absorption spectra.

The pH of the environment plays a critical role in determining the color of anthocyanins [45]. As reported in the literature, anthocyanins display different colors depending on the pH: they appear red in acidic conditions, violet or purple in neutral solutions, and blue in alkaline media [46]. This variability in colors [47] (Figure 14), is due to the transformation of the flavylium cation, which is red at pH 1-3. As the pH rises, the cation gets protonated, becoming a colorless pseudobase at pH 4-5, or forms an anionic quinonoidal structure (blue) at pH 7-8. A purple quinonoidal base is also observed between pH 6-7. At pH values above 9, these forms can further convert into chalcones, resulting in distinct color changes [48, 49].

**Figure 8.** UV-Vis spectra of purple flower at different pH**Figure 9.** UV-Vis spectra of red flower at different pH**Figure 10.** UV-Vis spectra of violet flower at different pH**Figure 11.** UV-Vis spectra of light pink flower at different pH

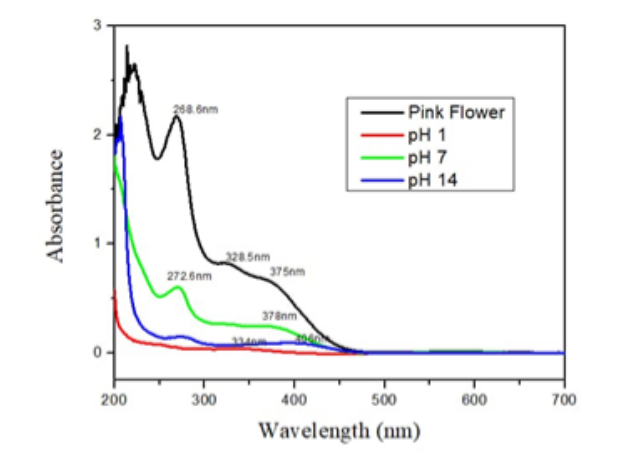


Figure 12. Uv-Vis spectra of pink flower at different pH

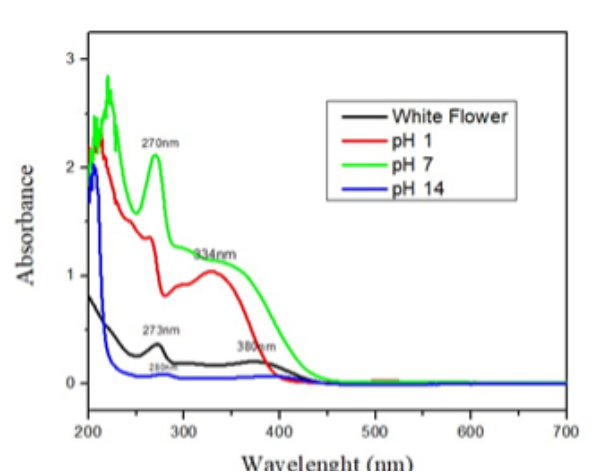


Figure 13. Uv-Vis spectra of light pink flower at different pH

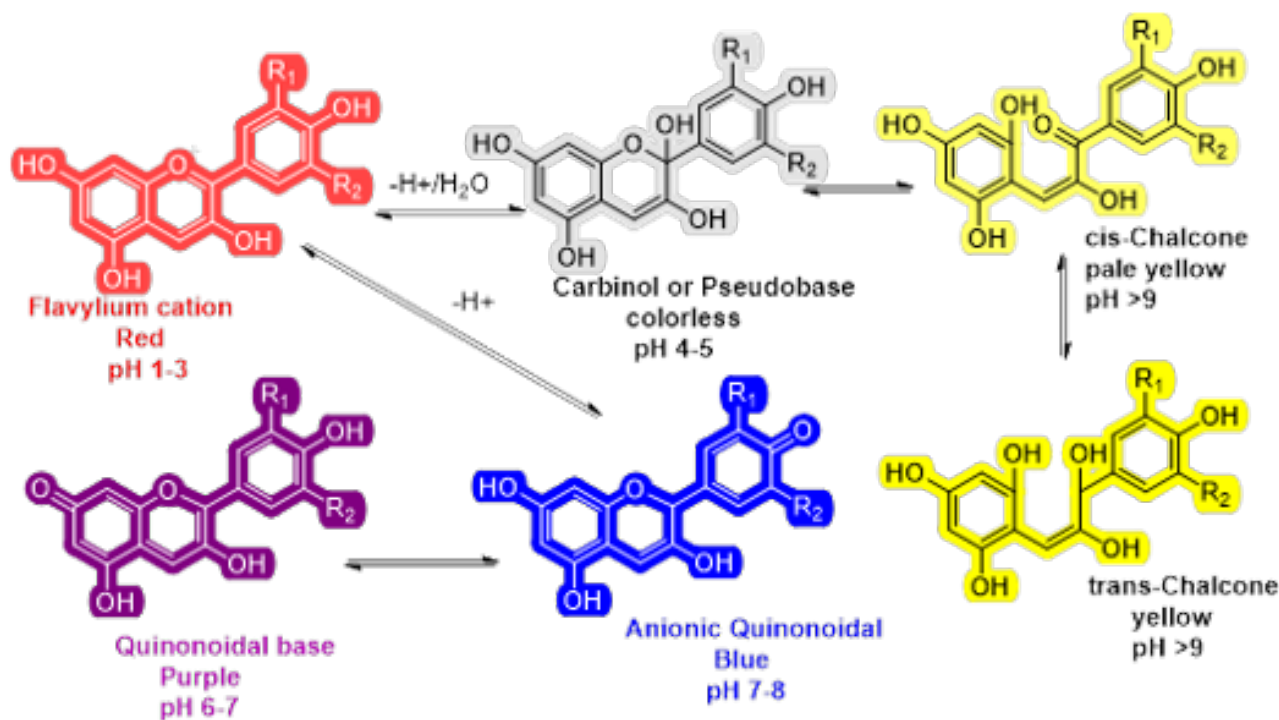
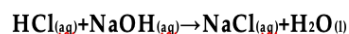


Figure 14. Different forms of Anthocyanins [42]

### Comparison with Synthetic Indicators

After testing the Vinca extract across a range of pH values, the purified extract was used as a pH indicator and compared to synthetic indicators such as methyl red, methyl orange, and phenolphthalein. In the titration procedure, 10 mL of acid was mixed with a few drops of the respective indicator and titrated with a base from the burette. The resulting data is summarized in Table 3. Additionally, the pH at the endpoint of each titration was calculated using equations 1-6. The neutralization reaction for this titration is as follows:



$$\text{Moles of NaOH} = \text{Molarity} \times \text{Volume} \quad (1)$$

$$\text{Moles of HCl} = \text{Molarity} \times \text{Volume} \quad (2)$$

but, end point shows that amount of acid is little less than base, so acid is limiting reactant

$$\text{Excess moles of NaOH} = \text{moles of NaOH} - \text{moles of HCl} \quad (3)$$

$$\text{Molarity or concentratin of OH ions} = \frac{\text{Excess moles of NaOH}}{\text{total volume of solution}} \quad (4)$$

$$14 = \text{pH} + \text{pOH} \quad (5)$$

$$\text{pH} = 14 + \log [\text{OH}]^- \quad (6)$$



**Table 3.** Comparative titration data of Vinca and Synthetic indicators

Titration	Indicator	Color change	Mean±SD	pH
NaOH/HCl	Methyl Red	Red to Yellow	9.91±0.100	10.339
	Methyl Orange	Orange to Yellow	9.94±0.115	10.350
	Phenolphthalein	Colorless to Pink	9.83±0.058	10.348
	Purple Vinca	Purple to dark-yellow	9.96±0.200	10.351
	Red Vinca	Red to dark-yellow	9.86±0.173	10.348
	Violet Vinca	Violet to dark-yellow	9.79±0.115	10.346
	Pink Vinca	Pink to lime yellow	9.81±0.100	10.346
	Light pink Vinca	Greenish-brown to yellow	9.77±0.058	10.346
	White Vinca	Colorless to light-yellow	9.95±0.058	10.351
HCl/NH <sub>4</sub> OH	Methyl Red	Red to Yellow	11.20±0.112	10.38
	Methyl Orange	Orange to Yellow	11.31±0.015	10.386
	Phenolphthalein	Colorless to Pink	11.22±0.11	10.381
	Purple Vinca	Purple to dark-yellow	11.30±0.05	10.383
	Red Vinca	Red to dark-yellow	11.27±0.114	10.382
	Violet Vinca	Violet to dark-yellow	11.16±0.112	10.38
	Pink Vinca	Pink to lime yellow	11.38±0.058	10.385
	Light pink Vinca	Greenish-brown to yellow	11.44±0.117	10.386
	White Vinca	Colorless to light-yellow	11.48±0.013	10.387
NaOH/CH <sub>3</sub> COOH	Methyl Red	Red to Yellow	9.53±0.058	10.339
	Methyl Orange	Orange to Yellow	9.67±0.11	10.343
	Phenolphthalein	Colorless to Pink	10.40±0.013	10.36
	Purple Vinca	Purple to dark-yellow	9.83±0.017	10.347
	Red Vinca	Red to dark-yellow	9.79±0.0111	10.346
	Violet Vinca	Violet to dark-yellow	10.44±0.054	10.363
	Pink Vinca	Pink to lime yellow	10.57±0.013	10.366
	Light pink Vinca	Greenish-brown to yellow	10.41±0.11	10.363
	White Vinca	Colorless to light-yellow	10.62±0.055	10.368

The titration results using various indicators and Vinca extracts were compared across three different acid-base reactions: NaOH/HCl, HCl/NH<sub>4</sub>OH, and NaOH/CH<sub>3</sub>COOH. In the NaOH/HCl titration, the synthetic indicators methyl red, methyl orange, and phenolphthalein showed color changes from red to yellow, orange to yellow, and colorless to pink, respectively, with pH at the endpoint ranging from 10.338 to 10.350. The Vinca extracts exhibited similar color changes, with the purple Vinca transitioning from purple to dark yellow, and the red, violet, and pink Vinca extracts changing from red or violet to dark yellow or lime yellow. The white Vinca extract transitioned from colorless to light yellow, with endpoint pH values ranging from 10.346-10.351 and a mean pH of approximately 10.35.

In the HCl/NH<sub>4</sub>OH titrations, similar trends were observed, with synthetic indicators showing color changes from red to yellow for methyl red and from orange to yellow for methyl orange, while phenolphthalein shifted from colorless to pink. The Vinca extracts, including purple, red, violet, and pink, all transitioned to dark yellow, with the white Vinca extract changing from colorless to light yellow. The pH at the endpoint for all indicators

in this reaction ranged from 10.38-10.386, with a mean value of around 10.38.

For the NaOH/CH<sub>3</sub>COOH titrations, methyl red and methyl orange exhibited color changes from red to yellow and orange to yellow, respectively, while phenolphthalein changed from colorless to pink. The Vinca extracts followed a similar trend, with the purple, red, and violet Vinca extracts transitioning to dark yellow and the white extract changing from colorless to light yellow. The endpoint pH for this reaction ranged from 10.339 to 10.36, with the Vinca extracts showing endpoint pH values close to those of synthetic indicators, averaging around 10.346 to 10.368.

Overall, the Vinca extracts exhibited similar performance to the synthetic indicators in all titrations, with consistent pH readings at the endpoint and clear color transitions. The data also demonstrates that different Vinca extracts (purple, red, violet, pink, light pink, and white) can effectively serve as natural pH indicators, with color changes corresponding to the pH conditions of the titrations.

## CONCLUSIONS

In conclusion, Vinca flowers, with their wide range of vivid colors, have proven to be promising natural pH indicators. The striking color transitions of the Vinca extract across various pH levels demonstrate its potential for use in acid-base titrations. The study reveals that the color changes of Vinca extracts, derived through a cold extraction method with 70% ethanol, are highly responsive to pH fluctuations, similar to traditional synthetic indicators like methyl yellow, methyl orange, and phenolphthalein. The key anthocyanins in Vinca flowers are responsible for these color shifts, which occur due to the transformation of the flavylium cation and the presence of different anthocyanin structures at various pH values.

When tested across a pH range of 1 to 14, the Vinca extract displayed a vivid spectrum of color changes, ranging from red or pink in acidic conditions to yellow in alkaline solutions. This pH-dependent behavior is consistent with the known characteristics of anthocyanins. The extract's performance was evaluated through acid-base titrations, comparing its endpoint pH values to those of synthetic indicators. The results showed that Vinca extracts performed comparably to synthetic indicators, with endpoint pH values within a narrow range across different acid-base titrations. These findings highlight the potential of Vinca flowers as an eco-friendly and natural alternative for pH detection in chemical analysis, offering a sustainable solution without compromising accuracy or reliability.

## DATA AVAILABILITY STATEMENT

The authors confirm that the data that supports the findings of this study are available within the article. Raw data that support the finding of this study are available from the corresponding author, upon reasonable request.

## CONFLICT OF INTEREST

The author declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

## USE OF AI FOR WRITING ASSISTANCE

Not declared.

## ETHICS

There are no ethical issues with the publication of this manuscript.

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