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Exploring Water Henna Flower (*Impatiens balsamina* L.) Pigment as an Alternative Indicator for Acid-Base Titration

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Abstract

Acid-base titration is a common technique used in quantitative analysis in laboratories, with the indicator playing a crucial role in the process. However, synthetic indicators are often costly and can contribute to environmental pollution. As a result, there is a need for alternative, natural acid-base indicators that are affordable and easily accessible. One potential natural indicator is anthocyanin, a pigment found in plants. This study aims to develop an alternative acid-base indicator from the anthocyanin extract of water henna flowers and evaluate its performance in acid-base titrations. The process involved preparing the water henna flower extract, determining its dissociation constant (pI), and testing its effectiveness in acid-base titrations. The pI was found to be 4.22, and the extract exhibited a color change within the pH range of 3.22-5.22, making it suitable for use in acid-base titrations. The water henna flower extract successfully showed clear color transitions at the endpoint of base titrations and provided results comparable to those obtained using methyl orange, accurately determining HCl concentration and sodium bicarbonate levels. This suggests that water henna flower extract can be used as a cost-effective, easily prepared, and environmentally friendly alternative to synthetic indicators like methyl orange.

Keywords: Acid-Base Titration, Anthocyanin, Alternative indicator, Water Henna Flower Extract

1 Introduction

There are currently initiatives underway to promote green chemistry. Sustainable resources, less chemical use, and environmental friendliness are the goals of chemical research and allied subjects [1]. Titrations are a technique that uses visual endpoint detection to determine a substance's volumetric content [2]. An indicator is a material that is used in acid-base titrations. One of its functions is to provide a signal (a color shift) when one of the compounds involved in the reaction is present in excess. Synthesis indicators like phenolphthalein, methyl orange, and methyl red are chemical substances that are frequently employed as indicators in acid-base titrations. These indicators are costly, hazardous, and unfriendly to the environment. Other chemical substances that may take their place were needed to solve this issue and plant pigments are the solution [3].

Anthocyanins are a pigment that gives blue, red, or purple colors in plants. Anthocyanins can be use as food dyes [4], titration indicators [5], Sensitizer in Dye-Sensitized Solar Cells (DSSC) [6], and many others. Anthocyanins are found in plant tissue in the form of glycosides bound to one monosaccharide or two monosaccharides. Generally, anthocyanins can be dissolved in water and only when boiled with dilute acid, they decompose into anthocyanidins and monosaccharides. The color of anthocyanins is influenced by the surrounding pH; at low pH, it is red, while at high pH it is violet blue. The concentration of anthocyanins determines the color, and the more concentrated the redder the color [7]. The *Impatiens balsamina* L. plant, especially in the flowering part, is one of the plants known to contain anthocyanins. It has several flower colors, namely red, white, yellow, orange, and purple. The chemical content identified as pelargonidin 3-O-rutinoside, pelargonidin 3-O-(6″-Z-p-coumaroylglucoside)-5-O-glucoside, and pelargonidin 3-O-(6″-E-p-coumaroylglucoside)-5-Oglucoside as anthocyanins, and kaempferol 3-O-glucoside as flavonol [8]. The results of the study by Vankar & Srivastava (2010) showed that

the anthocyanin extraction content of *Impatiens balsamina* flower by the maceration method using methanol solvent was 336.56 mg/kg [9]. The method for determining anthocyanin can be used pH differential method [10]. Research conducted by Qoirunnisa and Asngad (2018) showed that the acid-base indicator paper of henna flower extract with 96% alcohol solvent displayed a color that tended to be stable during the storage process for up to 3 days [11]. However, the use of water henna flower extract as an indicator for titration has not yet been explored in detail.

This study aims to investigate the potential of water henna flower (*Impatiens balsamina* L.) extract as an acid-base indicator. The novelty of this research lies in the application of the anthocyanin extract directly in acid-base titrations and its comparative evaluation with synthetic indicators, focusing on dissociation constant, pH transition range, and titration accuracy. This study can create an alternative natural acid-base indicator from the anthocyanin content from henna flower extract. This alternative indicator has several advantages, including being easy to use and obtain and being more environmentally friendly.

2 Method

Water Hena flowers were collected from Bandung and determined plant identity at the Jatinangor Herbarium, Plant Taxonomy Laboratory, Department of Biology, Faculty of Mathematics and Natural Sciences, Padjadjaran University, Bandung.

2.1 Preparation of Water Henna Flower Extract

The water henna flower is cut and macerated with a ratio of 1:10 with a mass of 10 g of water henna flower and methanol added as much as 100 mL. tightly closed and left for 3 days, protected from sunlight and occasionally stirred every day. After 3 days, the extract is filtered, and the filtered results are stored in a dark bottle.

2.2 Determination of Indicator Dissociation Constants (pI) Extract

Some buffer solutions with various pH are added with 1 mL water henna flower extract until 10 mL. The each solution is measured by spectrophotometer UV-Vis (Shimadzu UV-1800) with scanning spectrum and checked pH by a pH meter (Mettler Toledo). The measurement result is related to the Handerson-Hasselbach equation and calculated with linear regression. The independent variable in this step was the pH of the buffer solution, while the dependent variable was the absorbance of the extract at corresponding wavelengths. Controlled variables included the concentration of extract (constant volume of 1 mL), buffer solution volume (9 mL), ambient temperature, and light exposure.

2.3 Application of the Acid-Base Titration using Extract as Indicator

Two hundred mg of sodium bicarbonate is placed in the Erlemeyer flask. Then it is dissolved using 10 mL distilled water and added 3 mL water henna flower extract. It is titrated with standardized HCl until a color change occurs from dark brown to red orange. It was compared with acid-base titration using methyl orange as an indicator. The independent variable in this step was the sodium bicarbonate, while the dependent variable was the HCl volume. Controlled variables included the concentration of extract (constant volume of 3 mL), distilled water volume (10 mL), ambient temperature, and light exposure.

3 Result and Discussion

Anthocyanins are polar compounds so can be extracted with polar solvents, such as methanol. The extract obtained is stored in a dark place, protected from light; this aims to keep the anthocyanin stable. The light affects the stability of anthocyanins in the formation and the rate of degradation, thus affecting the stability of the anthocyanin pigment contained in the extract and can increase the damage to the anthocyanin pigment [12].

The anthocyanins found in *Impatiens balsamina* L. include pelargonidin derivatives such as pelargonidin 3-O-rutinoside, which are responsible for the observable color shifts under varying pH conditions [8]. In acidic environments (pH < 3), the pigment appears red due to the dominance of the flavylium cation form. As the pH increases, the molecule transitions through several structural forms—quinonoidal base, carbinol pseudo-base, and chalcone—resulting in noticeable color changes from red to purple, then yellowish. This pH-dependent structural rearrangement underlies the functionality of anthocyanins as acid-base indicators [7].

The result of extraction is the determined value of Indicator Dissociation Constants (pI) Extract. The value of pI is an important parameter that indicates the degree of ionization of molecules in solution at different pH values. The pI value

determines the path of color change of the indicator which is very useful for determining the end point of a titration. Besides this, the pI value also determines the type of acid-base titration that will be applied (acidimetry or alkalimetry) [13].

Determination of pI extract is done by adding henna flower extract under different pH conditions, so that color changes are visible at various pH solution.

Figure 1 The color changes at various pH solution

The ionization equilibrium of the indicator as a weak organic acid can be explained by the following **Eq. 1.**

$$HIn \rightleftharpoons H^+ + In^- \qquad \text{(Equation 1)}$$

From the Handerson-Hasselbach equation, the pI value can be obtained. The absorptivity value of this acid-base indicator is highly dependent on the pH. In acidic conditions (low pH), the indicator will be in the form of HIn. While in basic conditions (high pH) the indicator will be in the form of In^-. If log $[In^-]/[HIn]$ and pH are made into a curve and regressed, the regression curve equation will be: y = Bx + A, With: y = log $[In^-]/[HIn]$; x = pH. Then pI can be determined when log $[In^-]$ / [Hin] = 0, where pI = pH. The absorbance results of each pH (**Table 1**) were then made into a regression equation which can then be calculated to determine the pI.

Table 1 The Measurement of Absorbance at λ 761 nm with Various pH

pH	A $_{761\ nm}$	$\log\frac{[In]^-}{[HIn]}$ (see Eq. 2)
4.86	0.497	-
5.98	0.494	0.176
6.48	0.470	0.016
6.73	0.342	0.002
7.19	0.467	0.014
7.67	0.411	0.004
12.58	0.496	-

$$\frac{[In]}{[HIn]} = \left|\frac{A-Aa}{Ab-A}\right| \qquad \text{(Equation 2)}$$

Aa = absorbance at acid condition (0.497)
Ab = absorbance at base condition (0.496)

The results of the log $[In^-]/[HIn]$ (y) and pH (x) curve obtained a linear regression equation: y = -0.0346x + 0.1462. Then pI can be determined when log $[In^-]$ / [Hin] = 0, where pI = pH. From the regression equation, the pI value is 4.22 so the color change trajectory of the natural indicator of water henna flower extract is in the pH range of 3.22-5.22. Because the pH trajectory is in acidic conditions, the type of titration that can be applied is acidimetry.

Acidimetry is a titration method to determine the levels of base solutions with acidic standard substances. The standard used here is 0.1 N HCl and the sample will be determined is sodium bicarbonate.

Before determining the levels of the sample, standardization of HCl is first performed. HCl has properties that make its concentration uncertain directly from the dilution process, so it must be standardized or its concentration standardized with a primary standard solution. Solution standardization is a process in which the concentration of a secondary standard solution is determined precisely by titration with a primary standard solution. The primary standard used is sodium carbonate, which is titrated to determine the concentration. In this test, natural indicators and synthetic indicators are used, namely methyl orange, as a comparison because it has the same range as the natural indicator, namely 3.2-4.4.

The result of HCl Standardization using methyl orange and Extract as an Indicator gave the same result, that was 0.968 N. The methyl orange indicator shows the results of the change in color at the end of titration from red to yellow (**Fig. 2**) [14]. The extract indicator shows the results of the color change from purplish brown to orange red (**Fig. 3**).

(a) (b)

Figure 2 Before (a) and after (b) end of titration for standardization HCl with the methyl orange indicator

(a) (b)

Figure 3 Before (a) and after (b) end of titration for standardization HCl with extract indicator

The determination of the content was carried out using a sample of sodium bicarbonate tablets with 1 tablet containing 500 mg of sodium bicarbonate. Indonesian Pharmacopoeia VI Edition states that sodium bicarbonate tablets contain sodium bicarbonate, $NaHCO_3$, not less than 95.0% and not more than 105.0% of the amount stated on the label [15]. The reaction of a standard solution of 0.1 N HCl with $NaHCO_3$ is as follows **Eq. 3.**

$$NaHCO_3 + HCl \rightarrow NaCl + CO_2 + H_2O \quad \text{(Equation 3)}$$

The sodium bicarbonate content results using the methyl orange indicator obtained a content result of 100.952% on the first day and 99.212% on the second day, while the extract indicator obtained a content result of 99.212% for the first and second days, so it can be concluded that the results of the content determination using natural and synthetic indicators meet the content requirements because they are in the range of 95 - 105 %. For color changes, there is a color change from light brown to red for the methyl orange indicator, whereas with the natural indicator the color change occurs from dark brown to red orange.

Compared to other natural indicators reported in literature, such as anthocyanin from purple sweet potatoes [5] or Thai yellow flowers [3], the extract used in this study demonstrated a similarly narrow but sufficient transition range for acid-base titration. Unlike Qoirunnisa & Asngad (2018), who utilized henna extract as paper strips, our study applied the extract in a direct liquid form, yielding more immediate and clearer color transitions suitable for quantitative analysis [11].

In the next research, this alternative indicator should explore the stability of this extract over longer storage periods and its formulation into dry or tablet indicator forms for practical field applications.

4 Conclusion

In determining the indicator dissociation constant (pI), the result was 4.22 so the color change trajectory of the natural indicator of the water hyacinth flower was in the pH range of 3.22-5.22. Based on pH range, the natural indicator of water henna flower can be used in acidimetry titration.

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