EVALUATION OF ANTIOXIDANT ACTIVITY ON PLUM FRUIT
(Prunus Domestica L.) SKIN EXTRACT APPLIED FOR NATURAL ACID-BASE INDICATOR

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Abstract: Plum peel was extracted through the maceration method with 100 mL ethanol. This process obtained 5% (50 µg/µL) skin extract. The UV-Vis spectrum of the extract displayed the major absorption peak at $\lambda_{\text{max}} = 526$ nm in acid media, this assigned by the electronic transition from flavylium which as characteristic of anthocyanin compounds. The of anthocyanins stability on temperature effect showed the absorbance decreases with the heating time, while the level of pigment color loss, or the percentage of color loss, gradually increases. The pigment was maintained at 100°C for 100 min with the loss rate reaches 58.5%. Therefore, The loss of anthocyanin color in the plum peel is relatively stable at lower temperatures. Then, The plum peels extract shown the red color at pH 1-3, almost colorless at pH 4-7, and violet color at pH 12. Finally, plum peels extract was potentially used as the acid-base indicator.

Keywords: acid-base, antioxidant, anthocyanin, indicator, plum

Introduction

Indonesia is one countries that has tropical rainforests which are rich in biodiversity. Since a long time ago, plants have been used to fulfill the needs of human life in the area of food, even ethnombotany has developed the use of plants by certain communities such as the use of traditional medicines, poisons, dyes, and others. Along with the development of research in the field of natural materials, nowadays the use of plants is increasingly widespread, one of the exciting studies is the use of several types of plants as acid-base indicators (Soltan, 2013).

The indicator of acid-base is a complex compound that can react with acids or bases with a change in color according to the concentration of hydrogen ions through the process of titration (Reshetnyak et al., 2013; Zoromba, 2017; Sukhanov et al., 2016; Petrossyan et al., 2018; Udugala-Ganehenge et al., 2015; Baldigo et al., 2018). Indicators used in strong acid-base titrations are usually synthetic indicators, such as phenolphthalein (PP) indicators. This indicator is a synthetic indicator that sold on the market at a relatively high price. This indicator can cause chemical pollution, limited availability, and high production costs.

Besides, to change the use of synthetic indicators, indicators of natural ingredients have been found, for example from the flower of blood leaf or blood
leaf (Iresine herbstii), Opuntia ficus indica (L.), hibiscus (Hibiscus rosa sinensis L.) and sheets of Adam Hawa boat (Rhoeo discolor)(Macuwele et al., 2016). Almost all types of plants that produce color can be used as natural indicators because they can change color in an atmosphere of acid or base. Also, research shows several types of growths that can be used as natural indicators in acid-base titration such as purple cabbage (Brassica oleracea), purple sweet potato (Ipomoea batatas), red beet (Beta vulgaris), hibiscus (Hibiscus rosa-sinensis), Roselle flower (Hibiscus sabdarifa) and others (Kahlert et al., 2016). Previous research has shown that the ethanol extract of hibiscus flower (Hibiscus rosa-sinensis) can be used as an acid-base indicator wherein acidic conditions it is red and alkaline states are blue (Xiong et al., 2018; Kamneva et al., 2018; Serra-Mora et al., 2016). The application of using natural indicators in a practicum on chemistry subjects in schools can improve students' understanding of the concept of an acid base.

One of the plants that have the potential to produce dyes is plum (Prunus domestica. L.) which are ripe with blackish purple skin, and ripe plums contain flavonoids. This anthocyanin pigment is a dyestuff producer in plants and is very sensitive to acidity (pH) and heat (Aal et al., 2018; Uranga et al., 2018; Hsiao & Hsieh, 2018). This plum plant has fruit like apples with red fruit skin color and turns purple-black when it is ripe as seen in Figure 1.

So far no research has been found on plum peels can be used as a natural indicator in the titration process. This problem is what underlies the conduct of this research. In this study, an ethanol extract of Prunus domestica. L as a natural indicator of acid-base titration will be tested. Examination of the ethanol extract of plum peels on acid-base titration was carried out to see the pH range. The pH range can be seen when there is a significant pH change which is marked by a change in color on the titrate. An evaluation was carried out using comparative indicators namely phenolphthalein (PP) and methyl red (MM) indicators. The study of the anthocyanin activity of plum peel was observed with the UV-Vis spectrum.

Material And Methods

Material

The Prunus domestica (L.) Skin, doubly distilled water used in all experiments, ethanol 96% (C₂H₅OH). Potassium hydrogen phthalate (C₈H₅KO₄), potassium dihydrogen phosphate (KH₂PO₄), sodium tetraborate (Na₂B₄O₇), sodium hydroxide (NaOH), and sodium bicarbonate (NaHCO₃).

Extraction of Plum Peels

The skin of the plum was separated and then washed and then dried to dry using 62 cmHg vacum at a temperature of 50°C for approximately 4 hours. Then
the dried plum peels are crushed to powder, then macerated using 96% ethanol. Furthermore, ethanol was separated using a rotary vacuum evaporator at 45°C.

**Preparation of Buffer Solutions pH 1-12**

The buffer solutions were prepared at pH 1-12. These ranges supposed to monitor the color change in the PANi solution. For pH 1-2, this solution made only by HCl with suitable concentration. Then, the buffer solution with pH 3-5 was prepared by dissolving 10.21 g potassium hydrogen phthalate in 0.1 M HCl with appropriate volume. Next, pH 6-8 (buffer solution too) made by mixing 6.81 g potassium dihydrogen phosphate with the proper amount of 0.1 M NaOH. Then, for pH 9-10 of buffer solution, 4.77 g sodium tetraborate dissolved into 0.1 M HCl reach to pH 9, but 0.1 M NaOH to pH 10, with appropriate volume. Finally, 0.01 M NaOH need to make the solution with pH 12.

**Ultraviolet (UV) Spectroscopy Detection**

The Thermo Scientific Evolution 220 Series used to quantify the sample, which ethanol was used to calibrate the instrument. Furthermore, 0.1 mL of each sample diluted with 10 mL of ethanol and 1 mL of the sample was measured and placed in the cell. The absorbance of the extract was determined within the visible region at 300–900 nm.

**Discussion**

**The Extraction Process of Plum Peels**

Plum peel was washed with clean water, then dried in a vacuum dryer with a temperature of 50°C at a pressure of 62 cmHg for 4 hours. The dried plum peel was crushed with a blender, then sieved with a 40 mesh sieve so that the dry plum peel powder was obtained. A total of 20 g of dried plum peel powder was extracted with 100 mL ethanol by maceration method accompanied by stirring using a magnetic stirrer at room temperature for 3 hours. The mixture was then filtered successively using Whatman filter paper No. 4 and 1. The recovered residue was extracted with 100 mL ethanol. The filtrate from both extractions was combined, then dried using a rotary vacuum evaporator at 45°C. The dry extract obtained was then dissolved in ethanol to obtain ethanol extract from the skin of plum fruit 5% (50 µg/µL).

**Figure 1.** Extract solution of fresh plum peels
**pH Effect on The Anthocyanins Stability of Plum Peels Extract**

The UV-Visible absorption spectrum of anthocyanins in plum peel was recorded between wavelengths of 300 to 900 nm with different pH controls. The anthocyanin extracts showed a very different color change at pH 1 to 4, where the smaller pH showed the extracted color became redder (figure 2). The UV-Vis spectrum of these three solutions showed a significant absorption peak at $\lambda_{\text{max}} = 526 \text{ nm}$. The peak occurs due to the electronic transition from the electron which was stabilized by the cation of flavylium on the Oxygen atom (Ryu & Koh, 2018). On the other hand, a slight peak also appears at $\lambda_{\text{max}} 358 \text{ nm}$, and the intensity of this transition was quite low, so it can be ascertained that was the electronic transition between electrons around benzene B (Abdel-Aty et al., 2018; Chen et al., 2018). This result confirmed that anthocyanins from the plum peels may contain the same structural unit with cyanidin, peonidin, or malvidin (Ryu & Koh, 2018).

![Figure 2](image-url)
Furthermore, at pH 7 to 12, the plum peel extract shown purple (Figure 3). Observation through UV-Vis shows a shift in intensity at $\lambda_{\text{max}} = 526$ nm to $\lambda_{\text{max}} = 585$ nm, this uptake increases with increasing pH. Also, the same thing also happened at $\lambda_{\text{max}}$ in the area of 358 nm which shifted to 383 nm. This peak experienced an increase in intensity with increasing degrees of acidity. The UV-Vis spectra showed that pH values strongly influenced the stability of anthocyanins from the plum peel. At $3 < \text{pH} < 7$, extracts of plum peel lost color, this illustrates that anthocyanin stability is present at $\text{pH} < 3$, this is a characteristic of anthocyanin compounds. Anthocyanins from plum peel are stabilized in acidic solutions ($\text{pH} 3$, from purple to red), but not stable in neutral or alkaline solutions ($\text{pH} > 3$, from colorless to purple).

![Figure 3](image)

**Figure 3.** (a) The color change of the extract at pH 7, 9, 11, and 12, then (b) its spectrum of UV Vis.

The intensity shift at $\lambda_{\text{max}} = 526$ nm to $\lambda_{\text{max}} = 585$ nm can be explained as the loss of the presence of the flavylium cation. Based on figures 2 and 3, the addition of acid makes the dehydration of the -OH group in ring C. The loss of this group makes the resonance stability of positive ions in ring C so that the stable state of the charge is at the atom in the chain. The addition of bases causes...
the H atom in the phenolic group in ring A to be released. So that the anthocyanin structure at high pH will be in a state that does not contain positive ions anymore. This process causes the formation of quinones on ring A. The existence of quinones causes a shift in the electronic transition at $\lambda_{\text{max}} = 526$ nm to $\lambda_{\text{max}} = 585$ nm (figure 4) (Hsiao & Hsieh, 2018).

![Diagram](https://example.com/diagram.png)

**Figure 4.** pH-dependent conformational rearrangement of the anthocyanin molecule

*Effect of Temperature on the Stability of Anthocyanins From Plum Peels*

Five anthocyanin stock solutions from plum peels (50 μg / ml) at pH = 3 were stored at different temperatures, from 20°C to 100°C. Samples were taken periodically in every 15 minutes to 100 minutes and absorbance was determined at 526 nm. Based on Figure 5, it was seen that absorbance decreases with the heating time, while the level of pigment color loss, or the percentage of color loss, gradually increases — besides, the level of the most obvious casualty of pigment color at higher temperatures. When the pigment was maintained at 100°C for 100 min, the loss rate reaches 58.5%. Color loss at 100°C shown distinctly more elevated than that at 80°C. The loss of anthocyanin color in the plum peel was relatively stable at lower temperatures.
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**Figure 5.** Profile of the color stability of anthocyanins at various heating temperatures.

*Acid-Base Titration with Plum Peels Extract as Indicator*

The application of acid-base titration using the plum peels extracts indicator has been applied and was presented in table 2. This experiment also used several standard indicators such as Phenolphthalein (PP) and methyl red (MR), as a comparison. In titrating 10 mL of 0.1 M NaOH solution using 0.1 M HCl solution, a bright color changed from pink became colorless using the Phenolphthalein (PP) indicator, the color loss was observed when the pH had dropped past 8.00. Furthermore, in the red methyl color changed from yellow to orange when the pH has become 5.26 and became very bright red when the pH has passed 4.00. The use of plum peels indicator observed violet color when HCl has not yet been added, the color change appears to turn colorless~pink between the pH range 7-4 slowly and becomes red when the pH has passed 3 towards 1.

**Table 1.** Performance of the plum peels as the indicator

| Titration | Indicator          | Color               | pH       |
|-----------|--------------------|---------------------|----------|
| HCl       | Phenolphthalein    | Pink to colorless   | 12.02 – 5.77 |
|           | Methyl Red         | Yellow to pink      | 12.12 – 5.26 |
|           | Plum Peels         | Violet to pink      | 12.52 – 3.34 |
|           | Plum Peels         | Violet to red       | 12.20 – 1.02 |
| NaOH      | Phenolphthalein    | Colorless to pink   | 2.95 – 9.37  |
|           | Methyl Red         | Light Red to yellow | 3.02 – 8.85  |
|           | Plum Peels         | Pink to light grey  | 2.92 – 5.05  |
|           | Plum Peels         | Red to Violet       | 1.07 – 12.02 |
| CH₃COOH   | Phenolphthalein    | Colorless to pink   | 2.95 – 9.37  |
|           | Methyl Red         | Light Red to yellow | 3.02 – 8.85  |
|           | Plum Peels         | Pink to light grey  | 2.92 – 5.05  |
|           | Plum Peels         | Red to Violet       | 1.07 – 12.02 |

Similar results were also shown in the titration of 10 mL of 0.1 M CH₃COOH solution with 0.1 M NaOH solution. The use of the plum peels indicator displayed red changes to pink~colorless when pH has entered the value of 4 and above, adding further NaOH after the endpoint of the titration indicates the violet color on the indicator. Therefore, the plum peel indicator has the potential to be used as an excellent acid-base indicator, because it can work in acidic and primary regions.
Conclusions

The UV-Vis spectrum of the plum peels extract displayed the major absorption peak at $\lambda_{\text{max}} = 526$ nm in acid media, this assigned by the electronic transition from the electron which was stabilized by the cation of flavylum on the Oxygen atom. This phenomenon was a characteristic of anthocyanin compounds. When the pigment was maintained at 100°C for 100 min, the loss rate reaches 58.5%. Color loss at 100°C shown distinctly higher than that at 80°C. The loss of anthocyanin color in the plum peel is relatively stable at lower temperatures. The plum peels extract displayed the red color at pH 1-3, almost colorless at pH 4-7, and violet color at pH 12. The plum peels extract was also applied directly to natural acid bases that displayed same color change with the pH 1-12 control. Therefore, plum peels extract was potentially used as the acid-base indicator.

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