Utilization of Anthocyanin Extracted from Pletekan (*Ruelliatuberosa* L.) in Determination Soil pH

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**ABSTRACT**

Pletekan (*Ruelliatuberosa* L.) is a flowering plant that grows wild. Pletekan has blue-purple flowers, containing the anthocyanin as its specific color. Anthocyanin is an organic material that can be used as an indicator of natural acid alkali, because the color of anthocyanin can ensure specific color changes when it reacts with materials at a certain pH as well. This research aimed to utilize anthocyanin extracted from pletekan as a determinant of soil pH. The used method was maceration extraction by soaking fresh pletekan flowers using maceration solvent (*methanol acidified by HCl 1%*) for 2-3 days. Concentrated anthocyanin extracts needed to be diluted with extracts that had not been concentrated so that the resulted color changes were more specific. The anthocyanin mixture was applied to pH 1-12 buffer solution and in acid and alkaline soil samples. The soil was dissolved using aquadest at a ratio of 1:2, the soil was left to settle to get water. The color change in the tested water of soil sample was adjusted to the color of the buffer solution and pH meter. Results showed that color change of soil sediment-water which was dropped by natural pH indicators needed more indicator drops such as 2-3 levels than was color changes caused by the buffer solution.

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1. INTRODUCTION

Pletekan (*Ruelliatuberosa* L.) is a weed that belongs to the family of Acanthaceae and is known as a "cracker plant". Weed is a plant which is not desired on agricultural land because it can be harmful due to competition between cultivated plants and weeds such stunted growth so that the time to start producing longer, decrement of the quantity and quality of crop production, disruption of labor productivity, being nest of pests and disease (Muryasani, 2017). Because the existence of pletekan can interfere with the growth of cultivated plants, pletekan is eradicated from the land.

Pletekan is a blue-purple flower, 5-5.5 cm long, solitary or there are 1-3 flowers per branch (Chotani et al., 2010). Pletekan has a blue-violet flower containing anthocyanin (Kampeerapappun, 2010). Anthocyanins are known as pigments that cause flowers red to bluish purple. These pigments are not only found in flowers but also various parts of plants such as fruit peels, fruit flesh, bulbs, seeds, leaves, etc. (Lestario, 2017). Anthocyanin pigments have a high percentage of degradation, and this is caused by high sensitivity to various factors, namely temperature, pH changes, availability of oxygen, and light (Joseph, 2018). Based on various studies, anthocyanin-containing plants can be used as natural indicators of pH determination in chemicals and food ingredients. However, it cannot be applied as an indicator of determining soil pH.

The majority of farmers use the land as a medium for crop cultivation, while soil pH plays a role in plant growth and development. To gain maximum crop yields, the soil must have an appropriate pH range (*ideal*) with cultivated plant commodities. Certain plants have a certain ideal pH (Hanafiah, 2014). However, most farmers do not measure soil pH before planting. The range of soil pH values causes this problem in a land area challenging to determine (Esington, 2015). The pH meter cannot be reached easily by farmers. Therefore, this study is intended to utilize anthocyanin extracted from pletekan as a determination indicator of soil pH range. Through the color change of anthocyanin extract, the soil pH can be determined directly.

pH is related to nutrient availability and nutrients absorption by roots, which in turn affects plant growth. Individual plants have a specific ideal pH. For example, the perfect pH for potato plants ranges from 4.8 to 6.5; rice ranges from 5.5 to 6.5; corn ranges from 5.5-7.5; while sugar cane ranges from 6.0 to 8.0. (Hendra, 2014). In alkaline soils, the decrement of pH can be done by adding sulfur, while in acid soils, an increment of pH can be done by adding calcium.
2. MATERIALS AND METHODS

This research was conducted at the Land Resources Laboratory, Faculty of Agriculture, UPN "Veteran" East Java, Surabaya, from April to July 2019. The materials used in this research were fresh pletekan flowers, methanol, 1% HCl, acetic acid, sodium acetate, NH₃, NH₄Cl, KCl, HCl, aquadest, and aluminum foil. The equipment used in this research was an analytical balance, volumetric flask, mortar, beaker glass, measuring cylinder, stirring rod, filter funnel, erlenmeyer flask, water bath, UV-Vis spectrophotometer, test tubes, drop pipette, measuring pipette, and film bottle.

Plant Material
Fresh pletekan flowers obtained from Gresik and Surabaya were collected in the Laboratory of Land Resources. Flowers were kept in cold and dark storage until processed.

Anthocyanin Extraction using Maceration Method
Fresh pletekan flowers were separated from the stem, then mashed using a mortar. Mashed flowers were weighed and macerated in maceration solvents (methanol acidified by HCl 1% with a volume ratio of 10:1) at beaker glass at a ratio of 1:4 (gram : ml). The mixture was stirred for a few moments. Beaker glass was tightly closed by aluminum foil and stored in dark storage at room temperature for 2-3 days. The mixture was filtered by using a filter paper. The remaining solids in filter paper were washed with a maceration solvent until a clear solution was obtained. The resulted filtrates (unconcentrated extract) was evaporated in a water bath until a concentrated extract was obtained.

Measurement Maximum Wavelength
Measurement of the maximum wavelength of anthocyanin extract using unconcentrated extract. The absorbance of the unconcentrated extract was measured at the wavelength range 400-560 nm by using a UV-Vis spectrophotometer.

Determination of Total Anthocyanin Content (TAC) using pH Differential Method
The determination of TAC used a concentrated extract. The TAC determination is expressed as the cyanidin-3-glucoside equivalents. 2 ml of concentrated extract was diluted to 50 ml with a buffer solution of pH 1 (KCl-HCl), and 2 ml of concentrated extract was diluted to 50 ml with a buffer solution of pH 4.5 (sodium acetate - HCl). The absorbance of each diluent was measured at the wavelength 510 nm and 700 nm using a UV-Vis spectrophotometer.

The absorbance (A) was calculated using this equation:

\[ A = (A_{510\text{nm}} - A_{700\text{nm}}) \times \text{pH}1 - (A_{510\text{nm}} - A_{700\text{nm}}) \times \text{pH}4.5 \]

The total anthocyanin content (TAC) was calculated using this equation:

\[ \text{TAC (mg/L)} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times l} \]

Where \( A \) is the absorbance, \( MW \) is the molecular weight of cyanidin-3-glucoside = 449.29 g/mol, \( DF \) is the dilution factor (2 ml sample is diluted to 50 ml, \( DF = 25 \)), \( \varepsilon \) is the extinction coefficient of cyanidin-3-glucoside = 26900 L/(mol.cm), and \( l \) is the path length = 1 cm.

Determination of pH Track
The concentrated extract was diluted with an unconcentrated extract with a volume ratio of 1:10. 3 ml of each pH 1-12 buffer solution was put into a test tube, then added with 10 drops of anthocyanin extract. Color change was observed carefully. The acid buffer solution was made from a mixture of acetic acid and sodium acetate and alkali buffer solution was made from a mixture of NH₃ and NH₄Cl.

Soil Test
The concentrated extract was diluted with an unconcentrated extract with a volume ratio 1:10. Ten grams of soil sample was dissolved with 20 ml of aquadest in a bottle film, then shake well for a few minutes. The mixture is left for a few minutes until the soil settles. A small amount of clear water on the surface is taken, put into a test tube, added with 10 drops of anthocyanin extract, and homogenized. The results of the color change were equated to the color of the pH track, and soil samples in the film bottle were measured using a pH-meter.

3. RESULTS AND DISCUSSION

3.1 Anthocyanin Extract
The extraction process is the process to obtain the desired substance. 150 grams of mixed flowers were macerated in 600 ml of maceration solvent. Flowers are mashed because of the smaller particle size, the larger surface area of particles, finally, more anthocyanin that can be dissolved into solvent (Gustriani, 2016).

The solvent color changes from colorless to red. According to Zulfajri (2017), the solvent experiences a color change which indicates the solvent has pulled the extract. The flower maceration process was carried out under acidic conditions with HCl 1%. According to Wahyuningish (2016), the addition of acid serves to dissolve anthocyanin pigments and denaturation plant cell membranes.

The mixture was filtered, and the remaining solid was washed with 400 ml maceration solvent. Washing is done to obtain the remaining extracts that have not been pulled by the solvent. The unconcentrated extract is evaporated using a water bath for 1 hour. The concentrated extract was obtained as much as ± 150 ml. Figure 1 shows the results of the unconcentrated extract and the
3.2 Measurement of Maximum Wavelength

The results showed that the maximum wavelength of anthocyanin extract was 540 nm with absorbance 1.659 (Figure 2). Harvard Forest Team (2011) reported that the anthocyanin absorption spectrum is of maximum value in the wavelength range of 450-570 nm.

3.3 Determination of Total Anthocyanin Content

Determination of Total Anthocyanin Content (TAC) using the pH differential method. The pH differential method is used to see the comparison of anthocyanin compounds produced at different pH, which is pH 1 and pH 4.5. pH 1 is used because anthocyanin is stable at pH below 4 and less stable at pH 4.5.

2 ml of concentrated extract was diluted in pH 1 and 4.5 of buffer solution, then the absorbance of each diluent was measured at the wavelength 510 nm and 700 nm by using a UV-Vis spectrophotometer. According to Utami (2016), the wavelength of 510 nm is the maximum wavelength for cyanidin-3-glucoside, while the wavelength of 700 nm is to correct the deposits that are still present in the sample. If the sample is clear, the absorbance at a wavelength of 700 nm is 0.

In determining TAC, the sample dilution factor (DF) must be determined in advance by dissolving the sample in a pH 1 buffer solution then measuring the absorbance until the absorbance is less than 1.2 at a wavelength of 510 nm (Putri, 2015). The specified dilution factor is 25 times. Based on the calculation, the average TAC was 209.821 mg/L (Table 1).

| Replication | Buffer pH 1.0 | Buffer pH 4.5 | TAC (mg/L) |
|-------------|---------------|---------------|------------|
|             | λ 510 nm   | λ 700 nm | λ 510 nm | λ 700 nm |           |
| 1           | 0.576      | 0.005      | 0.114    | 0.003    | 192.075   |
| 2           | 0.572      | 0.005      | 0.105    | 0.002    | 193.745   |
| 3           | 0.581      | 0.006      | 0.102    | 0.006    | 200.009   |
| 4           | 0.599      | 0.005      | 0.102    | 0.010    | 209.612   |
| 5           | 0.600      | 0.008      | 0.107    | 0.012    | 207.525   |
| 6           | 0.608      | 0.007      | 0.098    | 0.016    | 216.711   |
| 7           | 0.630      | 0.003      | 0.099    | 0.003    | 221.722   |
| 8           | 0.628      | 0.002      | 0.014    | 0.009    | 221.722   |
| 9           | 0.614      | 0.003      | 0.100    | 0.008    | 216.711   |
| 10          | 0.609      | 0.003      | 0.094    | 0.011    | 218.381   |
| **Average** |             |             |          |          | **209.821** |

Figure 1. (a) uncutyent extract, (b) cencenetrated extract

Figure 2. Wavelength of anthocyanin extract

Table 1. Total Anthocyanin Content
3.4 Determination of pH Track

Figure 3 shows the pH track of anthocyanin from pH buffer 1 (right) to pH buffer 12 (left). Buffer solution change color to pink and orange in acid pH (1-6); violet in neutral pH; blue, green, and yellow in alkali pH. Color changes that occurred at each pH buffer will produce a color different. This happens because anthocyanin stability is greatly influenced by pH so anthocyanins will be forming derivative compounds (Gustriani, 2016).

Concentrated anthocyanin extracts need to be diluted with extracts that have not been concentrated so that the resulting color changes are more specific.

3.5 Soil Test

The results of soil pH measurements using natural pH indicators (anthocyanin plettress extract) showed discoloration of soil sediment-water which was dropped by natural pH indicators needed more indicator drops such as 2-3 levels than was color changes caused by the buffer solution.

(Figure 4). This color reduction was caused by natural pH indicators containing HCl solvents that were acidic so that the color of the sedimentary water tested was likely to drop 2-3 levels rather than the color chart. The color chart used was the color chart in the buffer solution of pH 1-12, because it had a pH track with a clear color change.

4. CONCLUSION

The color change of soil sediment-water which was dropped by natural pH indicators, needed more indicator drops such as 2-3 levels than was color changes caused by the buffer solution. Buffer solution changed the color to pink and orange in acid pH (1-6); violet in neutral pH; blue, green, and yellow in alkali pH. The maximum wavelength of anthocyanin extract was 540 nm with absorbance 1.659, and hthe average TAC was 209.821 mg/L.

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