Dioscorea alata L anthocyanin extract methanol as a sensitive pH active compound

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Abstract. Anthocyanin from Dioscorea alata L has been successfully extracted using a methanol solvent and has been tested for its sensitivity to pH changes using a UV-Vis spectrophotometer in citrate and phosphate buffer solutions. The extracted anthocyanin showed positive flavonoid and phenol tests. The total yield of crude extract was 1.63% that is related to 3.34 x10^{-7} M or 16.72 mg /kg dried sample. The maximum absorption against citrate buffer is at a wavelength of 600 nm with a linear range toward pH 4 to 8, sensitivity = 0.121 and R^2 = 0.98. Furthermore, the maximum absorption for phosphate buffer occurs at a wavelength of 590 nm that has a response linear in the range of pH 6 to 8 and sensitivity = 0.296 (R^2 = 0.97). The optimum concentration of citrate buffer is 0.1M with a linear pH of 4-8 and sensitivity = 0.121 (R^2 = 0.98). The optimum buffer concentration for both citrate and phosphate buffer solutions were reached at 0.1M and 0.075 M, respectively. The extracted anthocyanin showed stable response properties against citrate and phosphate buffer for 140 minutes during the test period. This anthocyanin is potential to be used as an active substance to developed optical pH sensors and biosensors based on the determination of pH change.

1. Introduction
Currently, the natural compounds used in several fields, especially in the areas of food, beverages and medicines are being a concern of researchers because it is safe than synthetic compounds. Not only the fields mentioned above, but natural compounds also have as a consideration in developing analytical methods such as sensor and biosensors that was aimed for the analysis of food and clinical samples. Natural compounds can be used as an active material; for example, the use of anthocyanin for monitoring fish freshness. The anthocyanin is abundant available in many parts of plants such as leaves, flowers, fruits and tubers [1].

Anthocyanin is a subclass of flavonoids and is also known as an antioxidant. As mentioned above, tubers are a source of anthocyanin. Wild yam plant tubers (Dioscorea alata L.) is purple tubers. Previous works reported that the purple plants contain high levels of anthocyanin [2,3]. The level of anthocyanin that has been reported from purple plants is about 31 mg /dry weight. Other sources are 21 mg / dry weight of black potatoes [4] and 26.5 mg/100 gram dry weight for black rice [5].

In addition to anti-oxidants, anthocyanin can change colour according to the pH of its environment [6]. Then, the nature of anthocyanin against pH changes properties is promising to further application such as in developing optical pH sensors. This compound is safe when it is applied to measure pH in...
situ analysis for food and clinical sample. In this work, anthocyanin was extracted from *Dioscorea alata* L, and the characterization of its properties, sensitivity toward pH has been studied in citrate and phosphate buffer solutions in the pH range 4-8. The work is a primarily study to investigate the ability of anthocyanin from *Dioscorea alata* L as an active substance pH change for further application.

### 2. Reagents and instruments

The instrument used in this study is the UV-Visible Spectrophotometer (Shimazu 1800), pH meter from Thermo Orion Star A2111 and FTIR spectroscopy. Chemicals used in this research are analytical grade, which includes; monopotassium phosphate (KH$_2$PO$_4$) and dipotassium phosphate (K$_2$HPO$_4$), which were purchased from Merck, pectin, ethanol absolute and CaCl$_2$ were obtained from Sigma-Aldrich. Methanol and acetic acid were purchased from Fluka.

#### 2.1. Anthocyanin extract

A total of 2 kg of tuber samples from *Dioscorea alata* L was cleaned and chopped into small pieces. These pieces were air-dried for two days until completely dry. The sample was inserted into a 250 ml chemical glass and then macerated sample using 1000mL methanol solvent for ± 24 hours at room temperature 25°C. The sample is then filtered, so that filtrate and residue are obtained. The filtrate is heated at 50°C until the solution becomes concentrated (± 50 ML) to remove the remaining solvent until an anthocyanin extract is obtained [7].

#### 2.2. Flavonoid test

The anthocyanin extract is analysed using phytochemistry with a flavonoid test based on the Shinoda method. 0.5 mL of anthocyanin extract samples were placed on a spot plate and a small piece of Mg ribbon is added. After several minutes, 0.5 mL of concentrated HCl is added. The formation of the colour change of orange to red characterizes positive flavonoid compounds [8].

#### 2.3. Phenol test

0.5 mL of anthocyanin extract was placed on the ppot plate and added with 0.5 mL methanol and a FeCl$_3$. The resulting discolouration was observed [9].

#### 2.4. FTIR characterization

Anthocyanin extract from the tuber *Dioscorea alata* L was further characterized using FTIR spectroscopy.

#### 2.5. The determination of $\lambda_{\text{max}}$ of anthocyanin at various citrate and phosphate buffer pH

3 mL of anthocyanin extract is taken and diluted with 20 mL of ultrapure water. 1 ml of this solution was added to 3 mL of buffer citrate of pH variation 4; 4.5; 5; 5.5; 6; 6.5; 7; 7.5 and 8. Their absorbance was further measured at a wavelength of 400-700 nm. Similar treatment was also done against the 0.1 M phosphate buffer at pH variation of 4; 8; 5; 5.5; 6; 6.5; 7; 7.5; 8; 8.5 and 9 [10].

#### 2.6. The study of Anthocyanin sensitivity towards various phosphate buffer and citrate buffer concentrations

1 mL of anthocyanin extract is taken and inserted into a reaction tube. Citrate buffer solution is added to the reaction tube in varying concentrations of 0.01 M; 0.03 M; 0.05 M; 0.075 M and 0.1 M. Each variation of the concentration was measured for its absorption at their respective Max$\lambda$. The same treatment was also done against the phosphate buffer [10].

#### 2.7. Stability of anthocyanin in citrate and phosphate buffers

1 mL of anthocyanin extract is added with 0.1M citrate buffer solution at pH 5 and 8 followed by absorption measurement. The same treatment is also conducted using 0.075 M phosphate buffer solution. The absorbance was measured from 0 to 140 minutes.
3. Results and discussions

3.1. Extraction anthocyanin from Dioscorea alata L

Dioscorea alata L. was extracted using methanol as a polar solvent. Therefore, it is suitable for the extraction of flavonoids, including anthocyanins. The process of maceration aims to attract the desired content of metabolites. The extract of Dioscorea alata L. is solid purple, as seen in Figure 1b. The Total Gross amount of extract obtained in this study was 1.63% with a total concentration of 3.34 x10^{-7} M or 32.544 mg/2000 g dry material.

Figure 1. (a) Dioscorea alata L. tuber (b) Dioscorea alata L. methanol extract

Anthocyanins mean a secondary metabolite of flavonoids. The anthocyanin extract from Dioscorea alata L shows positive results of flavonoids (Fig.1. a) when tested using concentrated Mg and HCl powders. The presence of flavonoids is shown with the formation of orange to red colour [8]. The change in colour proves that there is a covalent bond between magnesium ions and the OH phenolic group of the flavonoid’s compounds [11]. Next, the presence of flavonoids is further proved with a qualitative test of phenol using FeCl₃ reagent Figure 2. (b). Extracts of the tuber Dioscorea alata L, which is orange in colour turned into the black after a few drops of FeCl₃ reagent is added [9]. The reaction of flavonoids with HCl and Mg is shown in Figure 2. (a) and the phenol reaction with FeCl₃ is shown in Figure 2. (b)

Figure 2. (a) The reaction of flavonoids with HCl and Mg, (b) Phenol reaction with FeCl₃.

In addition to flavonoids test using Mg and HCl as well as phenol tests with FeCl₃, the anthocyanin extract of the tuber Dioscorea alata L. is also characterised using FTIR spectroscopy to locate the functional groups of anthocyanin. Analysis of the results is shown in Figure 3.
Figure 3. Profile spectra of FTIR of anthocyanin *Dioscorea alata* L.

Based on the IR spectrum data shown in Figure 3. It can be seen that the presence of *Dioscorea alata* L. tuber extract functional groups are indicated by absorption at wavelength 4500-500 cm$^{-1}$. The vibration of the O-H functional group is tied to wavelength 3385.7 cm$^{-1}$. At wavelength 2933.73 cm$^{-1}$ the presence of C-H is seen, while C = O carbonyl groups are present at wavelength 1726.29 cm$^{-1}$, C=C groups at wavelengths 1620.21 cm$^{-1}$ and the presence of the C-O ether group is exhibited at wavelength 1055.06 cm$^{-1}$. The IR spectra data of *Dioscorea alata* L. tuber extract showed the presence of a carbonyl group, while in an anthocyanin structure carbonyl groups absent. The carbonyl group is suspected to be a tannin compound, which is a derivative of phenolic compounds that possess carbonyl groups. The structure of anthocyanins and tannins-class compounds can be seen in Figure 4.

Figure 4. Structure of tannin.

3.2. Determination of anthocyanine $\lambda_{max}$ in citrate and phosphate buffer

The determination of $\lambda_{max}$ of the methanol extract of *Dioscorea alata* L. is conducted using UV-Vis spectrophotometer by measuring the absorbance of the methanol anthocyanin extract. The anthocyanin extract was added with a citrate buffer solution 0.1 M at various pHs. The same treatment is also done using 0.1M phosphate buffer at various pH. The absorption of each extract is measured at a wavelength range of 400-700 nm test results, as shown in Table 1.
Table 1. Determination of $\lambda_{\text{max}}$ of *Dioscorea alata* L tuber extract.

| A $(\text{nm})$ | Citrate buffer | | A $(\text{nm})$ | Phosphate buffer |
|----------------|----------------|----------------|----------------|----------------|
| 420 6.0-8.0 | 0.1005 | 0.9888 | 400 6.5-8.0 | 0.5856 | 0.9148 |
| 440 5.5-8.0 | 0.0724 | 0.9922 | 420 6.0-7.5 | 0.2711 | 0.9529 |
| 510 6.0-8.0 | 0.0225 | 0.9745 | 470 6.0-7.5 | 0.1176 | 0.9837 |
| 560 6.0-8.0 | 0.0528 | 0.9853 | 580 6.0-8.0 | 0.2114 | 0.9625 |
| 570 5.0-8.0 | 0.0630 | 0.9919 | 590 6.0-8.0 | 0.2946 | 0.9741 |
| 600 4.0-8.0 | 0.1210 | 0.98 | 610 6.0-7.5 | 0.4505 | 0.9979 |
| 610 6.0-8.0 | 0.1625 | 0.9915 | 620 6.5-7.5 | 0.394 | 0.9971 |

Results showed that the maximum wavelengths of *Dioscorea alata* L anthocyanine extract in citrate buffer were at the wavelength of 600 nm. At this wavelength, anthocyanin sensitivity value is 0.121 with an $R^2$ value of 0.98 at pH range 4-8. Next, the maximum wavelength using phosphate buffer is 590 nm with a pH range 6-8, the sensitivity of 0.2946 and $R^2$ value of 0.974. Then, a good correlation value ($R^2$) is 0.8-1 [12] while a good sensitivity is measured based on the slope of the calibration curve with an $R^2$ value approaching 0.99 [13]. Based on this, the maximum wavelength when using citrate buffer is at 600 nm and phosphate buffer at 590 nm, which will be used for absorbance measurement.

3.3. Effect of citrate and phosphate buffers concentration against anthocyanins *Dioscorea alata* L characteristics

Maximum wavelength is determined based on the highest sensitivity value and the most extensive pH range at a wavelength of 400-700 nm. The determination of the buffer solution concentration effect is performed using citrate and phosphate buffer. Buffer concentrations were varied at 0.01 M; 0.03 M; 0.05 M; 0.075 M and 0.1 M. The absorption of each concentration is measured at the buffer’s respective $\lambda_{\text{max}}$. The sensitivity value of anthocyanins in each buffer concentration is shown in Tables 2 and 3.

Table 2. Effect of citrate buffer concentrations towards sensitivity.

| No. | Buffer Concentration (M) | Linear range of pH | Sensitivity | $R^2$ |
|-----|--------------------------|--------------------|-------------|-------|
| 1.  | 0.01 M                   | 6.5-7.5            | 0.019       | 0.8650 |
| 2.  | 0.03 M                   | 6.0-7.0            | 0.038       | 0.9816 |
| 3.  | 0.05 M                   | 6.0-7.5            | 0.0301      | 0.9915 |
| 4.  | 0.075 M                  | 5.5-7.5            | 0.0272      | 0.9754 |
| 5.  | 0.1 M                    | 4.0-8.0            | 0.1210      | 0.9800 |

Table 3. Effect of phosphate buffer concentrations towards sensitivity.

| No. | Buffer Concentration (M) | Linear range of pH | Sensitivity | $R^2$ |
|-----|--------------------------|--------------------|-------------|-------|
| 1.  | 0.01 M                   | 7.0-9.0            | 0.0585      | 0.9850 |
| 2.  | 0.03 M                   | 7.0-8.5            | 0.0907      | 0.9990 |
| 3.  | 0.05 M                   | 7.0-8.5            | 0.1289      | 0.9591 |
| 4.  | 0.075 M                  | 6.5-8.5            | 0.1372      | 0.9922 |
| 5.  | 0.1 M                    | 4.8-8.0            | 0.1837      | 0.8711 |

Based on Tables 2 and 3, the optimum buffer concentration was selected based on the widest responsive pH range, highest sensitivity and $R^2$ value approaching 1. Therefore, citrate buffer is best at 0.1 M concentration with a pH range of 4-8, sensitivity value of 0.121 and $R^2$ value of 0.98. On the other hand,
buffer phosphate exhibits the best response at a concentration of 0.075 M, pH range of 5-8, the sensitivity of 0.1372 and R² value of 0.9922.

3.4. Stability study of Dioscorea alata anthocyanin extract

The anthocyanin extracts were added each with phosphate buffer and citrate buffer at pH 5 and 8. Their absorption is then measured at a period of 0 minutes to 140 minutes. The result of the colour stability of the anthocyanins extracts in citrate and phosphate buffer (pH 5 and 8) are shown in Figure 5 (a) and (b).

![Graph showing stability study of anthocyanin extract](image)

**Figure 5.** Stability study of anthocyanin extract of *Dioscorea alata* L in (a) citrate buffer and (b) phosphate buffer.

Based on the graphs shown in Figure 5 (a) and (b). It can be concluded that the colour stability of anthocyanins extracted from *Dioscorea alata* L. between 0 to 140 minutes showed stable measurements. The stability can be observed from the constant absorption rate at acidic and alkaline pH.

4. Conclusion

Anthocyanin was successfully extracted from the *Dioscorea alata* L tuber and exhibit pH-sensitive characteristics when tested with phosphate and citrate buffers solution at acidic and basic pH. The extract of anthocyanin also showed sensitivity and linearity towards change in pH. Therefore, this anthocyanin extract can be employed further as an active ingredient in the construction of an optical pH sensor.

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