**Ruellia tuberosa** L Anthocyanin extract as a pH sensitive substance

E Safitri¹,³, N Afifah¹, Khairi¹, Lelifajri¹, Nazaruddin¹, Susilawati¹ and N D Sani²

¹Department of Chemistry, Faculty of Mathematics and Natural Sciences, Universitas Syiah Kuala, Banda Aceh 23111, Indonesia
²Sanichem Resources Sdn. Bhd. No 7 & 7A Jalan Timur 6/1A Mercato Enstek, Bandar Estek 71060, Negeri Sembilan, Malaysia

E-mail: e.safitri@unsyiah.ac.id

**Abstract.** Anthocyanin from the flower Ruellia tuberosa L was successfully extracted by maceration using methanol. The total extract obtained was 19.22% with a concentration of 1.503 mg/L correspondingly. Retention time was analyzed using Thin Layer Chromatography and an Rf value of 0.43 was achieved. The analytical determination of functional groups was conducted using FTIR. The sensitivity of anthocyanin towards phosphate buffer pH is 0.222 at the range of pH 6 - 8 with R² = 0.996 at maximum wavelength of 635 nm. On the other hand, its sensitivity towards citrate buffer is 0.022 at pH range 6 - 8 with a linearity of R² = 0.999 at 625 nm maximum wavelength. The anthocyanin showed good in sensitivity and dynamic range in 0.1 M Phosphate buffer solution.

1. Introduction

Anthocyanin is known as a pigment that is derived from the phenolic compound group and is soluble in water. This pigment is available in glycosylated form [1]. Anthocyanin is also well known in the medical field as an important polyphenol compound that can prevent diabetes [2], cancer and obesity [3]. In fact, anthocyanin has been used as an anti-inflammatory agent [4].

The properties of anthocyanin are very much affected by temperature, light and pH. anthocyanin displays different structures at different pH [5]. This attribute confers anthocyanin with pH sensitivity which enables it to become an active substance that can detect change in pH. This phenomenon will prompt a colour change that can be witnessed visually and can be quantitated using certain instruments like UV-Visible Spectrophotometer. Anthocyanin has an added advantage over other synthetic organic dyes that also has pH sensitive properties such as Nile Blue ETH 5294 [6]. The anthocyanin is also easy to obtain, relatively cheap to acquire and is available in a variety of colours.

One of the sources of anthocyanin is flowers. This study attempts to analyze the properties of anthocyanin extracted from Ruellia tuberosa L (R. tuberosa L) which is a blue coloured wildflower. To this point, there has not been any study on the anthocyanin extract of this flower. Here anthocyanin extract is obtained through maceration using methanol. FTIR was used to determine the functional groups of the anthocyanin while the sensitivity of anthocyanin towards pH was tested using phosphate and citrate from an acidic pH to a base.

³ To whom any correspondence should be addressed.
2. Reagents and instruments

The instrument used in this study is the UV-Visible Spectrophotometer (Shimazu 1800), pH meter from Thermo Orion Star A2111, FTIR spectroscopy, UV lamp and G_{60} F_{245} thin layer chromatography silica gel. Chemicals used in this research are analytical grade, which include; 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. Butanol, metanol and acetic acid were purchased from Fluka.

2.1. Anthocyanin extract

Fresh R. tuberosa (200 g) samples were macerated using 85 mL methanol as solvent for ± 24 h at room temperature of 25°C and filtered until filtrate and residue were obtained. The filtrate was heated at 50°C until the solution becomes ±50 mL. The anthocyanin extract was tested using Thin Layer Chromatography (TLC) and FTIR spectroscopy. The TLC was conducted using G_{60} F_{245} as the stationary phase with the mobile phase n-butanol: acetic acid : water (4:1:5). The elution patterns were easily visible under UV-light.

2.2. The determination of \( \lambda_{\text{maks}} \) of Anthocyanin at various citrate and phosphate buffer pH

1 mL of Anthocyanin extract was poured into a reaction tube and added with 9 mL of 0.1 M phosphate buffer solution at various pH. The absorbance of this solution was measured at wavelengths 500 - 640 nm. This procedure is repeated on 0.1 M citrate buffer as well.

2.3. The determination of Anthocyanin absorbance at various phosphate buffer concentration

1 mL of anthocyanin extract was poured into a reaction tube. Various concentrations of phosphate buffer (0.05, 0.03, 0.05 and 0.1 M) were added in a number of reaction tubes containing 1 mL of the extract. The absorbance of the various phosphate buffer concentrations (pH 6, 6.5, 7, 7.5 dan 8) was measured at \( \lambda_{\text{maks}} \) until the sensitivity value for pH determination was obtained.

3. Results and discussions

3.1. Anthocyanine extract from the flower R. tuberosa L

Anthocyanin from the flower R. tuberosa L exhibits different colours in acidic or basic pH [7]. The maceration of R. tuberosa L flower with methanol produces a brownish red coloured extract with a yield of around 19.22% and concentration of 1.50 mg/L in accordance with a study conducted by Ref. [8]. Qualitative analysis of the anthocyanin extract using TLC indicated that anthocyanin from R. tuberosa L extract is a cyanidine-3-glucoside compound with an \( R_f \) value of 0.43. This finding is consistent with the study by Ref. [9] using anthocyanin extract from the flower Verbena. Functional group analysis using FTIR (Figure 1) shows a widening of O-H group frequency with medium intensities in the wavenumber regions of 3333.63 cm\(^{-1}\) dan 3291.70 cm\(^{-1}\). The strong and sharp vibration of C-H group is seen in the wavenumber region of 2949.70 cm\(^{-1}\) and 2838.62 cm\(^{-1}\). In addition, the presence of anthocyanin compound in the extract is proven by the aromatic C=C group found in the wavenumber region of 1644.57 cm\(^{-1}\) dan 1545.31 cm\(^{-1}\) which indicates the general characteristics of anthocyanin compound. The frequencies of group C-O are also visible at wavenumbers 1111.13 cm\(^{-1}\) and 1015.07 cm\(^{-1}\). The FTIR vibration patterns which exhibits the presence of anthocyanin in the form of cyanidine-3-glucoside is similar in findings reported by Ref. [10] and [11].

3.2. The determination of Antocyanin \( \lambda_{\text{maks}} \) at various phosphate and citrate buffer pH

The determination of the maximum wavelength of anthocyanin (Cyanidine-3-glucoside) is conducted using anthocyanin solution that has been diluted 10 times using deionized water. Results obtained showed that anthocyanin from the R tuberosa possess different sensitivities at different wavelengths and buffers.

Therefore, the maximum wavelength of 635 nm will be used throughout this study for the measurement of phosphate buffer absorbance at various pH. The change in the colour of anthocyanin at acidic and basic pH is due to protonation (H\(^{+}\)) and deprotonation (OH\(^{-}\)).
At acidic pH, anthocyanin changes colour to red. This is due to the structure of anthocyanin that has undergone protonation which releases H\(^+\) ions between C and H bonds and this forms flavium cations. At normal pH, anthocyanin undergoes deprotonation and hydration that cause anthocyanin to change to blue or colourless. The blue colour shows that anthocyanin is in the form of quinonoidal base while colourless anthocyanin is due to the pseudobase carbinol structure. At pH range 6 - 8, anthocyanin will undergo protonation and hydration until the dominant cyanidine structure becomes a kuinonoidal base and produces a bluish or purplish hue. At pH 8 and above, anthocyanin will undergo deprotonation to form a chalcone and turns yellow in colour [12].

The buffer that gives the best sensitivity is phosphate buffer as shown in Table 1. The effect of anthocyanin response towards various buffers at pH 6 - 8 is shown in Figure 2. Results show that anthocyanin is more sensitive towards buffer with a higher pKa value. The pKa value of citrate buffer is 6.40 while the pKa derived from phosphate buffer is 7.80 [13]. Based on the observation conducted, the colour of anthocyanin extract fades when diluted with citrate buffer compared to when it is diluted with phosphate buffer even at the same pH. Therefore, the change in the colour of anthocyanin due to the addition of phosphate buffer gives a higher absorbance compared to the addition of citrate buffer. Based on the sensitivity values, phosphate buffer is chosen as the best buffer for the next optimization.

**Table 1.** The determination of anthocyanin extracts sensitivities at different wavelengths.

| \(\lambda\) (nm) | pH 6 - 8 | Sensitivity | \(R^2\) | pH 6 - 8 | Sensitivity | \(R^2\) |
|-----------------|---------|-------------|-------|---------|-------------|-------|
| 500             | 0.1918  | 0.984       |       | 6 - 8   | 0.012       | 0.984 |
| 600             | 0.1444  | 0.995       |       | 6 - 8   | 0.009       | 0.994 |
| 625             | 0.2192  | 0.994       |       | 6 - 8   | 0.020       | 0.999 |
| 630             | 0.2128  | 0.997       |       | 6 - 8   | 0.019       | 0.953 |
| **635**         | **0.2224** | **0.996**   |       | **6 - 8** | **0.032**   | **0.982** |
| 640             | 0.2150  | 0.998       |       | 6 - 8   | 0.019       | 0.970 |
3.3. Anthocyanin absorbance at various buffer concentrations

As previously explained, anthocyanin from the flower *R. tuberosa* L gives a good sensitivity towards the change in pH when phosphate buffer is used. Next, anthocyanin sensitivity studies towards change in pH using phosphate buffer concentration at 0.01, 0.03, 0.05 and 0.1 M. The sensitivities of anthocyanin towards various concentrations of phosphate buffer are shown in Table 2.

| No | Concentration (M) | pH Range | Sensitivities | R² |
|----|-------------------|----------|---------------|----|
| 1.  | 0.01              | 6.5 - 8.0 | 0.0866        | 0.968 |
| 2.  | 0.03              | 6.5 - 8.0 | 0.1362        | 0.988 |
| 3.  | 0.05              | 6.5 - 8.0 | 0.1290        | 0.987 |
| 4.  | 0.1               | 6.0 - 8.0 | 0.2224        | 0.996 |

Based on Table 2, the buffer concentration with the best sensitivity is 0.1 M. As observed, the colour of anthocyanin that was diluted with 0.1 M phosphate buffer concentration is more intense compared to when it was diluted with 0.05 M phosphate buffer. The absorbance of anthocyanin at 0.03 M phosphate buffer concentration does not differ much with 0.05 M phosphate buffer. Therefore, the stability of anthocyanin’s colour at buffer concentrations 0.03 M and 0.05 M at a range of pH is almost the same. This phenomenon can give the conclusion that buffer concentration does affect anthocyanin’s colour stability. However, there has yet to be reports on the effects of buffer concentration towards anthocyanin absorbance.

4. Conclusion

Anthocyanin was successfully extracted from the flower *R. tuberosa* L and displayed pH sensitive properties when tested with phosphate and citrate buffers at acidic and basic pH. The extract of anthocyanin also exhibited sensitivities and linearity towards change in pH. Therefore, this anthocyanin extract can be utilized further as an active ingredient in the development of an optical pH sensor.

References

[1] Hock E K, Azrina A, Sou T T and See M L 2017 *Food and Nutrition Research* **61** 136-177
[2] Judit R H, Andrea N, Erika F, Gyöngyi D, Péter B, Ferenc G J A, Jérémie M, Gerhard W, László and Judit R 2016 *Food Chemistry* **194** 222–229
[3] Mihaela T, Nicoleta S A, Gabriela, Bahrim and Gabriela R 2016 *J. Food Eng.* **171** 200-207
[4] Wang L S and Stoner G D 2008 *Cancer Lett.* **269** 281-290
[5] Ummi K I, Ida I M and Ruzitah M S 2011 J. Appl. Sci. 11 (13) 2406-2410
[6] Uswatun H, Mita S, Rustam E, Muslem M, Nor D M S, Eka S, Lee Y H and Rinaldi I 2019 Biosensor 9 (60) 2-8
[7] Anggriani R, Ain N and Adnan S 2017 Jurnal Teknologi Pertanian 18 (3) 163-172
[8] Lee J, Durst R W, Wrolstad R E, Barnes K W, Eisele T, Giusti M M, Hache J, Hofsommer H, Koswig S, Krueger D A, Kupina S, Martin S K, Martinsen B K, Miller T C, Paquette F, Ryabkova A, Skrede G, Trenn U and Wightman J D 2005 J. AOAC Int. 88 (5) 1269-1278
[9] Toki K, Yamamoto T, Terahara N, Saito N, Honda T, Inoue H and Mizutani H 1991 Phytochemistry 30 (11) 3828-3829
[10] Jian C, Li-yi Z, Zhi-qiang W, Rui-meng S, Nan C and Ai-dong S 2016 Int. J. Food Prop. 19 (1) 1-12
[11] Anggistia M D, Widiyandari H and Anam K 2016 Jurnal Kimia Sains dan Aplikasi 19 (2) 50-77
[12] Andersen O M and Markham K R 2006 Flavonoid: Chemistry, Biochemistry, and Applications (Taylor and Francis Group CRS Press, Unites States of America)
[13] Silberberg M S 2007 Principles of General Chemistry (McGraw-Hill Higher Education, USA)

Acknowledgments
We acknowledge financial support from Universitas Syiah Kuala via grants Lektor Kepala (Contract Number 76/UN11.2/PP/PNDP/SP3/2019).