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Determination of Anthocyanin at Different pH from *Clitoria Ternatea* Using Maceration Method

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Abstract

Clitoria ternatea, popularly known as the butterfly pea flower, is a popular ornamental plant with significant medicinal significance. Butterfly pea is also known for its bright blue colour, which can be used as a natural food colouring in sweets and beverages. In this study, anthocyanin was extracted using a maceration process with distilled water as the solvent as it was simpler and less costly compared to other extraction methods. As a result, anthocyanin appears in purple, dark blue, and dark greenish hues depending on acidic, neutral, and alkaline pH, respectively. The UV-Vis spectrophotometer detected anthocyanin between 480 and 600 nm. The maximum absorbance of anthocyanin (8.50 A) occurs at pH 4, showing the presence of the highest concentration of anthocyanin in acidic conditions. The colour variations are caused by changes in anthocyanin's structural arrangement, which result in the creation of petunidin, delphinidin, and cyanidin. Thus, this study successfully demonstrated that pH affects the presence of anthocyanin in Clitoria ternatea.

1. Introduction

Clitoria ternatea is a perennial twiner and belongs to the family of Fabaceae (Lakshan *et al.,* 2019). It is commonly known as Telang (Malaysia), Aparajit, Aparajita, and Kakkattan (India), Dangchan (Thailand), Pokindong (Philippines), Lan hu die (China), Cunha (Brazil), and Kordofan pea (Sudan). *C. ternatea*'s flower petal is vivid blue, 1 to 2 inches long, and has a wavy-rimmed standard and a white centre (Ashraf *et al.,* 2023). *C. ternatea* has great bioactive compounds especially anthocyanin which is usually used as a natural colouring food additive. It is popularly used as food dyes in making *Nasi Kerabu* and *Kuih Tekan* respectively as a traditional food in Malaysia (Lijon *et al.,* 2017). A study conducted by Terahara et al. (1998) identified delphinidin glycosides, specifically eight anthocyanins (ternatins C1, C2, C3, C4, C5, and D3, as well as preternatins A3 and C4), which were recovered from the flowers of *C. ternatea*.

Anthocyanin, Fig.1 is a water-soluble flavonoid with blue, red, or purple pigments found in plants, especially flowers, fruits, and tubers, and has a diphenyl propane skeleton ($C_6C_3C_6$) (Rahayu *et al.,* 2023). Anthocyanin is considered one of the flavonoids although it has a positive charge at the oxygen atom of the C-ring of the basic flavonoid structure. In acidic conditions, anthocyanin appears as red pigment while in alkaline conditions, blue pigment anthocyanin appears (Huang *et al.,* 2023). Anthocyanin is widely used as an antidiabetic, anticancer, anti-inflammatory, and antimicrobial, as well as a treatment for cardiovascular diseases (CVDs) (Mattioli *et al.,*

2020) (Izirwan *et al.,* 2020). The colour, chemical degradation, and stability of anthocyanin pigment are highly influenced by environmental factors such as pH, temperature, and light (Enaru *et al.,* 2021).

Plant-derived extracts are utilized in diverse applications within the food, pharmaceutical, and cosmetic industries. The pH of the extraction solution significantly influences the efficiency and quality of anthocyanin extraction (Lan *et al.,* 2022). The challenge lies in figuring out the ideal pH level to reveal the presence of anthocyanin derivatives while maintaining their structural integrity and stability. Therefore, a study of the effect of pH on the presence of anthocyanin derivatives during the C. ternatea extraction process is essential. To enable the use of anthocyanin in a variety of industrial applications, this issue must be addressed to develop efficient and sustainable extraction procedures.

In this work, a maceration extraction technique was used to extract anthocyanin derivative from C. *ternatea* with distilled water as the solvent. The maceration method is a straightforward and inexpensive technique for extracting phytochemicals from plant parts. This extraction process relies solely on molecular diffusion. Suitable solvents that are compatible with anthocyanin such as water, hydrochloric acid, and sodium hydroxide are chosen based on the polarity of the target compound. The choice of solvent usually depends on factors such as solubility, and selectivity. An appropriate amount of time is given for the solvent to permeate the cell wall and dissolve the components found in the plant. Maceration is usually carried out at room temperature or slightly elevated temperature. The *C. ternatea* solution will exhibit a transition in colour, changing from red in an acidic solution to purplish, then blue, and finally green in a weakly alkaline solution. As a result, the purpose of this study is to isolate C. ternatea using distilled water as a solvent and detect the presence of anthocyanin derivatives in C. ternatea using a UV-Vis spectrophotometer, with a focus on the effect of pH.

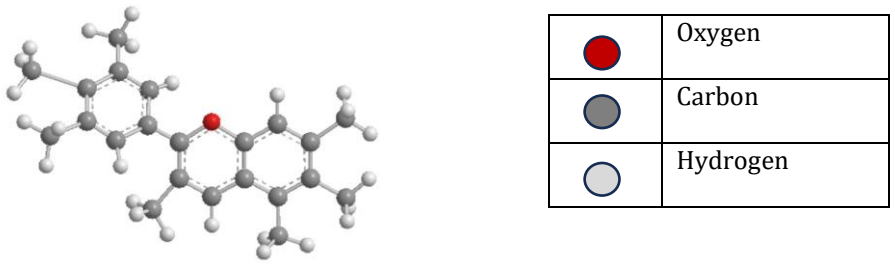

Fig. 1 *Structure of Anthocyanin*

2. Materials and Method

C. Ternatea flowers were collected from Shah Alam, Malaysia. The extraction was employed via maceration technique and the instrument used was UV-Vis spectrophotometer (U3900 Spectrometer, Hitachi, Japan) with a wavelength range of 480-600 nm. For the structural elucidation, the software used for drawing was ChemDraw 3D (2022).

2.1 Material Specifications

Hydrochloric acid (0.1M, brand R&M), distilled water, and sodium hydroxide (0.1M, brand R&M) were utilised.

2.2 Sample Preparation

The *C. ternatea* was arranged on trays and after that dried through natural sun drying. The sample was further crushed using a mortar and pestle until it reached a powdered consistency. Subsequently, the maceration technique was performed by measuring 5 g of the sample and immersing it in 100 mL of distilled water for 2 hours at room temperature. After that, the mixture was subjected to a filtration process by using filter paper, where, the filtrate was further analysed.

2.3 pH Value Determination

The filtrate extract was then transferred into three beakers, individually. The adjusted pH was carried out with hydrochloric acid and sodium hydroxide to get the pH values of 4, 7, and 10. According to a study by Ahlina et al. (2018), the pH evaluation of anthocyanin was analysed by using a UV-visible spectrophotometer with a wavelength of 480-600 nm.

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3. Results and Discussion

3.1 Colour based on pH

Fig. 2 shows that *C. ternatea* solution appears purple at pH 4, dark blue at pH 7, and dark greenish at pH 10. The colour degradation and chemical degradation are related to reversible equilibrium change between coloured and colourless forms of anthocyanins (Lan *et al.,* 2022). A similar study conducted by Koshy et al. (2022) found the extract solution containing anthocyanin displayed a diverse range of colours. At pH levels of 1-3 was red, at pH levels of 4-5 was purple, at pH levels of 6-7 was blue, at pH levels of 8-9 was green, and at pH levels of 10-12 was dark yellow. The observed variation in colour is due to structural modifications experienced by anthocyanin in different pH mediums (Poh *et al.,* 2019). Fig. 3 (a) illustrates the formation of petunidin as a derivative of anthocyanin under acidic conditions (pH4), whereby the functional group of CH_3 is transformed into CH_3-O and another CH_3 is converted into OH. Moreover, in neutral conditions (pH7), the existence of delphinin is observed by the conversion of CH_3 to OH groups. Conversely, under alkaline conditions (pH10), the presence of cyanidin is observed through the conversion of CH_3 to OH and another CH_3 to H.

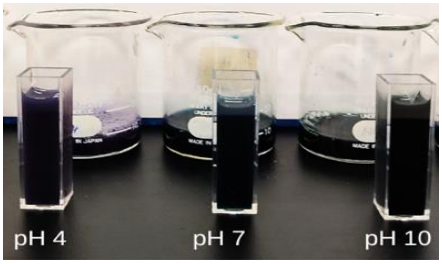
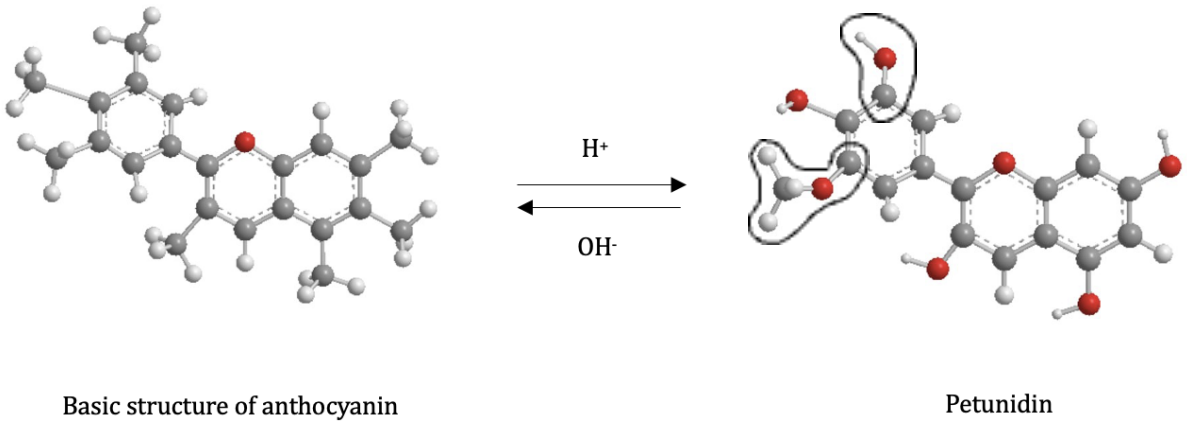
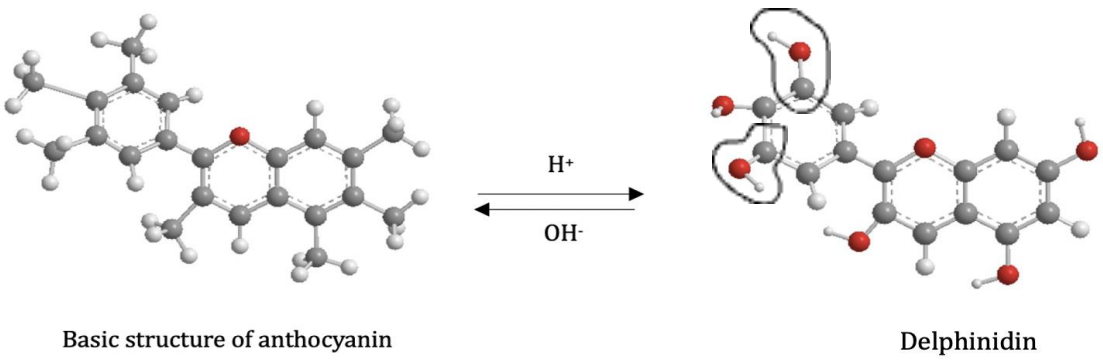


Fig. 2 *Discoloration of extracted C. ternatea at different pH*

(a) Acidic condition



(b) Neutral condition



(c) Alkaline condition

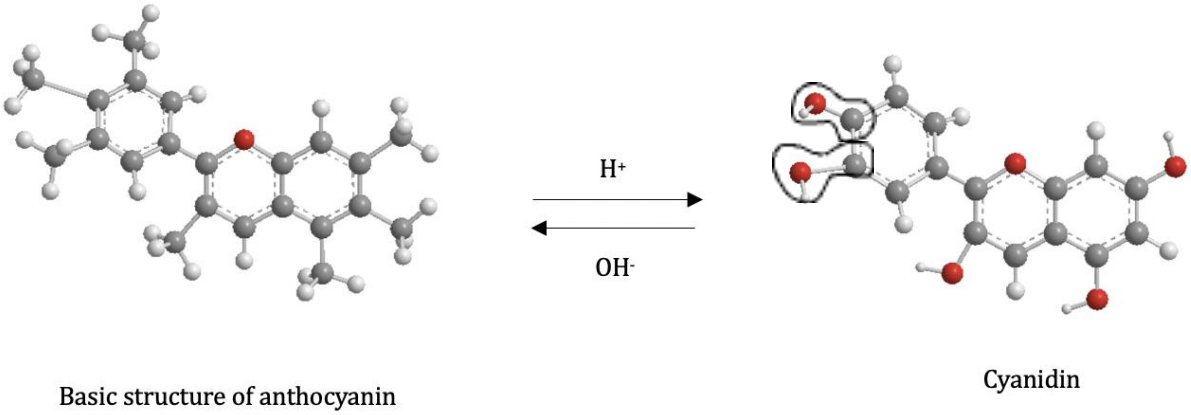


Fig. 3 *Mechanism of anthocyanin (a) pH 4 (b) pH 7 & (c) pH 10*

3.2 UV-Vis analysis

Fig. 4 interpreted that the anthocyanin active compound is identified at the range between 480-600 nm wavelength where the highest peak of each pH; is pH 4 (red curve), followed by pH 10 (black) and pH 7 (blue peak). Anthocyanin at pH 7 known as ternatin is blue and it is acylated-based delphinidin. Delphinidin is the main anthocyanin responsible for the deep blue turn to purple colours in the neutral solution (pH 7) (Thuy *et al.,* 2021) (Jeyaraj *et al.,* 2020). The wavelengths for anthocyanin are 570 nm for pH 10 followed by 565 nm at pH 7 and 560 nm at pH 4. According to Fig.4, the absorbance for pH 4 is the highest (8.50 A), compared to pH 10 (8.25 A) and pH 7 (6.60 A). It shows that anthocyanin is present highest in acidic conditions (pH 4) because the hydroxyls increase and methoxyl groups replace the hydroxyls (Koshy *et al*., 2022).

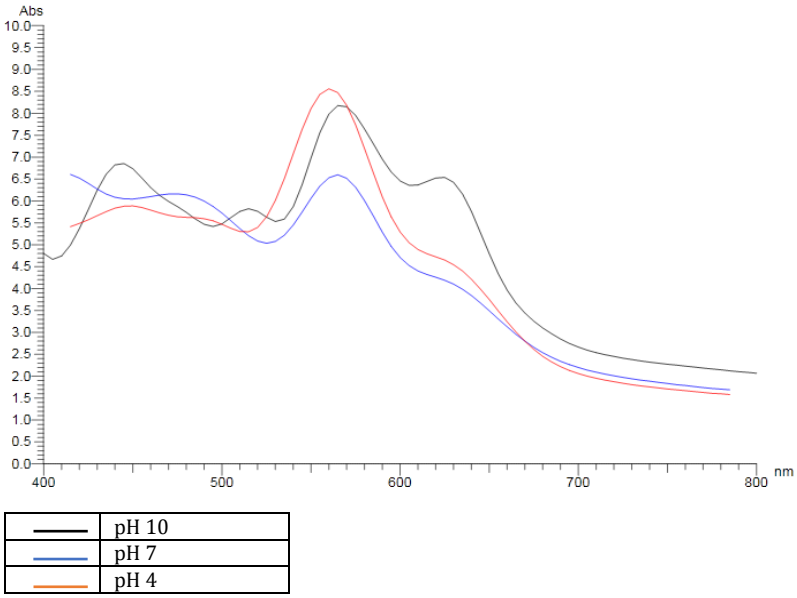


Fig. 4 *Absorbance spectra of anthocyanin at different pH*

3.3 Stability of anthocyanin colour based on pH values

Based on Table 1, anthocyanin has higher stability in an acidic medium compared to a base medium. The stability of anthocyanins is dependent on pH (Enaru *et al.,* 2021). From Fig. 3, the presence of β-ring in petunidin (pH4), delphinidin (pH7), and cyanidin (pH 10) structure indicates the existence of hydroxyl (-OH) and methoxyl groups (-OCH_3) that affect stability. The hydroxyl groups decrease anthocyanin stability in a solution, which affects the colour of anthocyanins depending on the pH of the solution. The absorbance of anthocyanin is affected by the substitution of the β-ring with either hydroxyl or methoxyl groups which changes the colour from red (peonidin) to bluish-purple (quinoidal base in delphinidin). Blue flowers of *Clitoria ternatea* contain delphinidin derivatives, although there are a few cyanidin-based anthocyanins that appear blue (Gençdağ *et al.,*

2022). Additionally, in acidic conditions, some of the anthocyanins appear red. Anthocyanin is more stable at lower pH. The solution appears red, in which it forms flavylium cations. The formed flavylium cations will help for the solubility of anthocyanin in water. The decrease in water concentration increases the rate of deprotonation of the flavylium cation, thus reducing colour stability (Koh *et al.,* 2020)

Table 1 *Colour changes in different pH*

pH	Condition	Absorbance	Colour
4	acidic / acid	8.50	purple
7	neutral	8.25	dark blue
10	alkaline/base	6.60	dark green

4. Conclusion

The determination of anthocyanin in the butterfly pea flower, *C. ternatea*, was achieved through colour changes at pH 4, pH 7, and pH 10. These colour changes were attributed to the presence of different anthocyanin derivatives (petunidin, delphinidin, and cyanidin) at different pH. The colour variation was caused by an alteration in the structural composition of anthocyanin, which was influenced by environmental pH conditions. Furthermore, the concentration of anthocyanin derivatives was highest at pH 4 (petunidin), as seen by the maximum absorbance value compared to pH 7 (delphinidin) and pH 10 (cyanidin) due to the presence of the hydroxyl and methoxyl groups. This study helps to contribute to the comprehension of plant chemical composition and offers valuable insights into its prospective applications in industries such as food, cosmetics, and pharmaceutical.

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Conflict of Interest

Authors declare that there is no conflict of interests regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: ***study conception and writing:*** *Alya Athirah Mohd Idris;* ***guidance and made corrections:*** *Alya Athirah Mohd Idris and Saliza Asman;* ***Reviewer:*** *Saliza Asman. All authors reviewed the results and approved the final version of the manuscript.*

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