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Research Paper

Complexation and Colour Stability of Anthocyanins from the Extracts of Java Plum Fruits (*Syzygium cumini*) with Selected Metal Cations (Al^{3+}, Fe^{3+}, and Ca^{2+}) at Various pHs

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Abstract

Anthocyanins are highly coloured polyphenols, a class of flavonoids, which are natural, non-toxic, and water-soluble compounds. Because of that, anthocyanins have been used in numerous applications, such as natural colorants for foods, beverages, and textiles. However, anthocyanins have some drawbacks regarding their stability towards temperature, pH, and light, leading to degradation. One method to improve anthocyanins' stability can be achieved through a complexation reaction of anthocyanins with metal cations. Thus, this study aimed to investigate the stability and colour profiles of anthocyanins extracted from Java Plum fruits (*Syzygium cumini*) complexed with Al^{3+}, Fe^{3+}, and Ca^{2+} ions in pH 1-12. Previously, anthocyanins from Java Plum fruits have been identified by LC-MS as cyanidin-3-O-glucoside ($[M]^+$, m/z 449.3), delphinidin-3-O-glucoside ($[M]^+$, m/z 465.3), and petunidin-O-glucoside ($[M]^+$, m/z 479.3). The ATR FTIR was also performed to confirm the functional group of anthocyanins. The colour of each solution at different pH was evaluated by UV-Vis Spectrophotometer at 200-800 nm. Complexation of anthocyanins with Al^{3+}, Fe^{3+}, and Ca^{2+} ions showed various colours at different pHs. The complexation of anthocyanins with Al^{3+} at pH 4-5 developed a blue colour. The intensity of the blue colour increases with an increase in Al^{3+} ion concentration. This investigation showed the potential application of anthocyanin-metal chelation for the textile industry due to the range of colour produced. Furthermore, the complexation of anthocyanins to produce blue colour can be an alternative in food industries as this colour is difficult to obtain in acidic pH.

Keywords
Anthocyanins, Java Plum, *Syzygium cumini*, Complexation

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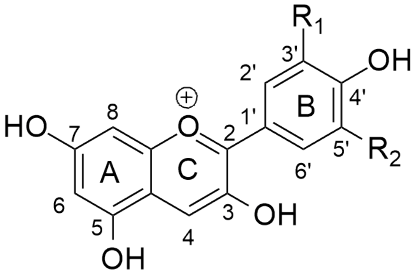
1. INTRODUCTION

Colour is an important aspect for humans when deciding on the desired products. Colour in chemistry occurs because a chromophore group in a molecule absorbs light at a specific visible wavelength of the electromagnetic spectrum, ranging from 400 to 700 nm, reflecting the rest of the wavelength. In general, the perceived colours, also known as complementary colours, are the opposite colours of the absorbed light (Araújo et al., 2018). Because of sustainability and safety issues, consumers demand more natural and sustainable colorants, which are considered safer than synthetic ones (Becerril et al., 2021). Additionally, natural colorants also possess health benefits. One of the natural sources of colorants originates from anthocyanins.

Anthocyanins, a class of flavonoids, are widely distributed in the plant kingdom. These natural, water-soluble, and non-toxic compounds have been known for displaying bright and vibrant colours, including red, purple, orange, and blue, to fruits, flowers, leaves, vegetables, and other parts of plants (Sigurdson et al., 2017). As such, anthocyanins have been mainly used as natural colorants in foods, beverages, smart packaging, textiles, and cosmetics (Alisaac et al., 2022; Mendoza et al., 2018; Oladzadabbasabadi et al., 2022). In plants, anthocyanins have a crucial role in several defensive mechanisms towards abiotic stress, including heat, light, water, salt, and/or biotic stress, such as insect and fungi attacks (Mannino et al., 2021). Additionally, anthocyanins have also been reported to display several potential benefits to human health, such as antioxidants, anti-inflammatory, anticancer, antitumour, and antibacterial (Li et al., 2017; Sandoval-Ramírez et al., 2018).

In nature, anthocyanins are biosynthesised via a flavonoid pathway in the cytosol and are mainly found in glycosides (Mannino et al., 2021). More than 700 anthocyanins have been reported in literature (Adaku et al., 2019). The aglycone form of anthocyanins, also known as anthocyanidins, is structurally based on the flavylium cationic form or 2-phenyl-1-benzopyrilium, consisting of one or more hydroxyl and methoxyl groups in different positions of the B-ring. There are



R1	R2	Anthocyanidin	Colour
H	H	Pelargonidin	Orange
OH	H	Cyanidin	Orange red
OH	OH	Delphinidin	Bluish red
OCH_3	H	Peonidin	Red
OH	OCH_3	Petunidin	Bluish red
OCH_3	OCH_3	Malvidin	Bluish red

Figure 1. The Main Structure of 6 Commonly Found Anthocyanidins in Ature. The Substituents on the B Ring, Either Hydroxyl (−OH) or Methoxyl ($-OCH_3$), Allow Different Anthocyanidins and Hues

six most anthocyanidins found in nature, namely cyanidin (Cy), delphinidin (Dp), malvidin (Mv), pelargonidin (Pg), peonidin (Pn), and petunidin (Pt), which account around 90% of total anthocyanins (Figure 1) (Alappat and Alappat, 2020; Olivas-Aguirre et al., 2016). The O-glycosyl moiety is attached to the structure of anthocyanidins mostly at the position of C-3 on the C-ring, but it can also attach to the position of C-5 and/or C-7 on the A-ring, improving the water solubility and stability of anthocyanins due to the addition of OH groups (Moriya et al., 2015).

Despite the interesting colour properties of anthocyanins, their colour stability is still challenging. Otherwise, it can be a hindrance to their application. Anthocyanins are known for their susceptibility to degradation by many factors, for instance, pH, temperature, light, water, and complexing agents, which also affect anthocyanins' stability (Sipahli et al., 2017; Zhang et al., 2020a). The anthocyanin's stability depends on its structure, for instance, the quantity of hydroxyl groups (−OH) attached to the B-ring, the quantity of sugar moieties linked to the C-ring, and the quantity of acylation on the sugar moiety (Teng et al., 2022; Zhang et al., 2020b). It is well documented that the structure of anthocyanins can change into many forms depending on the pH of solutions (Wathon et al., 2019, 2023b). The stable form of anthocyanins is mostly found in a flavylium cationic form (AH^+) in acidic solutions, giving red colour. Anthocyanins in hemiketal forms (B) are found colourless at pH 4-6. Purple quinonoid bases (A) and blue anionic quinonoid bases (A^-) can be found at pH 6-8. Furthermore, chalcones (C_E/C_Z) are yellow and can be found at higher pH.

There are many protocols to increase the stability of anthocyanins, including the chemical modification of anthocyanins, anthocyanin storage in a lower temperature and limitation for direct exposure to sunlight, co-pigmentation of anthocyanins, and complexation of anthocyanins. Some studies have reported the increased stability of anthocyanins through a complexation reaction with Al^{3+} (aluminium), Fe^{3+} (iron), Mg^{2+} (magnesium), Cr^{3+} (chromium), and Ga^{3+} (gallium) ions (Sigurdson et al., 2016, 2017). The complexation of anthocyanins with these cations resulted in a bathochromic shift and hyperchromic shift compared to their precursors without the addition of cations. However, many researchers conducted a complexation of anthocyanins only at certain pHs and did not thoroughly study the complexation and stability of anthocyanins at a broader range of pHs. To alleviate such problems, the stability of anthocyanins should be improved through a complexation reaction with selected metal ions at acidic to alkaline pHs. In this study, anthocyanins were obtained from Java plum fruits (*Syzygium cumini*), where the major anthocyanins found are cyanidin-3-O-glucoside (Cy3glc), delphinidin-3-O-glucoside (Dp3glc), and petunidin-3-O-glucoside (Pt3glc) (Wathon et al., 2023a,b). In this study, anthocyanins were complexed with Al^{3+} (aluminium), Fe^{3+} (iron), and Ca^{2+} (calcium) ions, as these metal ions are mainly used as mordants during the dyeing process using natural colorants in the textile industry. Those metal ions are commonly used in the production of Batik, a traditional cloth from Indonesia, using a wax-resist dying technique. Those metal ions are also known in Indonesia as alum ($Al_2(SO_4)_3$), tunjung ($FeSO_4$), and lime tohor ($CaCO_3$). However, Fe^{3+} ion was used instead of Fe^{2+} ion in this study because this ion achieves a more stable half-filled d subshell, increasing the stability of ions. Fe^{2+} could also promote a Fenton reaction with the presence of H_2O_2, producing hydroxyl radicals that give oxidative damage. Mordanting plays an important role in the dyeing process, especially those that use natural dyes, as the affinity of natural colorants to fabrics is not that great. The metal ions from mordants will make a bridge-like, connecting anthocyanins and fabrics, increasing the colour fastness.

To the best of our knowledge, literature reporting the complexation of anthocyanins from Java plum fruits is still limited. This study aimed to study anthocyanins' complexation reaction and colour stability with those selected metal ions. The colour stability of these complexes was assessed at pH 1-12 to discover the potential application, which requires a wide range of pHs. The colour quality of anthocyanin-metal complexes, such as the browning index (BI) and violet index (VI), was evaluated. On top of that, the source of anthocyanins in this study is derived from non-conventional fruits commercialised in supermarkets and regarded as "rarely worth eating" fruits by some due to their astringency. Hence, studying the complexation and stability of anthocyanins from Java plum fruits with selected metal ions is worth investigating.

2. EXPERIMENTAL SECTION

2.1 Materials

In this study, the extract containing anthocyanins was obtained from dried Java Plum fruits (*Syzygium cumini*), also known as Duwet, Jamblang, or Juwet in Indonesia. Anthocyanin-rich extracts were prepared from Java Plum fruits purchased from the local market in Indonesia and carefully dried in sunlight for two days as anthocyanins are sensitive to light and temperature as well. Dried fruits were stored in a freezer until further use. Ethanol, ethyl acetate, hydrochloric acid, $FeCl_3.6H_2O$, $AlCl_3$, and $CaCl_2$ were purchased either from Merck (USA) or Sigma Aldrich (USA). Solvents were bought HPLC and/or analytical grade and were directly used without any further purification. Amberlite XAD-7 resin (Sigma Aldrich, USA) was used as the SPE resin and treated with dionised water for one hour before use.

2.2 Methods

2.2.1 Extraction-Adsorption of anthocyanins from Java plum fruits

A sequential batch extraction-adsorption of anthocyanins from Java Plum fruits followed the procedures reported from the previous study with a slight modification in the concentration of acid used during the extraction (Wathon et al., 2019). Dried Java plum fruits were extracted with acidified water (0.01% v/v, HCl) using a solid-to-solvent ratio of 1:20 (w/v, dried Java plum fruits/ acidified water) at 60 °C for 3h. The crude extract was filtered and cooled down to room temperature for further purification using Solid Phase Extraction (SPE).

2.2.2 Purification of Anthocyanins using Solid Phase Extraction (SPE)

Crude anthocyanin extracts from the previous extraction were loaded onto an SPE column containing Amberlite XAD-7 resin which has been treated previously (Klisurova et al., 2019). Loaded SPE columns were washed with acidified water (0.01% v/v, HCl) to remove free sugars and phenolic acids, then washed with ethyl acetate to remove an initial fraction containing non-anthocyanin phenolics. Finally, the SPE column was eluted with acidified ethanol (0.01% v/v, HCl) to recover the anthocyanins. The post-SPE ethanol eluate was evaporated using a rotary evaporator. The anthocyanin-rich extract was redissolved in acidified water:ethanol solution (9:1 v/v, 0.1% v/v HCl) for analysis by double-beam UV-Vis spectrophotometer UV-1800, FTIR, and LC-MS.

2.2.3 Complexation of Anthocyanins with Al^{3+}, Fe^{3+}, Ca^{2+} Ions

The complexation reaction of anthocyanins with Al^{3+}, Fe^{3+}, and Ca^{2+} ions was conducted following the methods reported in the literature with a slight modification in a concentration of anthocyanin and metal ions (Li et al., 2016; Sigurdson et al., 2017). Anthocyanins extracted from dried Java Plum fruits were diluted to 0.1 mM in buffer pH 1-12. Individual metal salts ($FeCl_3.6H_2O$, $AlCl_3$, and $CaCl_2$) were dissolved in distilled water to achieve concentrations of 0.01 M and 0.1 M for $FeCl_3.6H_2O$ solution and of 0.1 M and 1 M for $AlCl_3$, and $CaCl_2$ solutions. These selected cations were then added to anthocyanin solutions. The pH of the solution was monitored throughout the analysis. Control samples without added salt solutions were also observed at each pH for comparison. All samples were equilibrated for 10 min at room temperature in the dark before further analysis to avoid any degradation. The light absorption of anthocyanin-rich extracts was determined at UV and visible region (200-800 nm) using UV-Vis spectrophotometer UV-1800. The spectroscopic data were used to determine the maximum wavelength (λ_{max}), brown index (BI), and violet index (VI).

Brown index (BI) is a number that shows the ratio of total anthocyanin loss, which is indicated by the development of brown colour (Muche et al., 2018). BI represents the proportion of yellow to red as measured by the anthocyanin-rich extract in a pH of 1-12 at wavelengths of 420 and 520 nm. BI was determined using the following equation (Sinela et al., 2017):

$$\text{Brown Index} = \frac{A_{420}}{A_{520}} \tag{1}$$

Meanwhile, the violet index (VI) shows the proportion of the blue to red colour ratio of the sample. VI was measured by mixing the anthocyanin-rich extract with a buffer pH of 1-12. The measurement was done at wavelengths of 520 and 580 nm. Afterwards, VI was determined using the following equation (Ndiaye et al., 2021):

$$\text{Violet Index} = \frac{A_{520}}{A_{580}} \tag{2}$$

2.2.4 UV-Vis Spectrophotometer

UV-Vis spectra were recorded using a double-beam UV-Vis Spectrophotometer 1800 (Shimadzu, the USA) at 200-800 nm.

2.2.5 Attenuated Total Reflection - Fourier-transform infrared spectroscopy (ATR FTIR)

ATR-FTIR spectra were recorded using an Agilent Cary 630 (Agilent, the USA). The spectra were registered at 2 cm^{-1} between 4000 and 400 cm^{-1}.

2.2.6 Liquid Chromatography-Mass Spectrometry (LC-MS)

LC-MS analyses were conducted at room temperature using an Advison C18 column (2.1 mm × 50 mm, 1.8 μm) at 20 °C with a flow rate of 0.3 mL/min. The mobile phase for this analysis consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in acetonitrile). The solvent system was set in gradient as follows: 90% A$\rightarrow$100% B 20 (0-10 min), 100% B (11-16 min), and 90% A (16-20 min). The mass spectrometer for ion detection was set as follows: ionisation mode used Electron Spray Ionisation (ESI) in positive mode, the mass spectrometry (MS) scan ranged from m/z 50 to 1800, the capillary temperature was kept at 250 °C, gas source temperature was set to 200 °C, and the capillary voltage was adjusted to 180 V. Positive mode was found to be more sensitive for anthocyanins and hence all analysis was performed in positive ionisation mode.

3. RESULTS AND DISCUSSION

Anthocyanins from dried Java Plum fruits (*Syzigium cumini*) were extracted and further purified using Solid Phase Extraction (SPE) as described previously (Wathon et al., 2019). This step is crucial in maintaining the purity of the extract as, through this purification, most non-anthocyanin polyphenols were removed from an SPE column by ethyl acetate elution. These non-anthocyanin polyphenols can interact with anthocyanins through co-pigmentation. Not only could it change the colour properties of anthocyanins, but it could also interfere with the complexation of anthocyanins with metal ions because there will be competition between co-pigments and metal ions. Furthermore, anthocyanins were recovered from the SPE column using acidified ethanol, and the extracts were referred to as the post-SPE ethanol eluate.

Characterisation of anthocyanins from the extracts of Java Plum fruits was done with UV-Vis, FTIR, and LC-MS. The maximum wavelength and absorbance of anthocyanin solution and anthocyanin-metal complexed solutions were measured at 400-800 nm. Complexation of anthocyanins was conducted with Al^{3+} (aluminium), Fe^{3+} (iron), and Ca^{2+} (calcium) ions, where the colour stability was analysed at various pHs ranging from acidic to alkaline (pH 1-12).

3.1 Identification of Anthocyanins from the Extracts of Java Plum Fruits (*Syzygium cumini*)

3.1.1 ATR FTIR Analysis

The results of spectra analysis using ATR-FTIR is presented in Figure 2. This analysis was conducted to identify the chemical composition in the extract of Java plum fruits based on their functional groups. The ATR-FTIR was recorded at 400-4000 cm^{-1}. According to the ATR-FTIR spectra of the extracts of Java Plum fruits, the characteristic of anthocyanins was shown by wavelength numbers of 3414, 2927, 2902, 2859, 1741, 1628, 1532, 1338, 1313, 1061 cm^{-1}. The presence of the aromatic rings was shown by the absorption band of 600-1000 $cmcm^{-1}$. The absorbance at 1061 cm^{-1} indicated the stretching vibration of the C−O−C esters. In contrast, absorbance at 1238 cm^{-1} was assigned to the stretching of the pyran rings, which are core of flavonoid compounds (C6-C3-C6). The absorbances at 1338 and 1313 cm^{-1} corresponded to the C−O deformation of phenols, whereas absorbances at 1628 and 1741 cm^{-1} belonged to the C=C and C=O groups for aromatic rings, respectively. The absorbances at 2927, 2902, and 2859 cm^{-1} indicated the C−H stretching. The broad band at 3414 cm^{-1} was assigned to O−H groups which belonged to the sugar vibrations and phenol. These functional groups analysis which are characteristics of anthocyanins is in agreement with results reported in literature (Jeyaram and Geethakrishnan, 2020; Swer et al., 2018), where they analysed anthocyanins from blueberries and wild Asian bird berries *(Prunus nepalensis* L.), respectively.

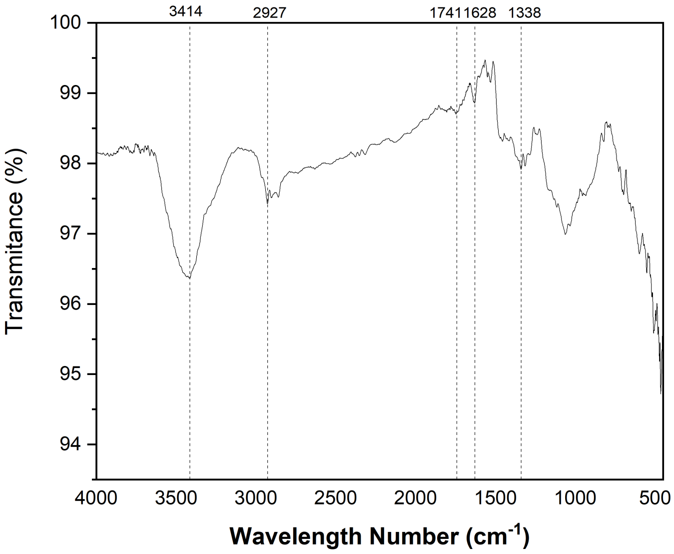


Figure 2. ATR-FTIR Spectra of Anthocyanins from the Extract of Java Plum fruits.

3.1.2 LC-MS Analysis

The post-SPE ethanol eluate was dissolved in the acidified water:ethanol (9:1, 0.1% v/v) to keep anthocyanins in the flavylium cationic form as previously studied (Wathon et al., 2023a). The LC-MS spectra analysis of anthocyanins from the extracts of Java Plum fruits is presented in Figure 3. The LC-MS analysis was conducted to identify the chemical composition present in the sample (Table 1). The LC-MS spectra from the previous study showed that anthocyanins from the extracts of Java Plum fruits were cyanidin-3-O-glucoside (M^+, m/z 449.3), delphinidin-3-O-glucoside (M^+, m/z 465.3), and petunidin-3-O-glucoside (M^+, m/z 479.3). Additionally, cyanidin-3-O-glucoside ($[M+H]^+$, m/z 450.3), delphinidin-3-O-glucoside ($[M+H]^+$, m/z 466.3), and petunidin-3-O-glucoside ($[M+H]^+$, m/z 480.3) were also detected as the results of ionisation in the electrospray ionisation spray (ESI). Positive mode has been found to be better in showing anthocyanin peaks than in negative mode. This finding is in accordance with the results reported in literature (Lestario et al., 2017; Tavares et al., 2016). However, diglycosides of anthocyanins were not identified in this study, which might be due to the result of deglycosylation (sugar removal) during the extraction and purification using SPE (Wathon et al., 2023b). Other anthocyanins, such as malvidin and peonidin, were also not detected. It might be geographical, nutrients, and climate that affects the biosynthesis of the Java Plum plants (Yang et al., 2018).

Colour profiles of Anthocyanins from the Extracts of Java Plum Fruits using UV-Vis Spectrophotometer Colour profiles

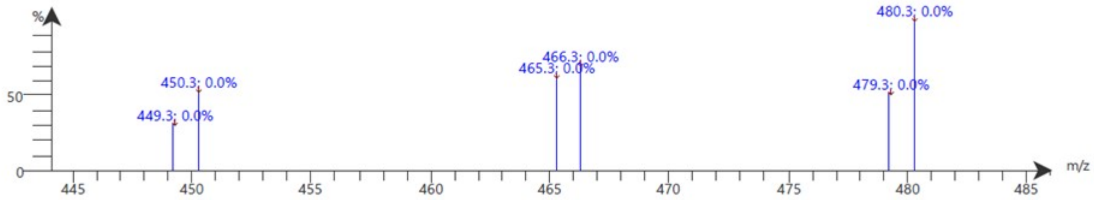
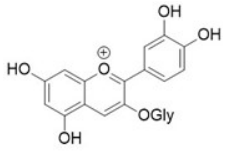
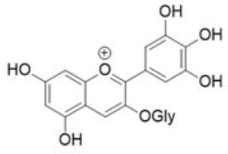
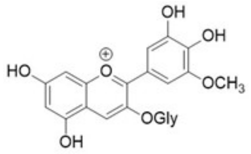


Figure 3. LC-MS Spectra of Anthocyanins from the Purified Extract of Java Plum fruits

Table 1. LC-MS Spectra Analysis of Anthocyanins from the Purified Extract of Java Plum Fruits

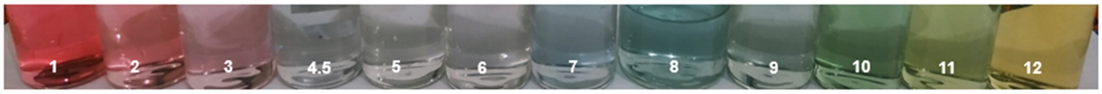
Experimental		Theoretical	Anthocyanin
$[M]^+$ m/z	$[M+H]^+$ m/z	$[M]^+$ m/z	
449.3	450.3	449.1	 **Cyanidin-3-*O*-glucoside**
465.3	466.3	465.1	 **Delphinidin-3-*O*-glucoside**
479.3	480.3	479.1	 **Petunidin-3-*O*-glucoside**

of anthocyanins from the extracts of Java Plum fruits were determined using UV-Vis Spectrophotometer (Figure 4). Anthocyanins can exist in various species depending on the pH of the solutions. At acidic pHs (1-3), anthocyanins are mostly found in red flavylium cationic forms (AH^+). At slightly acidic pH, anthocyanins are present in colourless hemiketal forms (B). At alkaline pHs, anthocyanins can present as a purple quinonoid base (A) or an anionic blue quinonoid base (A^-). At higher pH, yellow chalcones (C_E/C_Z) are mostly found (Mendoza et al., 2018). The combination of one or more anthocyanin species produces a different colour. For example, green is associated with a combination of blue quinonoid and yellow chalcone.

The intense colour of anthocyanins is due to the delocalisation of their π-conjugated electrons. The maximum wavelength of anthocyanins in red flavylium cationic forms (AH^+) was 516-517 nm in acidic pH 1 and 2. The most stable form of anthocyanins is found in this acidic pH. A bathochromic shift was detected at pH 1 to 12. Furthermore, anthocyanins at pH 1 and 4.5 can determine the class of anthocyanins. The results of this study showed that anthocyanins are classified as anthocyanins type A where there is low colour intensity because of the conversion of flavylium cationic form (AH^+) to hemiketal form (B) (Marpaung and Tjahjadi, 2020). Anthocyanins from the extracts of Java Plum fruits are not acylated. Hence, it adsorbs low or no light. At pH 1 and 4.5, the absorbance of anthocyanins can be used to determine the concentration of anthocyanins, which is also known as total monomeric anthocyanin content (TMAC) by the pH differential method (Lee et al., 2016).

3.2 Complexation of Anthocyanins from Dried Java Plum Fruits with Al^{3+}, Fe^{3+}, and Ca^{2+} Ions

In this study, anthocyanins from the extract of Java Plum fruits were complexed with selected metal ions such as Al^{3+} (alu-



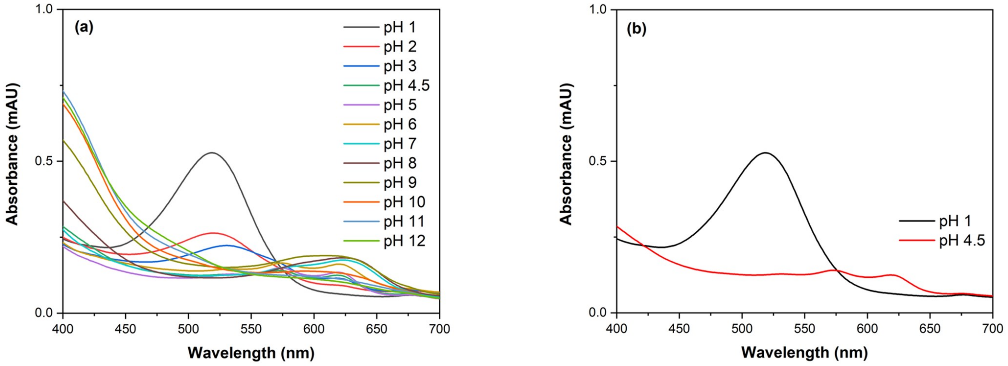


Figure 4. (a) Anthocyanins Profile of the Extract of Java Plum fruits Recorded with UV-Vis Spectrophotometer at 400-700 nm at pH 1-12. (b) Anthocyanin Absorption at pH 1 & 4.5 to Highlight the Type of Anthocyanin

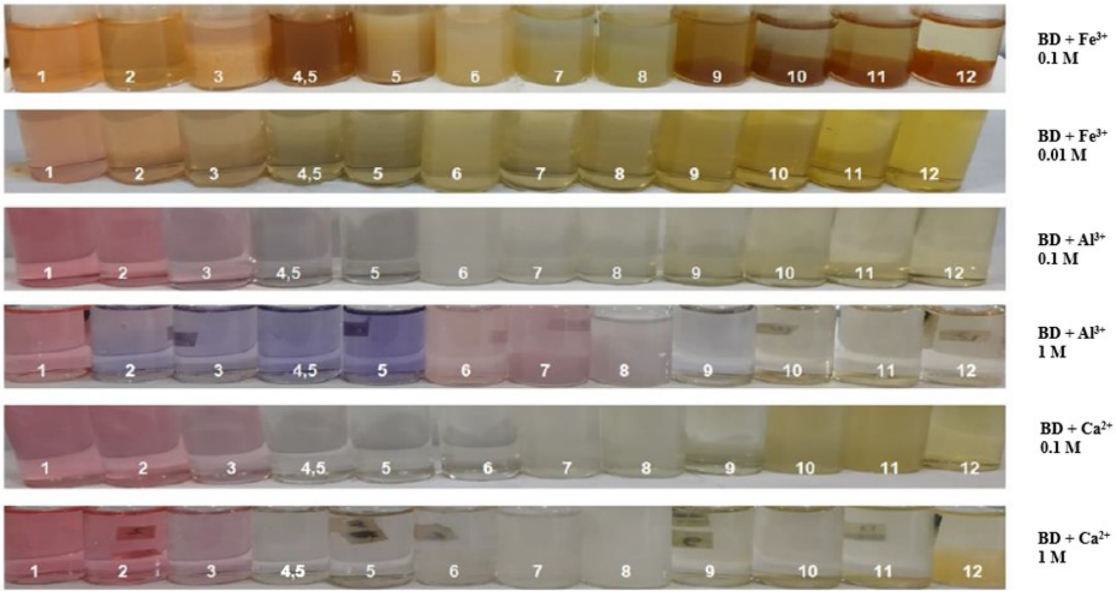


Figure 5. Colour Stability of Anthocyanin-Metal Complexes at pH 1-12 with Different Concentrations. BD: Java Plum fruit Extracts

minium), Fe^{3+} (iron), and Ca^{2+} (calcium) at pH acidic to alkaline (pH 1-12) to understand the colour changes and stability. These cations are commonly utilised as mordants or dye fixators in the textile industries. The purpose of this study was to mimic the mordanting process in the textile industry and explore the possible colours produced from the complexation of anthocyanins and metal ions at pH 1-12 (Figure 5). The absorbances of anthocyanin-metal complexes are shown in Figure 6. The concentration of ions was kept from 0.01 – 1 M. However, the colour library produced through this reaction can be potentially used as a reference to develop colours for various applications not limited to the textile industries. The differences in absorbance and maximum wavelength were recorded and compared. The brown index (BI) and violet index (VI) were also determined.

At lower pHs, anthocyanin-aluminium complexes were found to be red, as anthocyanins are mostly found in red flavylium cationic forms. Complexation of anthocyanins from the extracts of Java Plum fruit with Al^{3+} ion results in blue shades at pH 4 and 5 at both concentrations (0.1 M and 1 M). The stronger blue hues were obtained when the concentration of Al^{3+} was increased. Interestingly, the blue-colour develop-

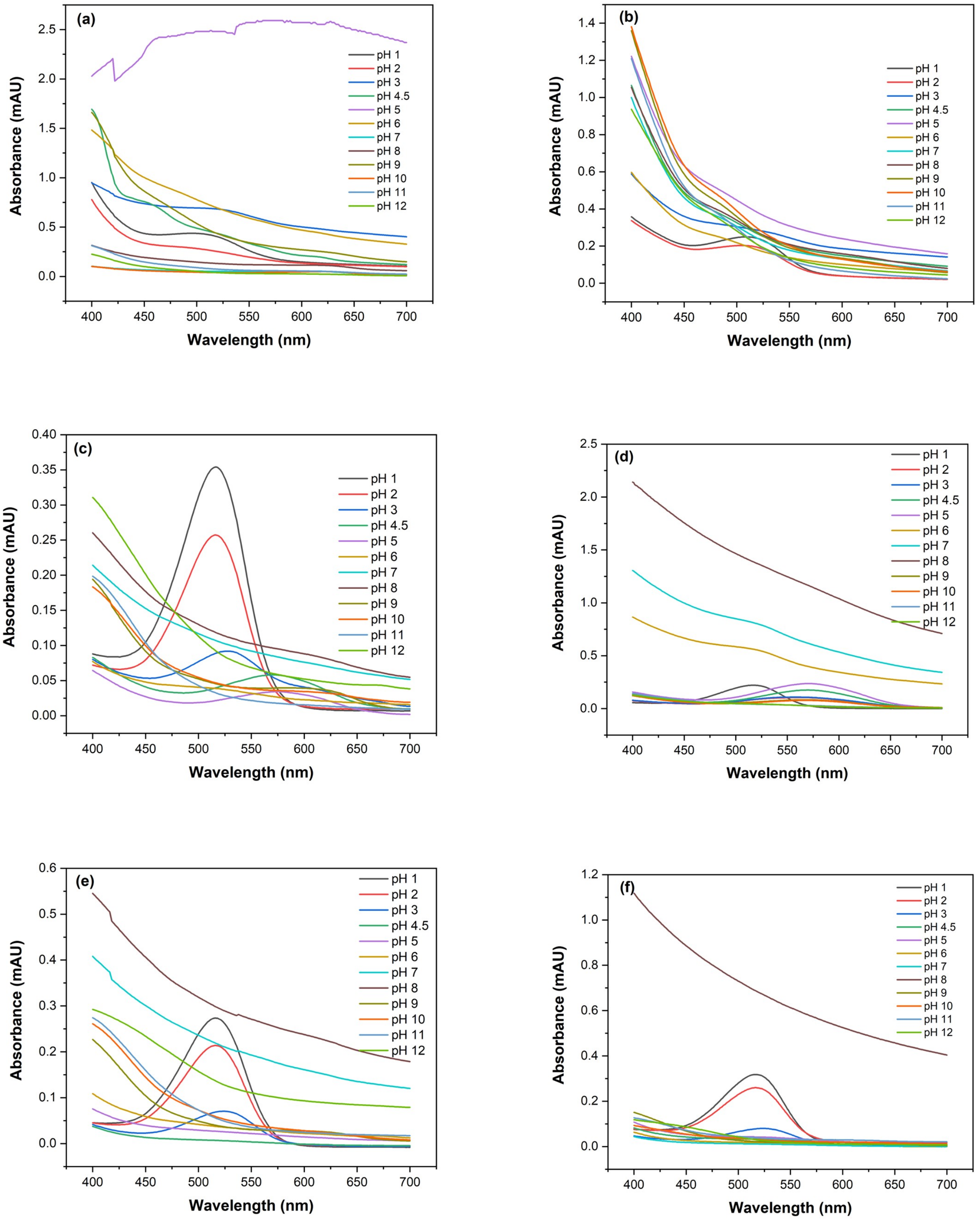


Figure 6. Absorbance of Anthocyanin-Metal Complexes. (a) ANC+Fe^{3+} 0.1 M; (b) ANC+Fe^{3+} 0.01 M; (c) ANC+Al^{3+} 0.1 M; (d) ANC+Al^{3+} 1 M; (e) ANC+Ca^{2+} 0.1 M; (f) ANC+Ca^{2+} 1 M;

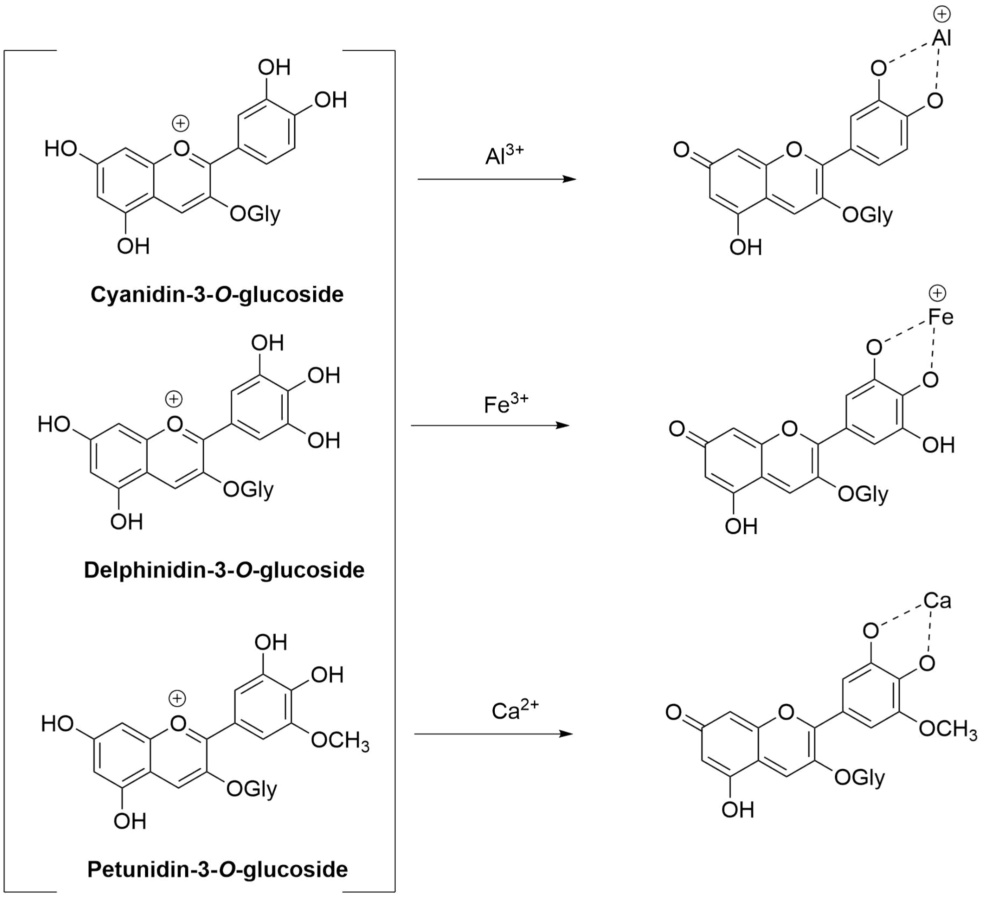


Figure 7. General Raction of Anthocyanin-Metal Cations (Al^{3+}, Fe^{3+}, and Ca^{2+}). Hydroxyl Groups on the B-Ring at 3' and 4' Positions Create a Complex with a Metal Cation

ment was observed even at acidic pH (2-3) for anthocyanin-aluminium complexes (1 M), where anthocyanins in red flavylium cationic form generally dominate. This finding agrees with the results reported by Sigurdson and Giusti (2014), where the anthocyanins from cyanidin derivatives complexed with metal Al^{3+} produce a violet-to-blue colour at acidic pHs. The blue-colour development for anthocyanins is usually caused by the formation of an anionic quinonoid base, which can be achieved at alkaline pH. However, the application of anthocyanins is limited at alkaline pH as it can lead to degradation. Therefore, this finding is interesting, where blue hues can be achieved at a slightly acidic pH. Additionally, for food safety, the pH is kept to 4.6. This finding shows that a new method in blue-colour development is promising in the textile industry and food and beverage industries. However, the application of aluminium in food and beverages is limited to 2 mg/kg body weight, according to WHO (Klotz et al., 2017).

There were no colours changes in pH during the complexation reaction at a lower concentration (0.1 M) of Al^{3+} solution. Colour changes in pH occur when the concentration of Al^{3+} is increased to 1M. A decrease in pH at pH 6 and 7 resulted in a pink colour solution, indicating the formation of anthocyanins in a flavylium cation. Sigurdson et al. (2016) also reported decreased pH at slightly acidic to neutral pHs due to increased Al^{3+} concentration. The decrease in pH occurs due to the release of hydrogen ions when anthocyanins chelate Al^{3+} metal. Then, Al^{3+} ions were hydrolysed in water, forming aluminium hydroxide and hydrogen ions, which make more H^+ species ($Al^{3+}(aq) + H_2O(l) \rightarrow Al(OH)^{2+}(aq) + H^+(aq)$). Consequently, it decreases the pH of the solution. This decrease in pH can be overcome by using a buffer with a higher concentration.

The second complexation was conducted with Fe^{3+} ions. Complexation of anthocyanin from Java Plum fruit extract with Fe^{3+} ions produced a yellowish-brown solution for both concentrations (0.01 M and 0.1 M). According to ionic radius amongst Al^{3+}, Fe^{3+}, and Ca^{2+} shown in Table 2. It shows that the size of Ca^{2+} ions is the largest compared to Fe^{3+} and Al^{3+} ions due to their lower positive charge, allowing for more electron shells around the nucleus. Meanwhile, Al^{3+} ions are the smallest among the three metal ions due to their higher positive charge, resulting in a stronger pull on the remaining electrons. Both ions are generally colourless, while Fe^{3+} ions show yellow colour. More precipitation was observed when anthocyanins were added with Fe^{3+} ions in higher concentrations. This is

Table 2. Comparison of Ionic Radius Amongst Al^{3+}, Fe^{3+}, and Ca^{2+}

	Al^{3+}	Fe^{3+}	Ca^{2+}
Ionic radius (pm)	53	60	100
Colour	Colourless	Yellow	Colourless

`https://www.wiredchemist.com/chemistry/data/metallic-radii`

in accordance with the data reported in literature where the reaction of anthocyanins and metal ions, especially with Fe^{3+} ions, forms anthocyanin metal chelate precipitates (Sigurdson and Giusti, 2014; Tachibana et al., 2014).

Furthermore, the complexation of anthocyanins from Java Plum fruit extracts was also conducted with Ca^{2+} ions with concentrations of 0.1 M and 0.01 M. Overall, the effect of complexation of Ca^{2+} ions with anthocyanins on the appearance of the violet-blue colour is not very visible. This is related to the type of anthocyanin found in Java Plum fruits, which are mono-glycosylated anthocyanins with aglycones of cyanidin, delphinidin, and petunidin. All anthocyanins found in Java Plum fruit were non-acylated anthocyanins. Another research group also reported a similar result where non-acylated anthocyanins do not show intense colour changes compared to acylated anthocyanins (Sigurdson and Giusti, 2014).

The interactions amongst anthocyanins or other polyphenols through co-pigmentation is because the aromatic nuclei of anthocyanins are flat, polarisable, non-polar surfaces, contributing to strong dispersion. Additionally, the polarity of OH groups of anthocyanins, especially in the B-ring, can make a complex with metal cations, acting as metal chelators (Dangles and Fenger, 2018). The formation of anthocyanin-metal complexes is often associated with the development of blue colour, one of the rarest natural colours to obtain in nature. The presence of ion-dipole electrostatic interactions mostly stabilises these anthocyanin-metal complexes than by electron pair sharing (Estévez et al., 2011). The anthocyanin-metal complexes are formed through the complexation of a quinonoid base with additional proton loss (deprotonation) from C3'−OH in the B-ring and positively charged metal ions (M^{n+}). The interactions are caused by the hydroxyl groups of anthocyanins (cyanidin (2 OH), delphinidin (3 OH), and petunidin (2 OH and 1 OCH_3)derivatives) in the B-ring with metal ions in a mildly acidic to neutral solution (Figure 7) (Dangles and Fenger, 2018). It is noted that each anthocyanin is depicted for each cation as an example of how anthocyanin forms a complex with cations. As anthocyanins are a class of polyphenols, they have more than a hydroxyl group (OH), and different deprotonation routes are possible. Deprotonation of anthocyanins is a key of anthocyanin-metal complexation which is shown by the value of pKa. pKa is a value describing the acidity strength of compounds. According to Arrhenius, acid is defined as a compound that releases an H^+ ion. The stronger the acids, the easier H^+ ions are released. The estimated pKa1 values of cyanidin, delphinidin, and petunidin were reported as 5.28, 4.97, and 5.38, respectively (León-Carmona et al., 2016). Further studies should be conducted to investigate the competition of cyanidin, delphinidin, and petunidin with metal cations.

3.3 Colour Stability of Anthocyanin-metal Ions Complexes

pH has an important role in anthocyanin stability, as has already been reported in many pieces of literature. Colour changes of anthocyanins at various pHs can be a reference for the application of anthocyanins as natural colorants in textile industries. Mordanting is a key technique in maintaining colours in fabrics, as it can act as a bridge between fabrics and dyes. However, the complexation study of anthocyanins from Java Plum fruit extract conducted at a wide range of pH levels is still limited. Therefore, the complexation of anthocyanins at acidic-alkaline pH was conducted. According to Figure 6, the $\lambda_{\text{vis-max}}$ shift shows how much the peak shifts due to the complexation of anthocyanins with metal ions at visible wavelengths (400-800 nm). The magnitude of the $\lambda_{\text{vis-max}}$ shift is known by the difference between the $\lambda_{\text{vis-max}}$ of the Java Plum fruit extract without complexation and the $\lambda_{\text{vis-max}}$ shift of the extract that has been complexed with metal ions.

Complexation of anthocyanins with Fe^{3+} and Ca^{2+} ions at both concentrations showed a hypsochromic shift. The highest hypsochromic shift was found at Fe^{3+} 0.1 M. This occurs because of the yellow-brown colour produced by the complexation reaction. Additionally, anthocyanins and Al^{3+} ions' complexation shows a bathochromic shift at pH 4.5 and 5 with concentrations of 0.1 M and 1 M. Apart from that, Al^{3+} ion complexation also occurs with a hypsochromic shift. In anthocyanin complexation, anthocyanins from the extract of Java Plum fruit show a hypsochromic shift rather than a bathochromic shift. These results are in accordance with previous similar research, where complexation of anthocyanins with Al^{3+} at pH 4 showed a bathochromic shift and hyperchromic effect, which indicated a shift from a red hue to a more purple-blue colour and colour intensification (Sigurdson et al., 2017). At pH 7, it shows a negative bathochromic shift (hypsochromic shift) and a slight colour change, as observed in Figure 3, to become slightly bluer or greener (Sigurdson et al., 2017).

The highest hyperchromic effect occurs when a solution of Fe^{3+} 0.1 M was added with anthocyanins at pH 5. This is consistent with the highest hyperchromic effect usually appearing at pH 5 due to the conversion of the colourless carbinol pseudo basic structure to a coloured structure due to the presence of metal ions (Sigurdson et al., 2016). The hyperchromic effect at Al^{3+} 1 M was also found to be higher than at Al^{3+} 0.1 M. Likewise for Ca^{2+} ions, the highest hyperchromic effect was observed at Ca^{2+} 1 M. Thus, it can be concluded that the higher the concentration, the greater the hyperchromic effect. Lambert-Beer Law states that the higher the concentration, the higher the absorbance ($A = \epsilon.b.c$). This finding is in accordance with the increase in absorbance with increased metal ion concentration (Sigurdson et al., 2016).

Table 3. Brown Index (BI) Anthocyanin Java Plum Fruit Extract Complexation with Metal Ions

	ANC	Ca^{2+} 0.1 M	Ca^{2+} 1 M	Al^{3+} 0.1 M	Al^{3+} 1 M	Fe^{3+} 0.01 M	Fe^{3+} 0.1 M
pH 1	0.42	0.17	0.23	0.24	0.24	1.17	1.59
pH 2	0.83	0.19	0.28	0.26	0.69	1.37	2.03
pH 3	0.90	0.41	0.46	0.71	0.70	1.65	1.23
pH 4.5	1.80	3.57	1.84	1.53	0.88	2.93	2.34
pH 5	1.44	2.10	1.81	2.06	0.82	2.44	0.89
pH 6	1.37	2.25	2.47	1.59	1.39	2.59	1.86
pH 7	1.72	1.64	2.64	1.78	1.45	3.06	1.61
pH 8	2.42	1.63	1.49	1.94	1.44	2.91	1.93
pH 9	2.97	4.71	5.67	3.42	1.62	3.66	2.97
pH 10	3.66	3.98	2.52	3.41	1.60	3.51	2.05
pH 11	3.63	4.51	2.84	5.51	2.80	4.30	3.21
pH 12	3.49	2.07	3.82	3.06	2.32	3.55	4.35

Note: ANC: anthocyanins; Ca^{2+} (calcium); Al^{3+} (aluminium); Fe^{3+} (iron)

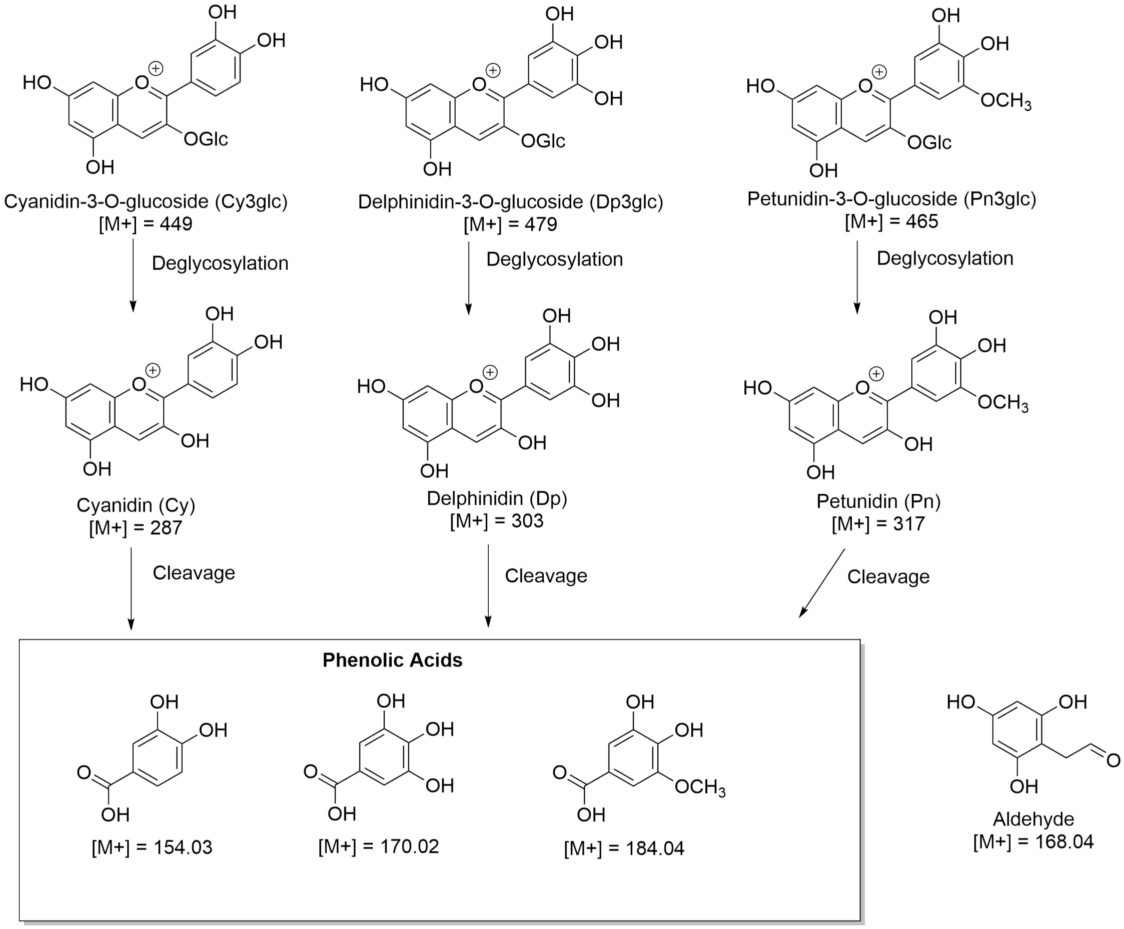


Figure 8. Proposed Degradation Reaction of Anthocyanins from Java Plum Fruit Extracts

Overall, the effect of the complexation with metal ions Fe^{3+} and Ca^{2+} produces less blue hues because the anthocyanins in Java Plum fruit include unacylated anthocyanins (cyanidin-3-O-glucoside (Cy3glc), delphinidin-3-O-glucoside (Dp3glc), and petunidin-3-O-glucoside (Pt3glc)). However, in this study, complexation with Al^{3+} 1 M produced a blue colour at a weak acid pH. The blue colour tends to indicate the appearance of metal chelation with acylated anthocyanins. Acylated anthocyanins from red cabbage and purple carrot extracts showed wider $\lambda_{\max}$ compared to metal chelates with unacylated antho-

Table 4. Violet Index (VI) Anthocyanin Java Plum Fruit Extract Complexation with Metal Ions

	ANC	Ca^{2+} 0.1 M	Ca^{2+} 1 M	Al^{3+} 0.1 M	Al^{3+} 1 M	Fe^{3+} 0.01 M	Fe^{3+} 0.1 M
pH 1	0.55	0.03	0.07	0.08	0.07	0.22	0.42
pH 2	0.60	0.03	0.12	0.10	1.14	0.26	0.60
pH 3	0.76	0.07	0.19	0.53	1.13	0.71	0.78
pH 4.5	0.60	0.07	0.66	1.38	1.57	0.62	0.57
pH 5	0.81	0.66	0.64	1.51	1.54	0.69	1.04
pH 6	0.83	0.69	0.47	0.66	0.68	0.63	0.74
pH 7	0.72	0.80	0.55	0.78	0.73	0.60	1.00
pH 8	0.55	0.85	0.82	0.81	0.82	0.64	0.88
pH 9	0.39	0.76	0.90	0.89	1.27	0.56	0.67
pH 10	0.25	0.58	0.68	0.79	1.31	0.51	1.10
pH 11	0.21	0.53	0.79	0.56	0.63	0.40	0.78
pH 12	0.21	0.75	0.46	0.65	0.50	0.48	0.71

Note: ANC: anthocyanins; Ca^{2+} (calcium); Al^{3+} (aluminium); Fe^{3+} (iron)

cyanins (Sigurdson et al., 2017).

Other aspects of anthocyanin-metal complexes that are interesting to study are the brown index (BI) and the violet index (VI). While the BI values indicate the degradation of anthocyanins as the browning is the sign of the degradation, the VI values show the proportion of red flavylium cationic form (AH^+) and blue quinonoid base (A). It should be noted that red and blue mix to form purple or violet. The data for BI and VI can be seen in Table 3 and 4. The measurement was conducted using UV-Vis spectrophotometer at visible light (400-800 nm) as mentioned in an experimental section.

The increasing brown index (BI) indicates the decrease of desirable colour (red, purple, or blue) and or the increase of undesirable pale-yellow colour (A_{420}) that is contributed by the formation of chalcone species. Higher BI (BI > 1) is mostly found in the complexation of anthocyanins with Fe^{3+} for both concentrations. This phenomenon was also observed for the complexation at a pH higher than 4.5. It occurred because anthocyanins in slightly acidic to alkaline conditions tend to be less stable and eventually will form yellow chalcones, leading to a ring opening. The formation of chalcone indicates the ring opening, which could further degrade anthocyanins into 3,4-hydroxybenzoic acid, also known as protocatechuic acid, with a molecular ion at m/z 139 and phloroglucinaldehyde with a molecular ion at m/z 155 (Patras et al., 2010). The Proposed degradation products of anthocyanins from Java Plum fruit extract can be seen in Figure 8.

Meanwhile, the VI was mostly shown by the complexation of anthocyanins with 0.1 M Al^{3+}(VI > 1). The VI was measured to determine the colour quality of anthocyanin at 580 nm, which represents purple, and at 520 nm, which represents red. The purple colour of anthocyanins is caused by the presence of quinonoid species (A or A^-), and the red colour of anthocyanins is caused by the presence of flavylium cationic form (AH^+). Both species are in equilibrium, which competes in kinetics and thermodynamics. An anthocyanin transformation from a flavylium cationic form to a neutral quinonoid ($AH^+ \rightarrow A$) resulted in the shift of the maximum visible wavelength (λ_{max}) absorption by 20-30 nm and by 50-60 nm for a neutral quinonoid to an anionic quinonoid ($A \rightarrow A^-$). These shifts were observed by the colour changes of anthocyanins from red to purple-blue (Wathon et al., 2019). Interestingly, higher VI (VI> 1) was observed for the complexation of anthocyanins with Al^{3+} at pH 4.5-5, which are generally found in colourless hemiketal. The higher VI indicates that more species are present in purple quinonoid species rather than in red flavylium cationic form.

4. CONCLUSIONS

Anthocyanins are sensitive to pH changes, transforming into respective species, including a flavylium cationic form (AH^+ at pH < 3), hemiketal form (B at pH 4-6), quinonoid base (A at pH 7-8), anionic quinonoid base (A^- at pH 8-9), and chalcone (C_E/C_Z at pH > 10). Complexation of anthocyanins from the extracts of *Syzygium cumini* fruits with Ca^{2+}, Al^{3+}, and Fe^{3+} at pH 1-12 showed various colours, increasing the stability of anthocyanins. This investigation showed the potential application of anthocyanins as this anthocyanin-metal chelation produced a wide range of shades that can be potentially utilised for textile dyes. Anthocyanins and anthocyanin-metal complexes are more stable at acidic pH, where anthocyanins are in a flavylium cationic form (AH^+). Blue colour development was observed on the complexation of anthocyanins with Al^{3+} ions at pH 4-5. The intensity of the blue colour increases with increased Al^{3+} ion concentrations. This finding can be extended to the food and beverage industry, where obtaining a blue colour at acidic pH is normally difficult.

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