# A Smart Naked-eye Detection for Fe(III) using Ethanol Extract of Purple Sweet Potato

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Received: 5 July 2022; Revised: 21 November 2022; Accepted: 14 December 2022

**Abstract**

Simple naked-eye detection of Fe(III) in a water sample by using crude anthocyanin extracted from purple sweet potatoes as a chelating agent was investigated in terms of both qualitative and quantitative detections. Anthocyanin was extracted in ethanol from a purple sweet potato which is a local plant in Thailand. The selectivity of this method for detecting Fe(III) was examined by testing with different metal ions and by regulating the pH of the buffer solution varying from buffer pH 1-12. It was found that Fe(III) responded with a color change at buffer pH 4-11. However, the disodium hydrogen phosphate/sodium hydroxide buffer pH 11 was chosen because it showed no color change from others interference metals. The detection procedure was to add 100 µL of buffer pH 11 and 40 µL of Fe(III) solutions (0.5-100 ppm) to 20 µL of ethanol extract of purple sweet potato solution. The qualitative detection was by observing the color change from light green to brown by the naked eye whereas the quantitative detection was measured by UV-Vis spectrophotometer. The results obtained from naked-eye detection were evaluated by comparing them with the detection results from the UV-Vis spectrophotometer. Our results show that crude anthocyanin used as a natural chelating agent could be employed for semiquantitative determination by naked-eye detection of 3 color shades depending on Fe(III) concentrations. Importantly, this work could be potentially applied to real water samples as a simple, rapid, low-cost, highly accurate and environmentally safe procedure.

**Keywords:** Naked-eye, Purple Sweet Potato, Anthocyanin, Fe(III) detection

## Introduction

Water is one of the prime necessities of life without which it is not possible to live. The human body is 70-80% water. Cells are 90% water, blood 75%, and bones 22% water (Kumar & Puri, 2012; Kumar, Kachwaha, Verma, & Patidar, 2019). Water is an inorganic, transparent, tasteless, odorless, and nearly colorless chemical substance, which is the main constituent of the Earth's hydrosphere and the fluids of all known living organisms (in which it acts as a solvent). Water contamination is due to a host of pathogenic agents and chemicals such as chloride, fluoride, nitrate, pH, calcium, and pesticides and water disinfectants, as well as by minerals such as magnesium, iron and toxic heavy metal ions, and water exhibiting TDS, alkalinity, hardness, all as consequential products of industrial and agricultural activities and natural leaching from soil, rocks, and atmospheric deposition and other human activities. Contaminated water is a hazard to human health in several regions of the world (Mahato, Mahato, Karna, & Balmiki, 2018). Of particular interest in this research is iron, a toxic metal if present beyond permissible limits.

Iron is the fourth most abundant element in the earth's crust and the most abundant transition metal in the human body (Weber, Achenbach, & Coates, 2006). Iron supports a range of physiological processes such as transporting oxygen, electron transfer, respiration and gene expression (Dixon & Stockwell, 2014). Iron

deficiency leads to anemia (Kew, 2014) whereas, excess iron can increase the production of reactive oxygen species (ROS), resulting in oxidative stress cascades that lead to lipid oxidation and DNA damage (Dixon & Stockwell, 2014; Chang, 2015). In this context, chronic exposure to elevated iron levels in common drinking water is a potential contributor to abnormal iron accumulation in the body. Accumulation of excess iron is a cause of several cardiovascular, neurodegenerative, and cancer diseases. Iron can be found in water samples in two forms; ferrous (Fe(II)) and ferric (Fe(III)). Fe(II) is water soluble, and Fe(III) is water-insoluble. Fe(II) can convert into ferric ions through oxidation by induction of air into water. Soluble iron can be found in deep wells whereas insoluble iron is found in aerated well water and surface sources, as well as the iron scale from unlined pipes and tanks. Iron contamination in water is generally due to the dissolution of ores, rusting and decomposition of water supply pipes. The World Health Organization recommends an upper limit of 0.3 ppm for total iron ions in drinking water (Weaver, 1961). There are various methods to determine iron metal ions such as photometric methods (Koronkiewicz, 2021) and electrometric methods (Hostetter & Roberts, 1919; King & Howaed, 1927). However, most of these methods need expensive equipment, are time-consuming and require trained operators. The determination of metal ions in environmental water samples using inexpensive methods is therefore of wide interest. To meet this challenge, color development is one of the popular methods which have been investigated (Khaodee, Aeungmaitrepirom, & Tuntulani, 2014; Samanman, & Suding, 2019; Baru, Samanman, & Fatoni, 2019; Silva, Mageste, Silva, Ferreira, & Ferreira, 2020). Various reagents used as chelating agents for the determination of metal ions must be synthesized (Ji, Zhu, Nie, & Du, 2019) and these reagents are usually not environmentally friendly. Synthesized compounds would be costly to use.

Anthocyanin is a bioactive water-soluble color pigment in the flavonoid group which has varying colors from red to blue and purple that differ depending on its type. In acidic conditions, anthocyanin appears as a red pigment while blue pigment anthocyanin exists in alkaline conditions. It is safe to be consumed thus it is often used as a natural coloring agent (Abbey, Fields, Mullane, & Tomaska, 2014; Damant, 2011). It is found in many plants such as the flowers of roses (Ogata, Kanno, Itoh, Tsugawa, & Suzuki, 2005), berries (Kang, Ko, & Chung, 2021), red cabbage (Khaodee et al., 2014; Majdinasab, Mohammad Hashem Hosseini, Sepidname, Negahdarifar, & Li, 2018) and purple sweet potato (Khaodee, Wongkiti, & Madang, 2018; Wulandari, Sunarti, Fahma, & Noor, 2018).

Purple sweet potatoes contain a natural anthocyanin food coloring pigment (*Ipomoea batatas*) that is an attractive purple-red color, with high anthocyanin content, high total phenol content, and high antioxidant activity (Steed & Truong, 2008; Oki et al., 2003; Yoshinaga, Yamakawa, & Nakatani, 2010). It is reported that the content of anthocyanin in purple sweet potatoes is significantly higher than that in ordinary orange-fleshed sweet potatoes (Xu et al., 2015), and is similar to other anthocyanin crops with high yields, such as blueberries, blackberries, cranberries, and grapes (Enicole, Maris, & Vanden, 2010). Purple sweet potato is also an important source of natural anthocyanin pigments because of its low cost (Gisela & Wilhelm, 2006). The anthocyanidin components of the purple sweet potato consist of pelargonidin, peonidin, malvidin, petunidin, delphinidin and cyanidin. While the components in each plant differ depending on its type (Montilla et al., 2010), several research groups have reported that the components of anthocyanin in the purple sweet potato are cyanidin and peonidin (Montilla et al., 2010; Khaodee et al., 2018)

This research focused on developing the simple naked-eye method for Fe(III) detection in terms of qualitative and quantitative determinations using anthocyanin extracted from purple sweet potato as a selective chelating

agent. Purple sweet potato is the best choice because it is a Thai local plant growing all year round. Naked-eye detection was the proposed method carried out together with UV-Vis spectrophotometer detection as a comparison method. The advantages of this approach are simplicity, rapidity, and low cost, with no requirement for sample preparation, and is an environmentally friendly detection method.

## Methods and Materials

The purple sweet potatoes were obtained in the local market in Narathiwat, Thailand. Acetic acid ($CH_3COOH$) was purchased from BDH laboratory supplies (Poole, UK). Ethanol was purchased from J.T. Baker (Phillipsburg, USA). Nitric acid ($HNO_3$), aluminum chloride ($AlCl_3.6H_2O$), and Manganese (II) sulfate monohydrate ($MnSO_4.H_2O$) were purchased from Loba Chemie (Mumbai, India). Iron (III) nitrate nonahydrate metal salts ($Fe(NO_3)_3.9H_2O$), and Lead (II) nitrate ($Pb(NO_3)_2$) were obtained from Qrec, New Zealand. Cobalt (II) nitrate hexahydrate ($CoSO_4.6H_2O$), Copper (II) sulfate pentahydrate ($CuSO_4.5H_2O$), Nickel sulfate hexahydrate ($NiSO_4.6H_2O$) and Zinc sulfate heptahydrate ($ZnSO_4.7H_2O$) were obtained from KemAus, Australia. The buffers pH 1-2 were prepared from hydrochloric acid-potassium chloride, pH 3-6 were prepared from acetic acid-sodium acetate, pH 7-9 were prepared from hydrochloric acid-disodium hydrogen phosphate, pH 10-11 were prepared from sodium hydroxide-disodium hydrogen phosphate and pH 12 was prepared from sodium hydroxide-potassium chloride. All buffers were prepared with deionized water treated with a reverse osmosis deionized system (Millipore, Bedford, USA).

**Crude anthocyanin extraction from purple sweet potato**

The purple sweet potatoes were washed, peeled, and sliced into small pieces. About 100 g of the potato pieces were crushed with a blender for 3 minutes and the crushed material was placed in a beaker with 200 mL of solvent (solvent: purple sweet potato = 1:2). The solvent consisted of ethanol 96%, acetic acid, and distilled water with a ratio of 25:1:5. The extract was drained with a white cloth filter. The filtrate was heated in a water bath at 50°C to vaporize the ethanol to obtain a more concentrated filtrate. The pigment filtrate then was filtered using a vacuum filter with filter paper and the filtrate was centrifuged at 3000 rpm for 15 min. The supernatant was then kept in a dark glass bottle in a refrigerator until required for further analysis. The crude anthocyanin was characterized by UV-Vis spectrophotometry.

**Standard metal ions preparation**

Standard metal ion solutions were prepared in 0.1 M $HNO_3$ and adjusted to pH 1-12 with the buffer solution. The ions of each metal ($Al^{3+}$, $Fe^{3+}$, $Cu^{2+}$, $Co^{2+}$, $Pb^{2+}$, $Ni^{2+}$, $Mn^{2+}$ and $Zn^{2+}$) in the concentration of 1000 ppm were diluted using the buffer for preparing the metal ion standard solutions at 100 ppm, except the Fe(III) standard solution was prepared with a broader range of concentrations, 0.50, 1, 5, 10, 20, 25, 50 and 100 ppm, for further analysis.

**Effect of pH for Fe(III) detection based naked-eye detection**

The pH of the buffer that was selective for Fe(III) was first tested. The color-developing method was observed by adding 100 µL of buffer pH 1-12 and 40 µL of each metal solution at 100 ppm followed by 20 µL of crude extracted anthocyanin solution. The color change was observed by the naked eye at the different pH levels and by relating the effect of each metal tested on the anthocyanin formation complexes.

Then, the condition that was selective for Fe(III) detection was chosen for further analysis of other parameters. The reagent volumes of the crude extract of anthocyanin and the metal interfering ions ($Ca^{2+}$, $Mg^{2+}$ $Na^{+}$ and $K^{+}$) in a concentration 10 times as high as that of the tested metal ion, were tested and the color changes were observed by the naked-eye.

**Quantitative determination of Fe(III)**

Semiquantitative determination was done under optimum conditions, the detection procedure was obtained by adding 100 µL of the disodium hydrogen phosphate/sodium hydroxide buffer pH 11 and 40 µL of Fe(III) solutions (0.5-100 ppm) followed by 20 µL of crude extracted anthocyanin solution. The color change was recorded with a digital camera and the limit of detection (LOD) was obtained at the lowest concentration which can be observed by the naked-eye. For quantitative determination, measuring the absorbance by UV-Vis spectrophotometer, the calibration curve was investigated using the same semi-quantitative determination procedure and increasing the volume of each component to be 12 times higher than those used in the naked-eye method (1200 µL of the buffer pH 11, 480 µL of Fe(III) solutions (0.5-100 ppm). Linearity was determined from the plot between the absorbance (y) and Fe(III) concentration (x) in the range where the correlation coefficient ($r^2$) was greater than or equal to 0.9900.

## Results

**Crude anthocyanin extraction from purple sweet potato**

Deep red-violet extracted anthocyanin solution was stored in the refrigerator and observed for a long time without any color change (inset Figure 1). The spectrum showed the maximum absorption wavelength at 535 nm (Figure 1).

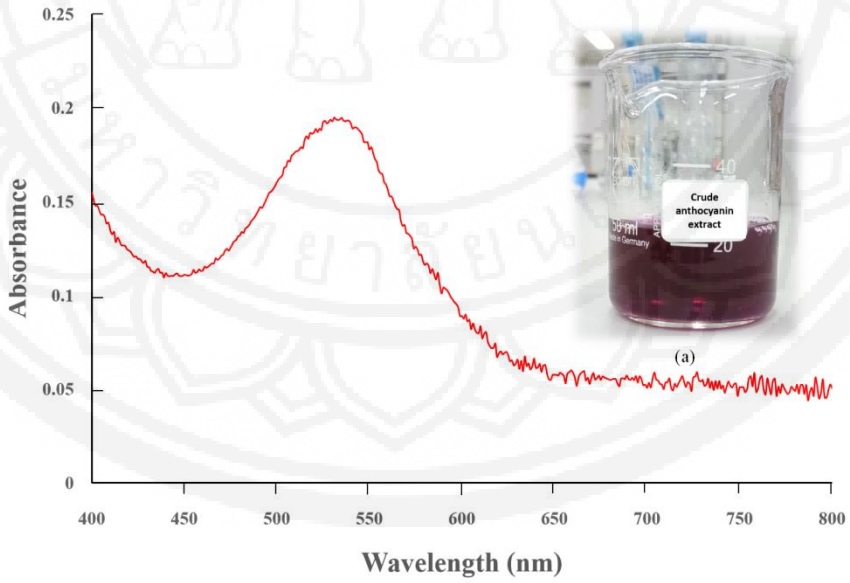


**Figure 1** Spectrum of crude extracted anthocyanin solution and deep red-violet color chelating agent extracted from purple sweet potatoes (inset).

**Naked-eye detection**

The pH of the solution is a major parameter affecting the anthocyanin structure. Anthocyanin in solution appeared as a pink-red color at pH 2-4, purple at pH 5-7, blue at pH 8-10 and green at pH 11-12. Complexation of anthocyanin and metal ions ($Al^{3+}$, $Fe^{3+}$, $Cu^{2+}$, $Co^{2+}$, $Pb^{2+}$, $Ni^{2+}$, $Mn^{2+}$ and $Zn^{2+}$ were investigated under the detection conditions using 100 µL of the buffer solutions pH 1-12 and 40 µL of Fe(III) solutions (0.5-100 ppm) followed by 20 µL of crude extracted anthocyanin solution. Anthocyanin extracted the clearly formed complexes and the color change could only be observed by the naked eye with $Al^{3+}$, $Fe^{3+}$ and $Cu^{2+}$ at wide pH ranges, pH 8-9 for $Al^{3+}$, pH 4-12 for $Fe^{3+}$ and pH 7-9 for $Cu^{2+}$ as shown in Figure 2(a). After the chelating agent interacted with some metal ions, a bathochromic shift occurred due to the interaction between the metal ions and the ortho-dihydroxyl group at the B-ring of the cyanidin molecule (Khaodee et al., 2014) as shown in Figure 2(b).

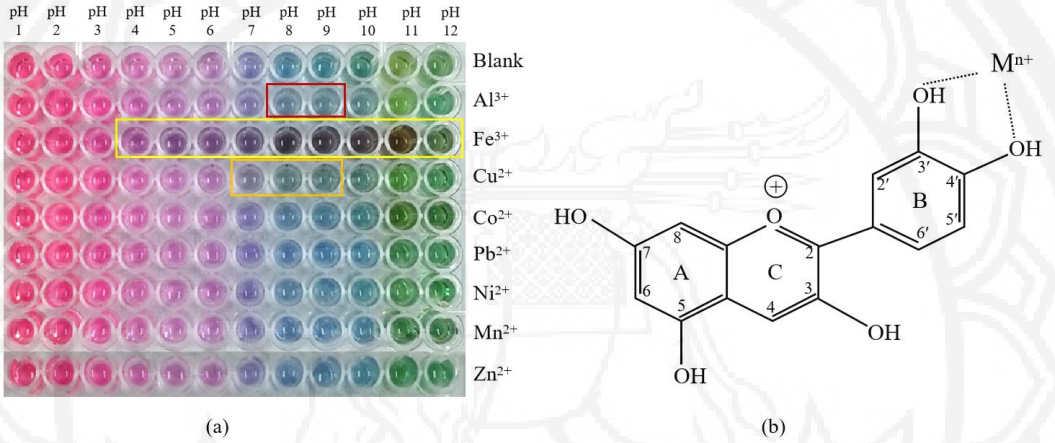


**Figure 2** (a) Qualitative determination of metal ions at different pH buffer solutions (b) Interaction between the metal ions and the ortho-dihydroxyl group

**Effect of reagent volume**

The color of the reagent might affect the color of the complex molecules, so the reagent volume should be considered when attempting to achieve the lowest concentration detectable. The reagent volume was studied between 10 and 50 µL under the detection procedure obtained by adding 100 µL of the buffer pH 11 and 40 µL of Fe(III) solutions (0.5-100 ppm). The result showed that the lowest concentration of Fe(III) was found at the reagent volume of 20 µL as presented in Figure 3

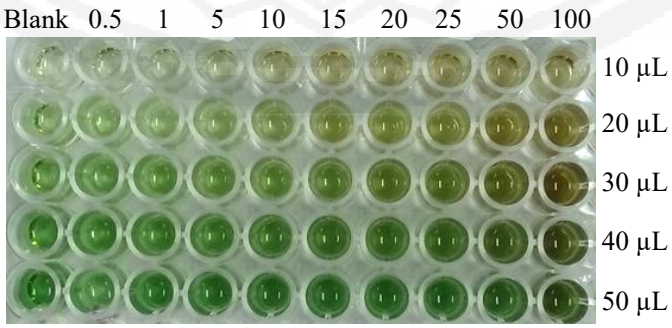


**Figure 3** Effect of reagent volume for determination of Fe(III) by naked-eye detection

**Effect of interfering ions**

It is well known that one ligand could potentially be complex with many cations. Therefore, the interfering ions should be considered. Fe(III) in the concentration of 120 ppm was prepared in the buffer pH 11. Metal ions ($Ca^{2+}$, $Mg^{2+}$ $Na^{+}$ and $K^{+}$) in the concentration of 1 and 10 times as high as that of the tested metal ion were prepared as interfering ions in Fe(III) solution. The results presented in Figure 4 show that no interference was found that could be observed by the naked eye after adding these to all interfering ions. This indicates that some cations would not affect naked-eye detection of Fe(III) in real water samples.

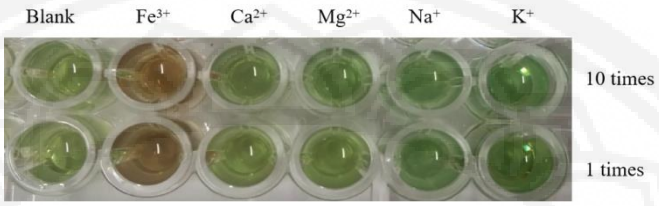


**Figure 4** Interfering effects of metal ions

**Quantitative determination**

Semiquantitative determination was obtained under the optimum conditions of qualitative determination by applying 100 μL of pH 11 buffer, and 40 μL of Fe(III) metal solutions (0.5-100 ppm) followed by 20 μL of anthocyanin solution. Naked-eye detection was performed as a semiquantitative determination of the division into 3 color shades depending on Fe(III) concentration ranges in Figure 5 and Table 1.

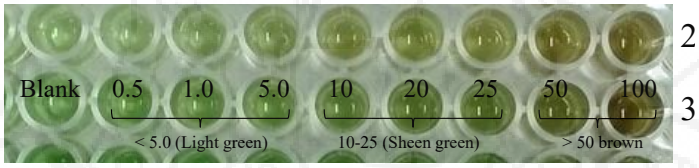


**Figure 5** shows the semiquantitative determination

**Table 1** Semiquantitative detection of Fe(III) by naked-eye detection

| Color shades | Concentration range (ppm) | LOD |
|---|---|---|
| Light-green | <5.0 | 5 ppm |
| Sheen-green | 10-25 | |
| Brown | >50 | |

The quantitative determination was investigated by UV-Vis spectrophotometer under the optimum conditions by applying 1200 μL of pH 11 buffer, and 480 μL of Fe(III) metal solutions (1.0-100 ppm) followed by 240 μL of anthocyanin solution. The calibration curve was obtained by plotting the maximum absorption wavelength at 475 nm and Fe(III) concentration range (1.0-100 ppm), as shown in Figure 6a, together with the color sensitivity of extracted anthocyanin solution against Fe(III) in various concentrations and the spectrum of the metal-complexes (inset Figure 6a). The relationship of a linear equation with the value of the equation of y= 0.0103x + 0.0102 for Fe(III) concentration range (1.0-20 ppm), with the correlation coefficient of 0.9939, showed that there was an increase in absorbance as the concentration of Fe(III) increases (Figure 6b). The relationship of a linear equation with the value of the equation of y= 0.0009x + 0.221 for Fe(III)

concentration range (25-100 ppm) with the correlation coefficient of 0.9715 indicated an increase in absorbance as the Fe(III) concentration increases to the optimum point until constant (Figure 6c).

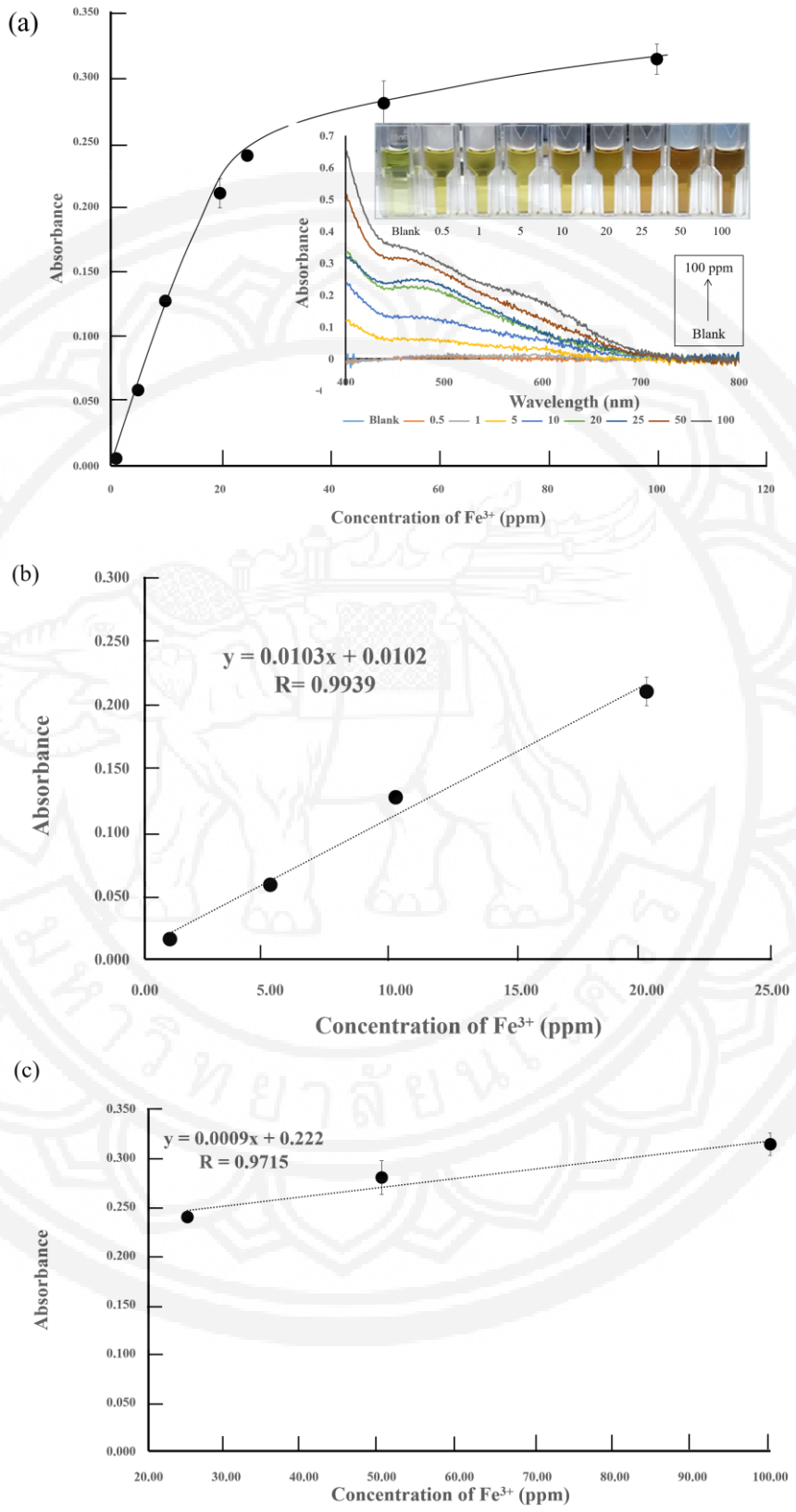


**Figure 6** The calibration plot of absorbance at 475 nm (a) $Fe^{3+}$concentration range of 1.0-100 ppm (b) 1.0-20 ppm and (c) 25-100 ppm

**Analysis of real samples**

Tap water and Laboratory wastewater samples were collected from the Faculty of Science and Technology at the Princess of Naradhiwas University, and groundwater was collected from Chana district, Songkhla province. These water samples were analyzed by using a UV-Vis spectrophotometer and naked-eye detection under optimum conditions. The water samples were filtered through 0.45 μm cellulose acetate buffer to remove some suspended particles which were preserved in 0.1 M $HNO_3$ solution. The filtrated water sample was divided into two parts to measure the Fe(III) using two methods. A semi-quantitative determination was done under optimum conditions, the detection procedure was obtained by adding 100 μL of buffer pH 11 and 40 μL of water sample followed by 20 μL of extracted purple sweet potato solution. For quantitative determination measuring the absorbance by spectrophotometer, the real water detection was investigated using the same procedure as semi-quantitative determination (1200 μL of pH 11 buffer, 480 μL of Tab water sample. The results shown in Table 2 indicate that both the UV-Vis spectrophotometer detection and the naked-eye detection were in agreement.

To validate the method, the recovery was tested by using the spectrophotometer to investigate the spiked sample that was recovered. A filtered water sample was used to prepare the spiked Fe(III) standards (3, 20, 90 ppm). The known amount of Fe(III) was spiked into the water samples. The color of the spiked water samples was developed under the optimum conditions as described in the quantitative determination of Fe(III). The semi-quantitative result of naked-eye detection was compared with the quantitative detection from the UV-Vis spectrophotometer. As shown in Table 2, the actual concentrations of Fe(III) are close to the naked-eye measurement and are in the same concentration range as the naked-eye detection. Thus, this method can be applied to determine Fe(III) in water samples without any interfering effects. The recoveries were calculated following the guidelines from EURACHEM (Eurachem Guide, 1998). The recovery values that were obtained (92-106%) were acceptable for testing water samples since the accepted recovery value was between 90-107% in the ppm level (Taverniers, Loose, & Bockstaele, 2004).

**Table 2** Comparison of Fe(III) determination in various samples by naked-eye detection and UV-Vis spectrophotometer using ethanol extract of purple sweet potato as colorimetric reagent

| Samples | UV-Vis spectrophotometer | | %Recovery | Naked-eye detection |
|---|---|---|---|---|
| | Add (ppm) | Found (ppm) | | |
| Tab water | 0.0 | ND | | ND |
| | 3.0 | 2.9±0.1 | 95.5±1.8 | <5 |
| | 20.0 | 18.5±0.4 | 92.5±1.8 | 10-25 |
| | 90.0 | 87.8±7.8 | 97.5±8.6 | >50 |
| Groundwater | 0.0 | 0.4±0.1 | | ND |
| | 3.0 | 3.2±0.3 | 94.8±8.8 | <5 |
| | 20.0 | 18.9±0.9 | 92.8±4.3 | 10-25 |
| | 90.0 | 86.7±5.9 | 95.9±6.5 | >50 |
| Laboratory wastewater | 0.0 | 6.0±0.2 | | 5 - 10 |
| | 3.0 | 8.9±0.4 | 94.3±12.4 | <5 |
| | 20.0 | 26.7±0.9 | 102.9±4.3 | 10-25 |
| | 90.0 | 101.5±12.2 | 106.0±13.6 | >50 |

ND = not detectable, ±SD (n=5)

## Discussion

### Anthocyanin extraction from purple sweet potato

At present, the main extraction method for purple sweet potatoes is solvent extraction which usually uses an acid solution for step-by-step extraction. The usual solvent extraction methods for purple sweet potatoes include water extraction, acidified water extraction, and acidified ethanol extraction (Lu, Shi, Wu, & Huang, 1997). The extraction method usually dissolves water-soluble purple sweet potato in an acidic solution by stirring, then extracts the target substance (Santos, Vegg, & Meireles, 2010). Purple sweet potatoes are not easily extracted and also are not stable in neutral or weak alkaline solutions, so acid solvents are frequently used in the extraction process. Acidic solvents can dissolve water-soluble purple sweet potatoes while destroying their cell membranes. The result shown in Figure 1 are in agreement with Khaodee et al. (2018), Kidmose, Edelenbos, Norbaek, and Christensen (2002), Steed and Truong (2008) who have reported on the presence of anthocyanin in purple sweet potatoes.

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. It is also called the flavylium (2-phenylchromenylium) ion. The general molecular structure of anthocyanin is shown in Figure 2. The intensity and stability of the anthocyanin pigments are dependent on various factors including the structure and concentration of the pigments, pH, light intensity, temperature, quality and presence of ether pigments together, metal ions, enzymes, oxygen, ascorbic acid, sugar and sugar metabolites, sulfur oxide etc (Laleh, Frydoonfar, Heidary, Jameei, & Zare, 2006; Mazza & Minitiati, 1993; Francis, 1989)

### Naked-eye detection

The pH of a solution is a major parameter affecting the anthocyanin structure. Anthocyanin solution appear in different colors at different pH levels, as shown in Figure 2(a). After the chelating agent interacts with some metal ions, the bathochromic shift occurs due to the interaction between the metal ions and the ortho-dihydroxyl group at the B-ring of the cyanidin molecule (Khaodee et al., 2014) as shown in Figure 2(b). Many research groups have reported the complex ratio as having different ratios depending on the metal ion types. The complex ratio of $Al^{3+}$-cyanidin was found to be 1:1 when investigated with a simple model using synthetic flavilium salts (Moncada et al., 2003). However, a 1:2 complex ratio was found in the case of complexation of $Al^{3+}$ with Cy 3,5-glucoside at pH 4.5, similar to the case of $Fe^{3+}$-Cy 3-glucoside complex reported by the same research group (Salinas, Esparza, Gómez, Santamaría, & Fernández, 2005). For the Cy 3-glucoside-$Cu^{2+}$ complex, the stoichiometry was 1:1 for a large excess of $Cu^{2+}$ ion as studied by Smyk, Pliszka, and Drabent (2008). The complex ratio for $Pb^{2+}$ was reported to be 1:2 at pH 4.2 (Ukwueze, Nwadinigwe, Okoye, & Okoye, 2009). The $Fe^{3+}$ metal and pH 11 buffer solution was chosen for further analysis because they provided a clear color change and were highly selective to Fe(III) solution. The developed colors were based on the interaction between Fe(III) and the chelating agent, causing the color change from white-green to brown that could be easily observed by the naked eye.

## Conclusion and Suggestions

We designed and extracted a natural chelating agent for the colorimetric detection of Fe(III). This work is

easy to operate by a non-expert operator and allows rapid detection with no complicated sample preparation for Fe(III) detection. Both qualitative and quantitative research was investigated. The method tested is an environmentally friendly detection method that uses a low volume for all reagents, and anthocyanin is also a common natural product that is easily extracted from several Thai plants. Finally, the use of a natural agent of anthocyanin as a chelating agent provides an efficient and accurate tool for directly determining the Fe(III) concentrations from various water samples, with minimal processing and equipment needs based on the naked-eye detection method.

**Acknowledgements**

This research was supported by the Faculty of Science and Technology, Princess of Naradhiwas University, Thailand. We thank Mr Roy I. Morien of the Naresuan University Graduate School for his editing of the grammar, syntax and general English expression in this document.

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