Determination of the Stability of Anthocyanins Extracts from *Plumeria rubra* Flower

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Abstract: Nowadays, natural dyes have been used widely in many sectors such as food industry. The intensive research also have been conducted to replace synthetic dyes with natural dyes as sensitizer for dye-sensitized solar cell (DSSC) as it is more economical and environmental friendly than synthetic dyes. However, it becomes a problem as natural dyes are less stable than synthetic dyes. This study was carried out to determine the optimal conditions for anthocyanins extraction from Plumeria rubra flower by using different extraction solvents, time and extraction temperatures. The light stability of Plumeria rubra flower extract was also investigated. The anthocyanin in Plumeria rubra was extracted by using simple extraction method. At various temperatures of 30, 35, 40, 45 and 50 °C, the anthocyanins concentration were found to be in the range of 0.5360 to 0.6178 mg L^{-1} in 95% ethanol solvent and 0.3044 to 0.6587 mg L^{-1} in water solvent. The total content of anthocyanins extracted at 40 °C using 95% ethanol solvent increased from 0.09086 to 0.79952 mg L^{-1} in the range of 2 to 24 hours extraction time. The optimum extraction condition of anthocyanins from Plumeria rubra was in 95% ethanol at 40 °C for 24 hours for extraction solvent, temperature and time respectively. The extraction efficiency under the optimum conditions of 95% ethanol as extraction solvent, extraction temperature of 40 °C and extraction time of 24 hours with the highest concentration of anthocyanin extract of 0.79952 mg L^{-1}. The degradation of anthocyanins pigment increased up to 72.65% as the exposure time increased from 0 to 24 hours. The stability of anthocyanins extract was significantly affected by the presence of UV light.

Keywords: Anthocyanin, *Plumeria rubra* flower, Simple extraction method

1. Introduction

According to Gratzel et al. (1991), a photovoltaic of dye-sensitized solar cells (DSSCs) was developed to convert a visible light energy to electrical energy (as cited by Calogero *et al.,* 2012). The innovation of this type of solar cell is it impersonate a photosynthesis process in plants (Shahid and Mohammad, 2013). A DSSC is consists of a semiconductor electrode, a counter electrode, an electrolyte and a sensitizer. The sensitizer can affect the efficiency of DSSC as it plays an important part in absorbing and transforming solar energy from sun into electricity (Zhou et al., 2011). A good sensitizer should absorb a wide range of light from the visible to the near-infrared (as cited by Calogero *et al.,* 2012). There are two types of dye can be used as sensitizers which are synthetic dyes and natural dyes. Synthetic dye such as ruthenium (Ru) complex is a transition metal coordination compound. It is one of the excellent photosensitizer for DSSC with high efficiency in transforming solar energy from sun into electricity (Chiba et al., 2006).

Natural dyes are derived from natural sources such as plants. A variety colour of flower, fruits and leaf of plant from red to purple shows that it contains various pigments such as anthocyanins, betalains, carotenoids and chlorophylls (Escribano and García, 2010; Jensen, 2011) can be used as a natural dye by using simple extraction procedure (Hao et al., 2006). The pigments that have been mostly reported which show a good potential as sensitizer in DSSCs is anthocyanins (Chien and Hsu, 2013; Calogero et al., 2012), betalains (Calogero et al., 2012; Hernandez-Martinez

et al., 2011; Zhang et al., 2008) and carotenoids (Hug et al., 2014). These pigment are focused mostly in research due to their higher conversion efficiency than chlorophyll (Zhou et al., 2011). Anthocyanins found mostly in higher plant like apple, cherry, eggplant, *Dianthus*, *Petunia*, *Rosa*, *Tulipa* and *Verbena* (Delgado et al., 2000). Figure 1 shows the basic chromophore of anthocyanins. The R_x at the chromophore could be OH, H or OCH_3 as it depends on considered pigment.

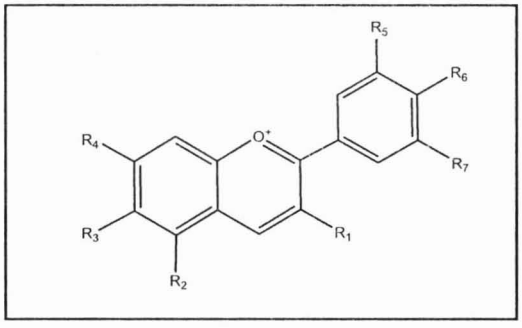

Fig. 1 The basic structure of chromophore anthocyanins pigments

Natural dyes are less efficient than synthetic dyes by several factor such as weak binding energy with TiO_2 thin film and low charge transfer absorption (Hug *et al.,* 2014; Chang *et al.,* 2010). Although natural dyes have lower efficiency than synthetic dyes, but natural dyes are cheaper, more environmental friendly with renewable and abundant source (San Esteban and Enriquez, 2013). Thus, it makes natural dye become an alternative sensitizer for DSSC. However, using natural dyes as alternative sensitizer become a challenge as it is less stable than synthetic dyes. The extensive study about the stability of anthocyanin as sensitizer has not been explored. The stability of natural dyes can be influence by several factors such as light exposure. The temperature affect the degradation rate of the pigments and the concentration of anthocyanins extract also varies with different solvents and extraction times. Thus, the purpose of this study is to determine the optimal conditions for extraction of anthocyans were also determined by assessing the influence of different extraction solvents, time and extracting temperatures for extracting the dye as natural sensitizer. The stability of anthocyanins extract from natural sources of *Plumeria rubra* was also investigated.

2. Experimental

2.1 Preparation of plant extracts with different extracting solvent

A freshly pluck of *Plumeria rubra* flowers was cut into pieces before dried it in oven at 65°C for ~10 hours. Dye solutions were prepared by grinding dried flowers into powder using pestle and mortar. One grams of powdered sample were separately extracted with 60 mL of different extracting solvents 95% ethanol and water for overnight (Kim *et al.,* 2013) in waterbath shaker at 80 rpm. An aluminium foil was used to cover the beaker to prevent loss of solvent through evaporation. The flowers were then filtered out from extract sample by using Albet 40 μm standard filter paper to acquire a clear dye solution.

2.2 Determination of optimal extraction temperature

The effects of extraction temperature of flower dye samples were controlled by using water bath without exposure to sunlight. The water baths were set up at four different temperatures of 35, 40, 45 and 50 °C. One sample extracted in room temperature of 30 °C was used as the control. The

solid residue were filtered out from dye extract using Albet standard filter paper (Hernandez et al., 2011). Concentration of anthocyanins extracts were analysed using pH differential method. The best optimal temperature was used in the next experiment.

2.3 Determination of optimal extraction time of anthocyanins

The extracted flower dye sample which has the highest total content of anthocyanins at optimal condition of extraction temperature and solvent were then determined for its optimal time of extraction (Lapornik et al., 2005). The dye sample extracted at room temperature 30 °C was used as control. Thus, dye samples were extracted at various time ranges at 3, 16 and 24 hours. The best optimal time was used in the next experiment.

3. Light stability of anthocyanins

About 15 mL of flower dye samples extracted using 95% ethanol at temperature of 40 °C for 24 hours were pipette into a closed capped vial to prevent sample from evaporated. The stability of dye samples was studied at room temperature with a presence of light for 1, 8, 16 and 24 hours. The sample that extract at 30 °C for 24 hours was used as a control. By using pH differential method the total content of anthocyanins extracts remained in samples after exposure were determined (Stanciu et al., 2010).

4. Quantitative analysis of anthocyanins

Total monomeric anthocyanins in flower extract were measured using spectrophotometric pH differential method. This method was conducted by preparing two types of buffers solution with different pH of 1.0 and 4.5.

Buffer of pH 1

About 1.86 g of KCl was weighted and added into a beaker containing 970 mL distilled water. The solution was stirred until solid KCl completely dissolved before adjusted it's pH to pH1.0 by added a drop wise of concentrated hydrochloric acid, HCl. The buffer solution was then transferred into a 1000 mL volumetric flask. Distilled water is added until it reached the calibration marks of flask.

Buffer of pH 4.5

About 54.43 g of CH_3COONa was weighted and dissolved in 960 mL distilled water. The pH of solution is adjusted to pH4.5 through addition of a drop wise of concentrated hydrochloric acid, HCl. The solution was transferred into a 1000 mL volumetric flask and completed the volume of 1 L using distilled water.

Procedure

About 1 mL of *Plumeria rubra* flower dye sample was diluted in each of two buffer solution of potassium chloride (KCl) buffer 0.025 M and sodium acetate (CH_3COONa) buffer 0.4 M in 10 mL volumetric flask. The absorbance of samples were read at $\lambda = 520$ nm and $\lambda = 700$ nm after been left for 15 minutes in room temperature. Blank sample used was distilled water.

The anthocyanins are expressed as cyanidin-3-glucoside equivalents. The concentrations of it are measured in mg/L using the equation as follow (1):

$$(A \times MW \times DF \times 10^3) / (\varepsilon \times b) \quad (1)$$

Where $A = (A_{520nm} - A_{700nm})pH1 - (A_{520nm} - A_{700nm})pH4.5$, MW is the molecular weight of cyanidin 3-*O*-β-(2″-glucopyranosyl-*O*-galactopyranoside) that is 611.0 g mol^{-1}, a dilution factor, DF was 2:10, b is path length in cm and molar extinction coefficient, ε is 26 900 L mol^{-1}. While 10^3 is a factor to convert gram to milligram (Suhad and Viorica, 2009).

4. Result and Discussion

4.1 Extraction solvents and optimum temperature

The extraction process of natural dye from *Plumeria rubra* flowers was carried out using different extraction solvent and extraction temperature within same period of time. Figure 2 show the total monomeric content of anthocyanins also found to be higher in ethanol extract than in water extract.

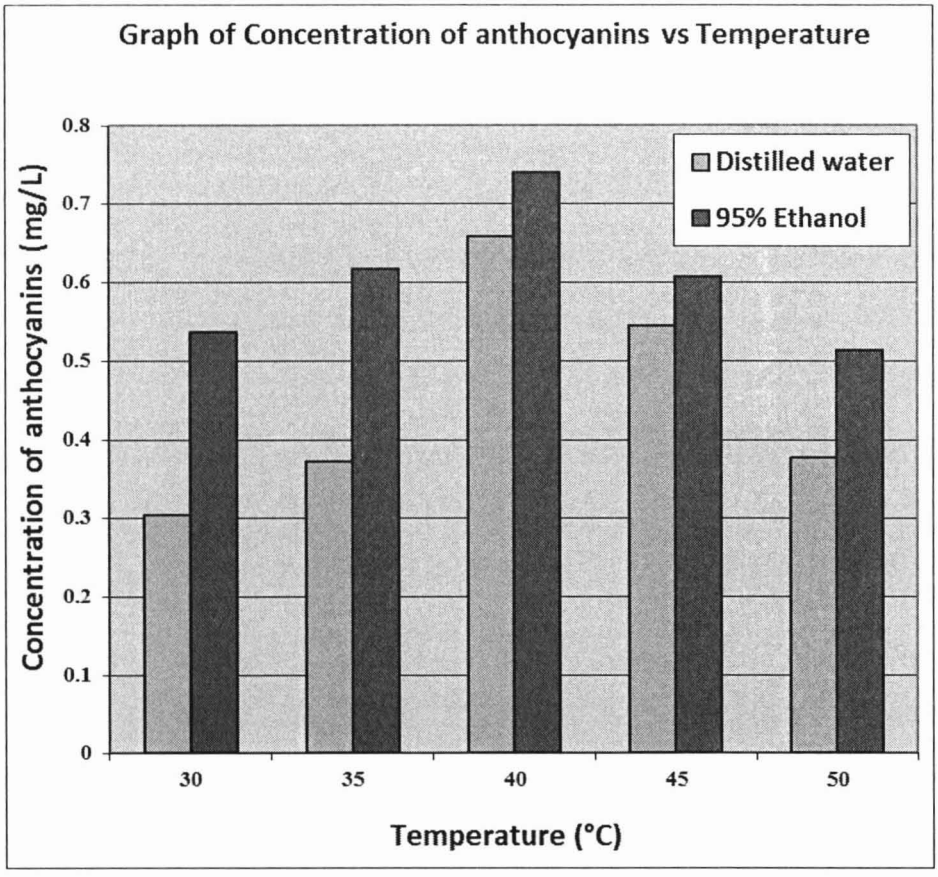

Fig. 2 Total contents of anthocyanins extracted using distilled water and 95% ethanol in different temperature for 16 hours

The different amount of anthocyanins obtained in different extraction solvents of 95% ethanol and water were cause by the different polarity of solvents. A polar anthocyanins can

diffused more rapidly into ethanol because it less polar than water and also because of ethanol solvent have same polarity with anthocyanins than water (Chaudhari, 2013). The less polar character of ethanol is efficient in degradation of seed and cell walls which help the released of anthocyanins and other polyphenols from cells (Lapornik et al., 2005).

The decreased in anthocyanins content at 40 to 50 °C indicate to the degradation of pigments has occurred in both samples. The percent degradation of pigments at extraction temperature 40 to 50 °C was 42.75% in water solvent while 30.68% in ethanol. As the temperature increased, the solvent's surface tension and viscosity decreased, leading to increased in mass transfer intensity and the decreased in extraction concentration as pigment's degradation occured (Maran et al., 2014). The degradation rate was higher than the extraction rate when the extraction temperature increased (Zheng et al., 2013). Therefore, only a small amount of anthocyanins can be recovered as the rest of it was degraded by high temperature.

The best extraction solvent for *Plumeria rubra* flowers extraction was 95% ethanol and the most favorable temperature for the extraction was at 40 °C. The data obtained was used for the next step in this experiment.

4.2 Optimum extraction time

Extraction time of sample was conducted using 95% ethanol at two constant temperatures of 30 °C and 40 °C for four different time range of 2, 8, 16 and 24 hours. Sample extracted at room temperature of 30 °C was used as a control sample. Figure 3 shows the relationship between anthocyanins and extraction factors where the anthocyanins recovery increase as extraction time increased.

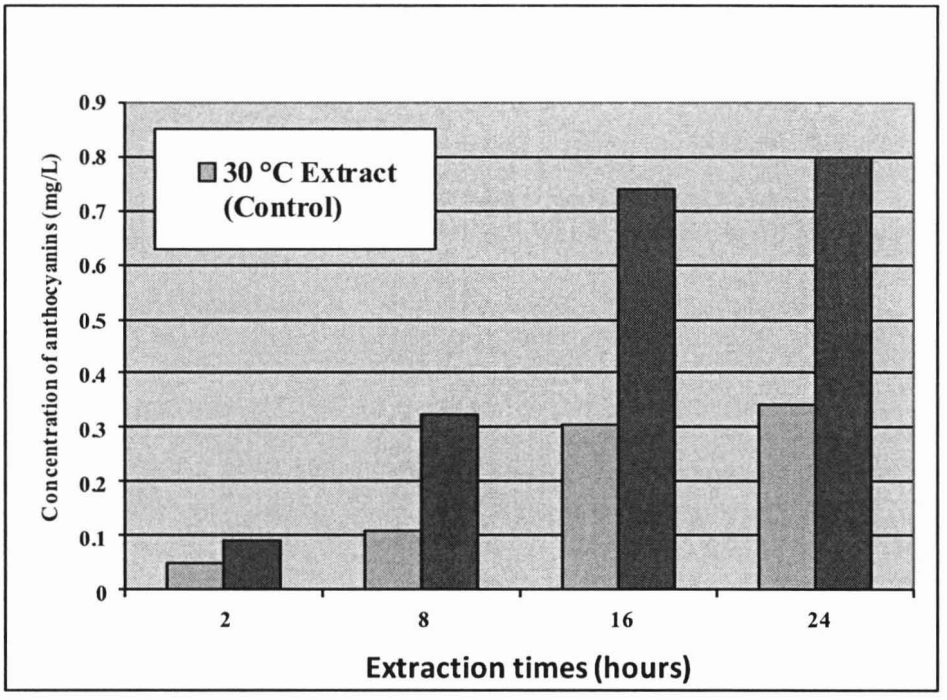

Fig. 3 Total contents of anthocyanins extracted in a two different extraction times

Extracted sample at 40 °C contained a higher concentration of anthocyanins than in sample extracted at 30 °C. At 24 hours, both 30 °C extract and 40 °C extract have a total anthocyanin content of 0.34071 mg L^{-1} and 0.79952 mg L^{-1} respectively. While fewer amounts of anthocyanins were recovered at lowest extraction time of two hours for both temperatures of 30 °C and 40 °C which only 0.04997 mg L^{-1} and 0.09086 mg L^{-1} respectively.

The concentration of anthocyanins extracted from *plumeria rubra* at 40 °C increased rapidly at the beginning of extraction time, but started to slow down toward the end, at 24 hours. At 30 °C extraction temperature, the rate of pigments extraction continued to increase slowly toward the 24 hours. The extraction rate of both extracts is different. Within time range of 2 to 16 hours, total anthocyanins recovered rose sharply and faster in 40 °C than in 30 °C extract. It is because more pigments were able to permeate into the solvent as extraction time increases. At 24 hour, maximum concentration of anthocyanins extracts can be seen. The total anthocyanins recovered in extract sample indicate the efficiency of extraction. Similar results were reported for the extraction time of anthocyanins using ethanol as a solvent (Lapornik *et al.,* 2005; Fan *et al.*, 2008).

4.3 Optimization extraction condition of *Plumeria rubra* flowers

The optimal condition of simple extraction methods for anthocyanins of *Plumeria rubra* flowers extracts was using 95% ethanol as extraction solvent, extraction temperature of 40 °C and extraction time of 24 hours. During the extraction of anthocyanins, controlling the extraction temperature is important to obtain an optimal total content of pigment. Although this may be the best condition for extraction of sample, increasing number of variables combines altogether can give a higher concentration of anthocyanins as it have slightly different optimum extraction condition (Fan et al., 2008).

4.4 Light stability of anthocyanins

The light stability of extraction time was determined by using optimal condition of dye extraction where its extraction temperature is 40 °C using 95% ethanol for 24 hours. The dye extracted at room temperature of 30 °C using 95% ethanol for 24 hours was used as control for this test. Extract dye were exposed into light in a range of times.

Figure 4 reflected a decreasing of anthocyanins concentration in both dyes as time for light exposure increase. Total content of antocyanins for 40 °C dye extract decrease from 0.79952 to 0.21865 mg L^{-1} after exposed for 24 hours in presence of light. While in control extract of 30 °C, concentration of anthocyanins decreased from 0.34071 to 0.16808 mg L^{-1}. Percentage of degradation of anthocyanins pigments in 40 °C extract was 72.65% and for 30 °C extract was 50.67%. The decrease anthocyanins concentration is due to alteration and destruction of its molecular structure by light (Jenshi et al., 2011). Similar pattern on decreasing of anthocyanins after light exposure had been reported by (Stanciu et al., 2010).

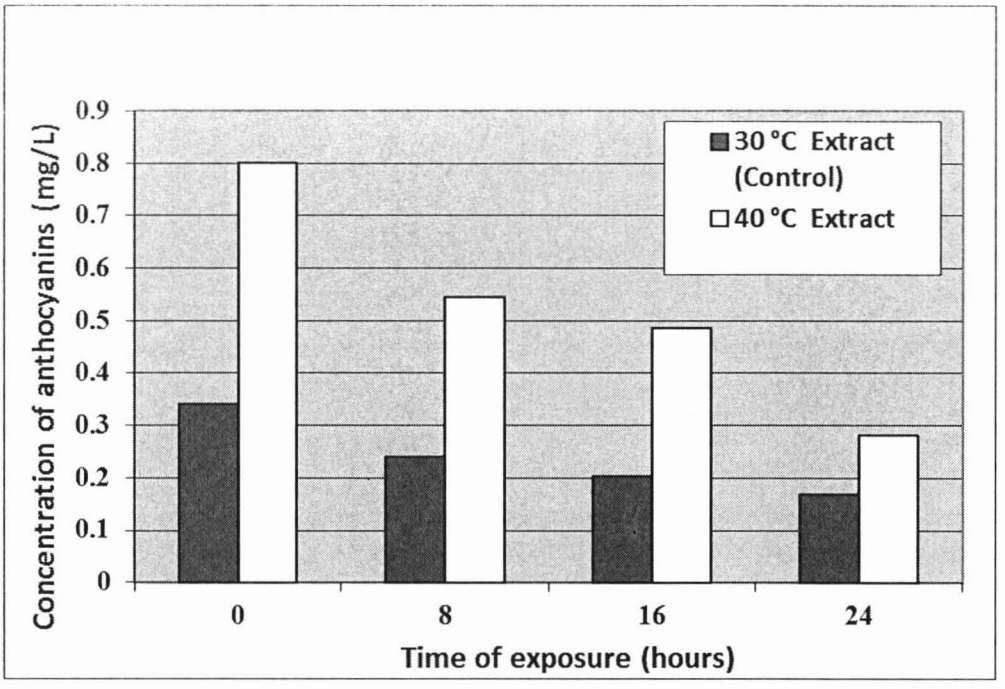

Fig. 4 Stability of anthocyanins in light exposure within a time range

5. Conclusion

In this study, the light stability of anthocyanins extracts from *Plumeria rubra* flowers and optimum conditions for extraction of anthocyanin were successfully analysed which suitable to be applied in DSSC. The anthocyanins extracts from *Plumeria rubra* flowers were found unstable when exposed to light. The amount of anthocyanins were rapidly decreases when increase its times of light exposure as anthocyanins pigments degrade up to 72.65%. The best solvent for anthocyanins extraction was 95% ethanol and it reached up to 0.7405 mg L^{-1} of total extracted pigment. While only 0.6587 mg L^{-1} of anthocyanins recovered in water.

The extracted anthocyanins at temperature of 40 °C were increase of up to 0.79952 mg L^{-1} when extraction time increases to 24 hours. Increasing extraction time at optimal temperature can enhance the efficiency of anthocyanins pigment extraction from petals of *Plumeria rubra* flowers. In this study the highest amount of pigment in extract of 0.79952 mg L^{-1} was obtained by optimal condition of 95% ethanol as extraction solvent, extraction temperature of 40 °C and extraction time of 24 hours.

6. References

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