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# Effect of Solvent pH, Microwave Power and Extraction Time on Microwave-Assisted Extraction of Hibiscus rosa-sinensis

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The objectives of the study were to evaluate the effect of solvent extraction pH, microwave extraction power and time on the yield of total anthocyanin and colour of Hibiscus rosa-sinensis (H. rosa-sinensis) extract and to evaluate the storage stability of total anthocyanin content and antioxidant activity of these extracts during storage. The factors studied were solvent pH (1.0 and 4.0), microwave power (400 W and 800 W) and extraction time (1 min and 3 min). Results showed that the highest total anthocyanin extracted was 9.56 ± 0.001 g cyanidin-3-glucoside when extraction was performed using solvent of pH 4 at 800 W and 3 min and followed by 8.33 ± 0.001 g cyanidn-3-glucoside using solvent of pH 2 at 800 W and 3 min. H. rosa-sinensis extract in pH 2 was in red while the extract in pH 4 was in dark red. For the 10 d storage stability analysis, H. rosa-sinensis extract of pH 2 and pH 4 at 800 W and 3 min were selected. During storage, the extract in pH 4 showed an increase in total anthocyanin content and radical scavenging inhibition percentage. On contrary, the total anthocyanin content and radical inhibition percentage for pH 2 extraction sample decreased during storage.

## 1. Introduction

Synthetic colorants have been widely used in food, beverage and pharmaceutical industries owing to their intensive, permanent and stable color characteristics. Synthetic food colorants which are also known as artificial food colorants (AFCs), are believed to cause allergic reaction and food intolerance in children (Feketea and Tsabouri, 2017) and also believed to be carcinogenic. Due to these health concerns, food and beverage industries are shifting to natural food colorants. Natural colorant is the color pigment extracted from plants or other natural materials. Natural colorant from plant is regarded as safe, organic, non-toxic and normally plant natural colorants possess antioxidant and other health beneficial properties.

Hibiscus rosa-sinensis (H. rosa-sinensis) flower or locally known as Bunga Raya, contains high amount of anthocyanin, particularly the red anthocyanin. Anthocyanin is one of the important groups of water-soluble pigments which can be found in various fruits and vegetables. Anthocyanin has high potential as natural food colorant to replace synthetic colorant (Aishah et al., 2013).

The potential of H. rosa-sinensis flower extract as natural food colorant has not been fully explored even though it has high content of anthocyanin and available abundantly all year round in Malaysia. However, anthocyanin, as other natural plant colorants, are unstable and susceptible to degradation due to its sensitivity towards light, oxygen, water etc. In order to develop the H. rosa-sinensis anthocyanin as natural colorant, it is important to evaluate its stability during storage. Microwave-assisted extraction has become an important extraction technique owing to its volumetric heating mechanism, moderate capital cost and suitability under atmospheric conditions (Chan et al., 2011). It is also considered as green technology as it requires low amount solvent for extraction and at the same time, the extraction process is faster compared to conventional

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methods available. In developing an efficient microwave-assisted extraction method, the understanding on the effect of solvent pH, microwave power and time on the process is important because an appropriate combination of these parameters contributes to internal superheating which leads to liberation of high amount of targeted compounds into the solvent. There are two objectives of this study. The first objective is to evaluate the effect of solvent extraction pH, microwave extraction power and time on the yield of total anthocyanin (TAC) and color of H. rosa-sinensis extract. The second objective is to evaluate the stability of microwaved H. rosa-sinensis total anthocyanin content and antioxidant activity in 10 d storage.

## 2. Materials and methods

### 2.1 Sample and solvent preparation

H. rosa sinensis flower was collected from the compound of Universiti Teknologi Malaysia. The flowers were placed on glass petri dish and dried at 60 °C for 24 h by using universal oven (Memmert). The dried flower sample was then ground to powder and kept in amber bottles for further analysis (Zaidel et al., 2017). The extraction solvents of pH 2 and 4 were prepared by adding citric acid into 30 % ethanol-distilled water mixture according to method described by Jusoh et al. (2017) with modifications.

### 2.2 Extraction of H. rosa-sinensis by using microwave-assisted extraction (MAE) technique

MAE of H. rosa-sinensis flower was performed using Sineo Microwave Synthesis Workstation (MAS-II Plus). This microwave unit is equipped with power, temperature and time controller. For each extraction, 0.4 g of dried H. rosa-sinensis was placed in a beaker and combined with 50 mL of extracting solvent. The factors studied were solvent pH (2 and 4), microwave power (400 and 800 W) and extraction time (1 and 3 min). The temperature of extraction was set at 60 °C. After the microwave extraction, the samples were filtered, and the crude extracts were kept in amber bottles for subsequent analyses. The extraction and storage of H. rosa-sinensis flower was performed according to Jusoh (2015) with modifications. All extractions were done in triplicates.

### 2.3 Total Anthocyanin content

The total anthocyanin content of the crude H. rosa-sinensis extract was determined using spectrophotometric pH-differential method described in Nawi et al. (2015). This method was using two buffer systems: potassium chloride buffer, pH 1.0 (0.025 mol/L) (125 mL of 0.2 mol/L potassium chloride and 375 of 0.2 mol/L hydrochloric acid) and sodium acetate buffer, pH 4.5 (0.4 mol/L) (400 mL of 1 mol/L sodium acetate, 240 mL of 1 mol/L acetic acid and 360 mL water). 0.1 mL of sample was mixed with 0.9 mL of buffer solution in cuvette. The absorbance was measured at 520 and 700 nm with distilled water as blank using a UV–visible spectrophotometer (Jenway). The absorbance difference between the pH 1.0 and pH 4.5 samples was calculated using Eq(1) :

$$A = (A_{520nm} - A_{700nm})_{pH1.0} - (A_{520nm} - A_{700nm})_{pH4.5} \quad (1)$$

The total anthocyanin content was calculated as gram cyanidin-3-glucoside according to the following Eq(2):

$$\text{Total anthocyanin (mg/L)} = (A \times MW \times DF) / \varepsilon \times 1 \quad (2)$$

Where MW (molecular weight) = 449.2 g/mol for cyanidin-3-glucoside, DF is the dilution factor, 1 = pathlength in cm, ε = 26,900 molar extinction coefficient in L/mol/cm for cyanidin-3-glucoside. All analyses were performed in triplicates.

### 2.4 Color

The color of the extracts was measured using a CR-10 color meter (Konica Minolta). The L, a and b values were measured by placing the sample bottle filled with 20 mL crude extract on sample surface aperture of the color meter. L value represent the lightness of the sample, where the scale from 0 - 50 indicates dark region and scale 51 - 100 indicates light region. The a value represents the red-green color scale, where positive a value indicates red and negative a value indicates green. The b value is a measure of yellow-blue color of the sample, where positive b value represents the yellow region and negative b value indicates blue color region (Hunterlab, 2012).

### 2.5 Stability test

2.5.1 Total Anthocyanin content

The crude extract with the highest TAC value from each pH, 4A and 4B were used for stability test. Both crude samples were kept in the amber bottles and stored at room temperature for 10 d. The TAC in the crude extracts was measured and calculated for every two days using method described in Section 2.2.

2.5.2 DPPH radical scavenging activity

The scavenging effects of the crude extracts 4A and 4B on DPPH radical were monitored using method described in Ge and Ma (2013) with minor modifications. 0.1 mL of the crude extract were mixed with 0.1 mL DPPH methanolic solution. The mixture was mixed well and incubated for 30 minutes at room temperature. The absorbance of mixture was measured at 517 nm using a UV-visible spectrophotometer (Jenway). The antioxidant capacity of the crude extract during storage was expressed as the percentage inhibition of DPPH based on the following formula Eq(3):

$$\text{Percentage inhibiton of DPPH} = \frac{\text{A}_{\text{control}} - \text{A}_{\text{sample}}}{\text{A}_{\text{control}}} \times 100\ \% \quad (3)$$

where $A_{control}$ is the absorbance of DPPH solution without extracted sample and $A_{sample}$ is the absorbance of DPPH solution with extracted sample.

## 3. Results and discussion

This section presented the results on the microwave-assisted extraction of Hibiscus rosa-sinensis and the effect of solvent pH, microwave power and extraction time were compared. The effect is determined through the total anthocyanin content, colour and stability.

### 3.1 Total Anthocyanin content

The effect of solvent pH, microwave power and extraction time on the total anthocyanin content (TAC) in crude H. rosa-sinensis extracts were studied. The results of TAC in the extracts are shown in Table 1. From the result, it can be deduced that microwave extraction power has significant effect on TAC extracted in crude H. rosa-sinensis extract. Crude extracts obtained via 800 W power have higher amount of TAC compared to 400 W extract. The mass transfer of intracellular bioactive compounds may have been accelerated by microwave power beyond 500 W (Alara et al., 2018). Results also showed that extraction the time has significant impact on the yield of TAC in the crude extract. All samples exposed to 3 min extraction produced extracts with higher amount of TAC than other extracts. Higher anthocyanin content was extracted by increasing extraction time however the anthocyanin yield stabilised once saturation point has been reached (Alvarez et al., 2017).

*Table 1: Total anthocyanin content (TAC) in microwave-assisted H. rosa-sinensis extracts*

| Sample | pH | Power (Watt) | Time (min) | Total Anthocyanin Content (g cyanidin-3-glucoside) |
|---|---|---|---|---|
| 1A | 2 | 400 | 1 | $7.53 \pm 0.01^{b}$ |
| 2A | | | 3 | $7.68 \pm 0.01^{b}$ |
| 3A | | 800 | 1 | $7.23 \pm 0.01^{a}$ |
| 4A | | | 3 | $8.33 \pm 0.01^{c}$ |
| 1B | 4 | 400 | 1 | $6.58 \pm 0.01^{a}$ |
| 2B | | | 3 | $8.02 \pm 0.02^{b}$ |
| 3B | | 800 | 1 | $6.90 \pm 0.01^{a}$ |
| 4B | | | 3 | $9.56 \pm 0.02^{d}$ |

Values are mean ± SD of triplicates

Values in columns for every sample bearing different superscript letters are significantly different ($P < 0.05$).

### 3.2 Color

Figure 1 shows the color of H. rosa-sinensis extracts, ranging from a different intensity of colours between two extraction solvent, which were of pH 2 and pH 4. It is clearly shown that samples 1A to 4A, which were extracted in solvent of pH 2 were red in color, while sample 1B to 4B, which were extracted using solvent pH 4, were in dark red color. The difference in color of the samples is due to the effect of pH on the chemical structure of anthocyanin . In pH 2, flavyium cation which possessed red color of anthocyanin dominates, violet quinoidal bases takes place in pH 4 (Ibrahim et al., 2011).

According to Vankar and Shukla (2011), the anthocyanin exist in four molecular species depending on pH that are red flavyium cation (pH 1-3), purple quinoidal anhydrobase (pH < 7), deep blue ionized base (pH < 8) and

yellowish chalcone (pH > 8). Similar color change observed in this was also observed in the work done by Vankar and Shukla (2011). The change in color happens due to structural change in flavyium cation.

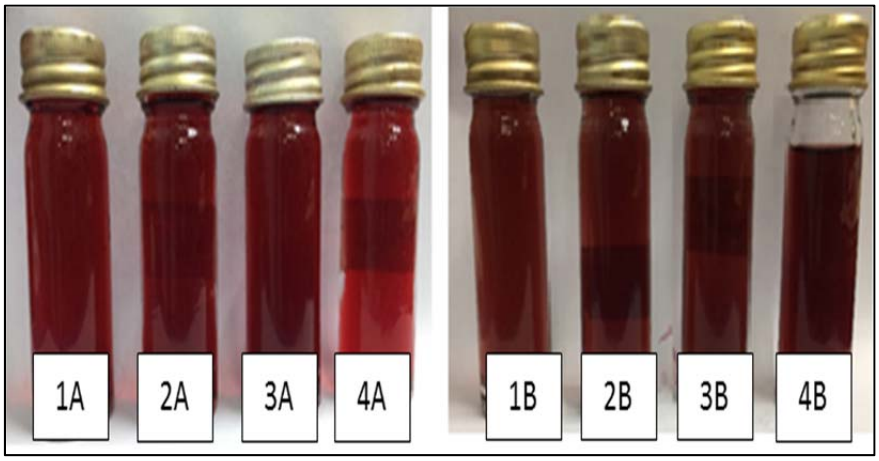


*Figure 1: Color of crude H. rosa-sinensis extracts with A label represents extracts in pH 2 and B label represents extracts in pH 4*

The color of the crude extracts in terms of L, a and b values are shown in Table 2. From this experiment, the L values of the crude extracts were between 21 and 24 which means that all the extracted samples were in dark region. The L values of pH 2 extracts were slightly higher than pH 4 extracts. This data is in agreements with Figure 1. All crude extracts have positive a value which implies they are red in color. The a values of pH 4 extracts were also lower than pH 2 extracts, indicating they are approaching to green region. All extracted samples were in yellow color region since all b values were positive. The pH 4 extracts have lower b values than pH 2 extracts, indicating they are approaching to blue color region. The difference in L, a and b values is probably due to different forms of anthocyanins that exists in the crude extracts due to effect solvent pH. Anthocyanins have four different forms that are flavyium (red), quinoidal (purple), carbinol (colorless) and chalcone (yellow) forms. In general, flavyium form predominates at pH 2 or below, colorless chalcone form dominates at elevated pH levels. Beside pH level, elevated temperature and prolonged exposure can also cause chalcone formation (McDougall et al., 2005).

*Table 2: L, a and b values for crude H. rosa-sinensis extracts*

| Sample | pH | Power (Watt) | Time (min) | L | a | b |
|---|---|---|---|---|---|---|
| 1A | 2 | 400 | 1 | $22.6^{a}$ | $1.1^{a}$ | 2.4 |
| 2A | | | 3 | $23.3^{b}$ | $1.4^{b}$ | 2.6 |
| 3A | | 800 | 1 | $21.0^{c}$ | $1.0^{a,d}$ | 2.5 |
| 4A | | | 3 | $22.7^{a}$ | $0.6^{e}$ | 2.3 |
| 1B | 4 | 400 | 1 | $21.8^{d}$ | $0.9^{d}$ | 2.3 |
| 2B | | | 3 | $22.2^{b}$ | $0.7^{f}$ | 1.8 |
| 3B | | 800 | 1 | $21.5^{c,d}$ | $0.8^{c}$ | 2.3 |
| 4B | | | 3 | $22.2^{a,d}$ | $0.6^{e}$ | 2.1 |

Values are mean ± SD of triplicates
Values in columns for every sample bearing different superscript letters are significantly different ($P < 0.05$)

### 3.3 Stability study

For stability study, only sample 4A (pH 2, 800 W, 3 min) and 4B (pH 4, 800 W, 3 min) were chosen based on the high amount of TAC in the extract obtained from each pH category. The result of the TAC stability over 10 d storage time is shown in Figure 2. From the result, the TAC was not stable during storage and it can be deduced that pH has significant influence on anthocyanin stability. From observation, TAC of crude extract in pH 2 decreased with time, while TAC in crude extract pH 4 increased with time. The degradation of anthocyanin during storage at lower pH was similar to Sui et al. (2014). As for pH 4 sample, the trend obtained

was plausibly due to certain types of anthocyanins which are more stable in pH 4 compared to lower pH (Fossen et al., 1998).
The difference in colour for sample 4A and 4B after 10 days storage is shown in Figure 3.

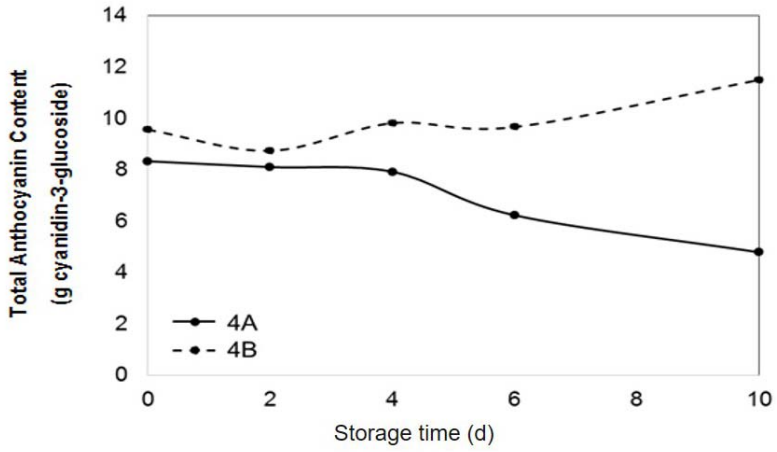


*Figure 2: Total Anthocyanin Content (TAC) of sample 4A (pH 2) and 4B (pH 4) during 10 d storage at room temperature*


*Figure 3: Color of sample 4A (pH 2) and 4B (pH 4) after 10 d storage at room temperature*

### 3.4 Antioxidant analysis via DPPH radical scavenging activity

Table 3 shows the result of antioxidant capacity of selected crude H. rosa-sinensis extracts 4A and 4B during 6 d storage at room temperature. The antioxidant capacity is measured by the inhibition percentage of DPPH free radicals by the extract. The antioxidant capacity or inhibition capacity of sample 4A was significantly lower compared to sample 4B during storage. In addition, the inhibition also decreased with storage time for 4A sample, however for 4B, there was slight increased with time. This observation is in agreements to TAC trend in Section 3.3.

*Table 3: Inhibition percentage (%) of H. rosa-sinensis in DPPH radical scavenging activity*

| Sample | pH | Inhibition percentage (%) | | |
|---|---|---|---|---|
| | | Day 0 | Day 2 | Day 6 |
| 4A | pH 2 | 27.65 | 20.18 | 14.99 |
| 4B | pH 4 | 93.39 | 94.67 | 94.70 |

## 4. Conclusions

From the presented results, it can be concluded that pH of solvent, power of microwave and extraction time have significant effect on the H. rosa-sinensis. Highest TAC in crude H. rosa-sinensis extract (9.56 g cyanidin-3-glucoside) was obtained when the MAE was performed using solvent of pH 4, microwave power of 800 W and the longest extraction time of 3 minutes. In terms of color of crude extract H. rosa-sinensis flower extracted using solvent pH 2 yielded red color extract while pH 4 yielded darker red color extract. From the storage study, it was observed that both extract, pH 2 and 4, were not stable. TAC pH 4 extract increased with time, while TAC pH 2 decreased with time. The antioxidant capacity of pH 4 extract increased slightly while pH 2 extract decreased significantly, during storage study.

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