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Comparison of Phenolic Constituent in *Hibiscus sabdariffa* cv. UKMR-2 Calyx at Different Harvesting Times

(Perbandingan Sebatian Fenolik dalam Kaliks *Hibiscus sabdariffa* kv. UKMR-2 pada Masa Penuaian Berbeza)

SITI AISHAH MOHD ALI*, CHE RADZIAH CHE MOHD & JALIFAH LATIP

ABSTRACT

The metabolic changes in the phenolics content of Hibiscus sabdariffa *cv. UKMR-2 were investigated at different harvesting times based on the High-Performance Liquid Chromatographic method using a photodiode array detector (HPLC-PDA) with gradient elution. The antioxidant activity was also determined using 2,2-diphenyl-1-picrylhydrazyl free radical scavenging activity (DPPH) assay. The cultivation was executed under a control condition, and the calyces were separately harvested at four maturation stages. UKMR-2 calyces were extracted with water via sonication (50°C, 30 min). HPLC-PDA analysis showed two predominant anthocyanins, namely delphinidin-3-O-sambubioside (2.07-2.47 mg/g DW) and cyanidin-3-O-sambubioside (0.55-1.01 mg/g DW). In addition, ascorbic acid (3.34-9.88 mg/g DW), caffeic acid (0.08-0.09 mg/g DW) and chlorogenic acid (0.50-0.65 mg/g DW) were also detected in all maturity stages. Cyanidin-3-O-sambubioside content increased as the calyx became more mature, whereas the delphinidin-3-O-sambubioside, ascorbic acid, and chlorogenic acid content declined towards calyx maturity. The antioxidant activity gradually increased as the calyx ripening progressed. However, the activities did not differ significantly between the stages ($p>0.05$). The high content of total phenolic, total anthocyanins, and free radical scavenging activity were detected at Stage 4 (28-30 DAA) in UKMR-2, suggesting that this stage is the most appropriate maturity stage for harvesting* H. sabdariffa *cv. UKMR-2 calyces compared to the other maturity stages.*

Keywords: Antioxidant; H. sabdariffa *cv. UKMR-2; maturity stages; phenolic content*

ABSTRAK

Perubahan metabolik bagi sebatian fenolik pada masa penuaian yang berbeza telah dikaji dalam Hibiscus sabdariffa *kv. UKMR-2 berdasarkan kaedah Kromatografi Cecair Berprestasi Tinggi menggunakan pengesan foto-diod (HPLC-PDA) dengan elusi kecerunan kepolaran. Aktiviti antioksidan juga ditentukan menggunakan asai penyah-radikal bebas 2,2-difenil-1-pikrilhidrazil (DPPH). Penanaman dilakukan di bawah keadaan terkawal dan penuaian kaliks dilakukan secara berasingan pada empat tahap kematangan. Kaliks rosel diekstrak dengan air secara sonikasi (50°C, 30 min). Analisis HPLC-PDA menunjukkan kehadiran dua sebatian antosianin iaitu delfinidin-3-O-sambubiosida (2.07-2.47 mg/g BK) dan sianidin-3-O-sambubiosida (0.55-1.01 mg/g BK). Selain itu, asid askorbik (3.34-9.88 mg/g BK), asid kafeik (0.08-0.09 mg/g BK) dan asid klorogenik (0.50-0.65 mg/g BK) juga dikesan dalam semua tahap kematangan. Kandungan sianidin-3-O-sambubiosida meningkat apabila kaliks menjadi lebih matang, sebaliknya kandungan delfinidin-3-O-sambubiosida, asid askorbik dan asid klorogenik menurun apabila kaliks mencapai kematangan. Aktiviti antioksidan menunjukkan peningkatan berterusan mengikut tahap kematangan. Walau bagaimanapun, ia tidak menunjukkan kesan signifikan antara tahap kematangan ($p>0.05$). Kandungan jumlah fenolik, jumlah antosianin dan aktiviti antioksidan yang tinggi dikesan pada Tahap 4 (28-30 DAA) bagi UKMR-2, mencadangkan tahap ini sebagai tahap kematangan untuk penuaian* H. sabdariffa *kv. UKMR-2 berbanding dengan peringkat kematangan yang lain.*

Kata kunci: Antioksidan; H. sabdariffa *kv. UKMR-2; kandungan fenolik; tahap kematangan*

INTRODUCTION

Hibiscus sabdariffa is well known in Malaysia as Roselle with a sour taste and reddish calyces. This subtropical plant is classified under the Family Malvaceae. It is an annual herbaceous shrub with a height of up to 2.5 m, tetraploid species characterized by smooth, cylindrical red stems, reddish veins and alternate 7.5 - 12.5 cm long green leaves (Husseina et al. 2010; Mohamed et al. 2007). Its habitat is variable, and it is relatively easy to grow and can be grown as part of a multi-cropping system (Da Costa Rocha et al. 2014; Eltayeib & Hamade 2014). The flowers are borne singly, usually yellowish and occur with dark red pigmentation at the centre. The calyx is red, consisting of five valves, each containing 3-4 kidney-shaped light brown seeds (Eltayeib & Hamade 2014; Mohamed et al. 2007). Roughly, the period spanning from the flowering stage for Roselle to complete maturation of the calyxes ranges from 30 to 40 days (Castro et al. 2004). According to Castro et al. (2004), the determination of calyx harvesting time is an important consideration to

produce optimum calyx yield and high quality in Roselle. Traditionally, the red fleshy calyxes are harvested before the seeds in the capsule have become completely dry and shatter. According to Copeland and McDonald (1995), the fleshy calyxes should be harvested according to the ripeness of the seeds, where the seeds' maximum dry mass is an indication of physiological maturity (PM). Similarly, McClaleb (1998) highlighted the importance of harvesting calyxes before the capsules become dry and exposed because it will cause deterioration to the quality of the calyxes.

Roselle plant, primarily the calyx, has long been used in folk medicine as a diuretic, mild laxative, digestive, antiseptic, sedative, emollient and treatment for cardiac and nerve diseases (Fasoyiro et al. 2005; Puro et al. 2014). Besides that, the red calyxes have been used for making jams, syrup, pudding, ice cream and beverages (Kouakou et al. 2015). Roselle calyces are rich in phenolic compounds and anthocyanins. The anthocyanins present in Roselle are essential for beneficial health effects associated with their antioxidant, antidiabetic, anti-hypertensive, anti-cancer agents, anticancer, cardio protective action and many other properties (Idris et al. 2012; Izatus Shima et al. 2017; Lislivia et al. 2017; Satirah et al. 2016). In addition to anthocyanins, Roselle calyces are also rich natural source of other chemical compounds including flavonoids, tannins, steroids, phenols, triterpenoids and alkaloids (Brahma et al. 2014; Ijeomah et al. 2012; Mungole & Chaturvedi 2011; Obouayeba et al. 2014). High-Performance Liquid Chromatography (HPLC) has been widely used in the identification and quantification of anthocyanins in the Roselle extract (Kouakou et al. 2015; Lislivia et al. 2017; Sukwattanasinit et al. 2007). Lislivia et al. (2017) and Sukwattanasinit et al. (2007) reported that delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside are two predominant anthocyanins that can be found in dried roselle calyces. It has also been reported that cyanidin-3-glucoside and delphinidin-3-glucoside as minor anthocyanins in *H. sabdariffa* dried calyces (Kouakou et al. 2015).

At present, various research on the phenolic compounds of Roselle has been attracting interest due to their increasing application in the food industry and their beneficial health gains. Research has shown that the content and distribution of phenolic phytochemicals might be affected by cultivar specificities, time of harvest, environmental factors, growing season and postharvest storage conditions (Bureau et al. 2009; Deshmukh et al. 2011). There are numerous references in the currently available literature to determine the effect of the maturity stage on the phenolic content of fruits and vegetables (Buta & Spaulding 1997; Goncalves et al. 2007; Miletić et al. 2012; Raffo et al. 2002; Ryu et al. 2016). For sweet cherries, the influence of ripeness due to the accumulation of anthocyanins has been analysed, and the findings have shown that the total levels of anthocyanins are considerably higher in ripe sweet cherries compared to partially ripe ones (Goncalves et al. 2007). According to Miletić et al. (2012), anthocyanins content in *Prunus domestica* L. (plum) had a significant effect on maturity, which indicates that the anthocyanins accumulation constantly occurs during fruit development.

Currently, there is scant information regarding the chemical differences in the Roselle calyxes between different maturity stages. Most current literature is focused on the phenolic contents of the ripe calyces. The metabolic changes in the phenolics content in Roselle calyces have not been correlated with the maturation stages using the chromatographic methods. Furthermore, several researchers have examined the antioxidant capacity of ripe *H. sabdariffa* extracts using different assays for analysis, but none have been carried out regarding the antioxidant capacities found in Roselle calyces at different maturation stages. In this study, the changes in different phenolics levels include the anthocyanin and antioxidant activity in *H. sabdariffa* var. UKMR-2 cultivated under controlled conditions had also been investigated at different stages of maturity.

Materials and Methods

Chemicals and Reagents

Gallic acid, ascorbic acid, chlorogenic acid, caffeic acid, Folin-Ciocalteu reagent, formic acid, methanol, sodium carbonate, potassium chloride, sodium acetate acquired from Merck (Darmstadt, Germany). Delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside purchased from Extrasynthese (France). Acetonitrile HPLC grade obtained from Fisher Scientific (USA). 2,2-diphenyl-1-picrylhydrazyl (DPPH) for the antioxidant assay purchased from Sigma Aldrich (USA).

Plant Material and Treatment

The plant cultivation of *H. sabdariffa* var. UKMR-2 was conducted at *Kompleks Rumah Tumbuhan* greenhouse, Universiti Kebangsaan Malaysia (UKM), Bangi from November 2016 until March 2017 where the seeds were obtained. The seeds were initially sown in trays filled with organic soil. After 2 weeks of sowing, the seedlings (6-8 cm in height) were selected and transplanted into polyethylene bags (20 × 20 cm) containing 20 kg of soil mixture (2:1:1) of topsoil, organic matter, and sand. All the plants received similar treatment per polyethylene bags including fertilization (NPK and organic matter), irrigation with 20% partial root drying (1.72 L/plant/day) and pesticide sprays. Irrigation treatment was done based on the method proposed by Nur Amirah et al. (2015) while weeds and insect pests were manually controlled. The UKMR-2 flower bud growth began at day 40 after the plants were transplanted, and the flowers were tagged at the beginning of the anthesis to obtain data at different maturity ages of the calyces.

EXTRACT PREPARATION

Calyces were collected at four different stages of maturity from five individual plants as biological replicates. The UKMR-2 maturation stages (Figure 1) were classified as follows: Stage 1 (7-9 day after anthesis, DAA), Stage 2 (14-16 DAA), Stage 3 (21-23 DAA), and Stage 4 (28-30 DAA). The collection of the calyces started at the end of January 2017 until March 2017. The fresh calyces were placed in a Ziplock bag, which was set in a cooler for transportation to the laboratory. Each calyx is washed with water, drained, followed by seeds removal and air dried at room temperature for three days until it achieved constant weight. The dried calyces were ground with mortar and pestle under liquid nitrogen, packed in a Ziplock bag and stored in a refrigerator at -20°C until they were subjected to extraction. The UKMR-2 calyces extract was prepared according to the method described by Chumsri et al. (2008). Briefly, the dried calyces were extracted using water at a ratio of 1:10. The extraction was conducted in a water bath at a constant temperature of 50°C for 30 min. The extracts were filtered using Whatman No.1 filter paper, and filtrates were dried using Alpha 1-2 LDplus freeze dryer (Martin Christ, Germany).

DETERMINATION OF THE TOTAL PHENOLIC AND TOTAL ANTHOCYANIN CONTENTS

The total phenolic contents (expressed as mg gallic acid equivalent (mg GAE)/g of dried weight) was determined using Folin-Ciocalteu assay as described by Waterhouse (2002), while the total anthocyanins content (expressed as mg cyanidin-3-glycoside equivalent per gram of dried weight) was measured using the pH-differential method with two buffer systems as described by Giusti and Wrolstad (2001).

DETERMINATION OF DPPH RADICAL SCAVENGING ACTIVITY

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity was measured according to the method described by Kouakou et al. (2015). Briefly, 100 µL from different concentrations of water extract solutions were added to 100 µL DPPH in methanol (0.1 mM) in a 96-well plate. The mixture was shaken and left to stand for 30 min in a dark environment. The absorbance was measured using the Epoch Microplate Spectrophotometer (BioTek, USA) at 517 nm. Ascorbic acid was used as a standard reference and determined using the same procedure. Each sample was measured in triplicate and averaged. The radical scavenging activity or inhibition percentage were calculated according to (1).

$$\% \text{ RSA} = (A_0 - A_1) / A_0 \times 100 \quad (1)$$

where A_0 is the absorbance of the control (DPPH without an extract solution); and A_1 is the absorbance of the sample (DPPH with sample).

HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY (HPLC) ANALYSIS

HPLC analyses were performed using the method described by Siti Aishah et al. (2019). Ten mg of freeze-dried extracts from the calyx were dissolved with 1 mL of 0.1% formic acid in water. The samples were sonicated for 5 min and filtered through a 0.45 µm PTFE membrane syringe filter (Gema Medical, Spain). Quantitative analysis was performed on the HPLC Waters e2695 separation module equipped with a degasser, an auto-sampler automatic injector and a Waters 2998 Photodiode Array Detector at multiple wavelengths. HPLC experiments were conducted using Purospher STAR RP-18e LichroCART column (250 mm × 4.6 mm × 5 µm). All the compounds were determined by standard reference calibration curves and were expressed as mg per g dried weight. The linear correlation co-efficient was > 0.996 for each compound. HPLC-PDA separation and identification were also carried out at a flow rate of 1 mL/min, injection volume 30 µL and 30°C column oven temperature. 0.1% formic acid in water and 0.1% formic acid in acetonitrile were employed as mobile phases A and B, respectively, in gradient elution as follows: 10 - 15% B (0-15 min), 15 - 90% B (15-25 min), 90 - 10% B (25-30 min) and 10% B (30-35 min). The chromatograms were monitored at 265, 320 and 520 nm.

STATISTICAL ANALYSIS

The data were expressed as a mean ± standard deviation of the three parallel measurements. Statistical calculations were carried out using Statistical Package for Social Science (SPSS) for Windows version 25.0 software (SPSS

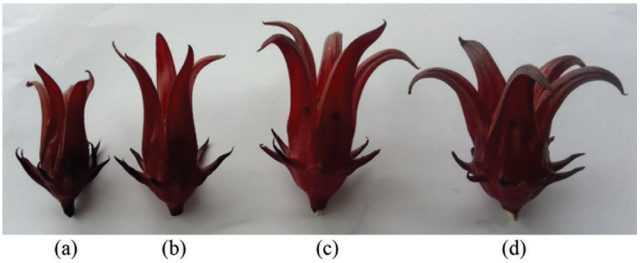


FIGURE 1. Profile of roselle calyx maturation stages: (a) Stage 1 (7-9 DAA); (b) Stage 2 (14-16 DAA); (c) Stage 3 (21-23 DAA); (d) Stage 4 (28-30 DAA)

Inc., Chicago, USA). Analysis of variance (ANOVA) was performed to determine the significant differences between values, defined at the 5% level ($p<0.05$). The correlation between the total phenolics content and antioxidant potential was calculated using Pearson correlation. The Tukey-Kramer test was used to compare the differences between the means.

Results and Discussion

Chemical Analysis

These are the findings from continuous study of the phenolic content of *Hibiscus sabdariffa* var. UKMR-2 calyces at different maturation stages. The analysis result of the total phenolic and anthocyanin contents was obtained from our previous investigation (Figure 2) (Siti Aishah et al. 2017).

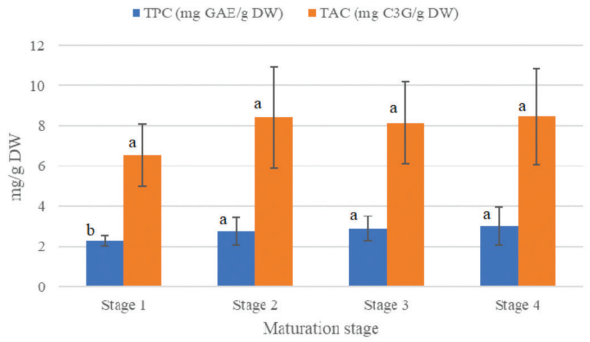


FIGURE 2. Changes in the TAC and TPC of Roselle calyces during different stages of development. The vertical bar represents the standard deviation. Different letters between the maturation stages bar indicate statistical difference using the Tukey-Kramer test ($p<0.05$)

The TPC and TAC show significantly increasing values as the development stages progress. The overall mean for TPC and TAC in Roselle calyces are 2.74 ± 0.63 mg GAE/g DW and 7.89 ± 2.53 mg C3G/g DW, respectively. The results also show that Stage 4 (28-30 DAA) produces the highest TPC and TAC content with 3.02 ± 0.95 mg GAE/g DW and 8.47 ± 3.38 mg C3G/g DW, respectively. These findings are similar to the total phenolic and total anthocyanin content observed by Ryu et al. (2016) in blackberry at different maturation stages. The heightenings of both TAC and TPC indicate that the accumulation of the phenolic compounds invariably occurs during the calyx development and ripening stages.

DPPH Radical Scavenging Activity

In this study, the antioxidant activities from different maturation stage were investigated using the DPPH scavenging assay or inhibition percentage and compared with ascorbic acid as a reference standard. The sequence for DPPH free radical scavenging activity of the Roselle extract is shown in Figure 3: Stage 4 > Stage 3 > Stage 2 > Stage 1. The DPPH free radical scavenging activity of the Roselle calyx shows it gradually increases as the calyx becomes more mature. Stage 4 shows the highest DPPH inhibition percentage at 56.6%, followed by Stage 3 at 54.7%, Stage 2 at 51.1% and Stage 1 at 47.7% at 0.2 mg/mL. The percentage of inhibition for ascorbic acid is 84.8% at 0.2 mg/mL. However, the antioxidant activities of the extracts from all the maturation stages do not differ significantly with each other ($p>0.05$). The TPC is closely related to the antioxidant activity. Correlation analysis was performed to examine the link between the antioxidant activities and TPC. The Pearson correlation coefficient (based on the TPC average quantified values and DPPH radical scavenging activity) is positively correlated ($p< 0.01$) with $R^2 = 0.545$. A strong correlation has also been reported in various studies on Roselle (Kouakou et al. 2015; Sharara 2017; Sukwattanasinit et al. 2007). The total phenolics content shows increasing progress throughout the ripening period. Moreover, the antioxidant activity observed in the extracts increases throughout the ripening process, suggesting that the phenolic content might contribute to the antioxidant activity in the Roselle calyxes.

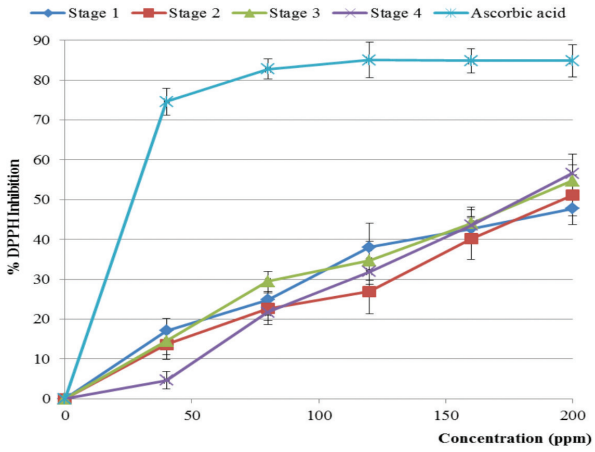


FIGURE 3. DPPH radical scavenging activities of roselle crude extracts. Ascorbic acid is used as a reference standard. The data represent the mean of three replicates ± of the standard deviation. The vertical bar represents the standard deviation

Chromatographic Profiling

Measurements of the phenolic contents, especially anthocyanins, have long been utilized as an indicator to determine the quality and processing of Roselle products. In this study, the phenolic compound was identified and quantified based on their retention times, which were compared with the standards reference. The representative HPLC chromatograms are given in Figures 4 and 5. Peaks are identified as: 1. Delphinidin-3-*O*-sambubioside; 2. Cyanidin-3-*O*-sambubioside; 3. Ascorbic acid; 4. Chlorogenic acid and 5. Caffeic acid.

Generally, two predominant anthocyanins are detected in all the stages with different concentrations in UKMR-2 calyx has a retention time of 6.15 min and 9.10 min. The peaks have been identified as delphinidin-3-*O*-sambubioside (1) and cyanidin-3-*O*-sambubioside (2). The overall range for anthocyanins in UKMR-2 calyx is 0.554 to 2.470 mg/g DW. Between the two anthocyanins, delphinidin-3-*O*-sambubioside shows the highest content in all the stages of calyx development. Cyanidin-3-*O*-sambubioside progressively increases up to 45.5% in content throughout the ripening stage. Compared to delphinidin-3-*O*-sambubioside, the content increases from Stage 1 to Stage 2 until it reaches 16.2% and gradually declines as the calyx becomes more mature. The presence of delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside in the calyx concur with previously reported findings of other Roselle varieties around the world (Bernal et al. 2016; Jafarian et al. 2014; Kouakou et al. 2015; Lislivia et al. 2017).

In addition, the analysis shows that only three non-anthocyanin compounds can be identified based on the standard reference materials. Peaks are identified as ascorbic acid (3), chlorogenic acid (4) and caffeic acid (5) with a retention time of 5.08, 11.55 and 15.52 min, respectively. All the compositions detected in Roselle calyces are strongly influenced during the ripening stages ($p < 0.05$). However, they show different trends: the highest ascorbic acid and chlorogenic acid content measured in the S2 are 9.883 and 0.654 mg/g DW, respectively, while the highest caffeic acid value recorded at the S3 stage is 0.090 mg/g DW. The highest level of ascorbic acid is found in the earliest stage of the calyx development, and the quantities decline rapidly during fruit ripening. The declines of the ascorbic acid and chlorogenic acid levels during the fruit ripening is paralleled with the decline in delphinidin-3-*O*-sambubioside levels. Caffeic acid is present at lower concentrations and has similar value in all the stages but is also noticeably increase during ripening.

According to Khafaga and Koch (1980), the acid content of the calyces increases during the growth but decreases when it reaches maturity or when it ripens. These results correspond with several studies which reported reductions of the phenolic compounds during ripening (Buta & Spaulding 1997; Macheix et al. 1990; Raffo et al. 2002). Macheix et al. (1990) study on plum reported that phenolic concentration is generally higher in immature fruits. Similarly, Buta and Spaulding (1997) reported that the higher levels of chlorogenic acid were found at the early stage of tomato fruit development and declined rapidly during fruit ripening. According to Miletic et al. (2012), fruit maturity development could be associated with biochemical changes that will modify the taste, colour, texture, and other quality fruit traits. In this study, it is shown that Roselle calyx harvested at different maturity stages has marked effects on the phenolic content. Only cyanidin-3-*O*-sambubioside and ascorbic acid show significant differences in the content throughout the calyx development stages (Table 1). All

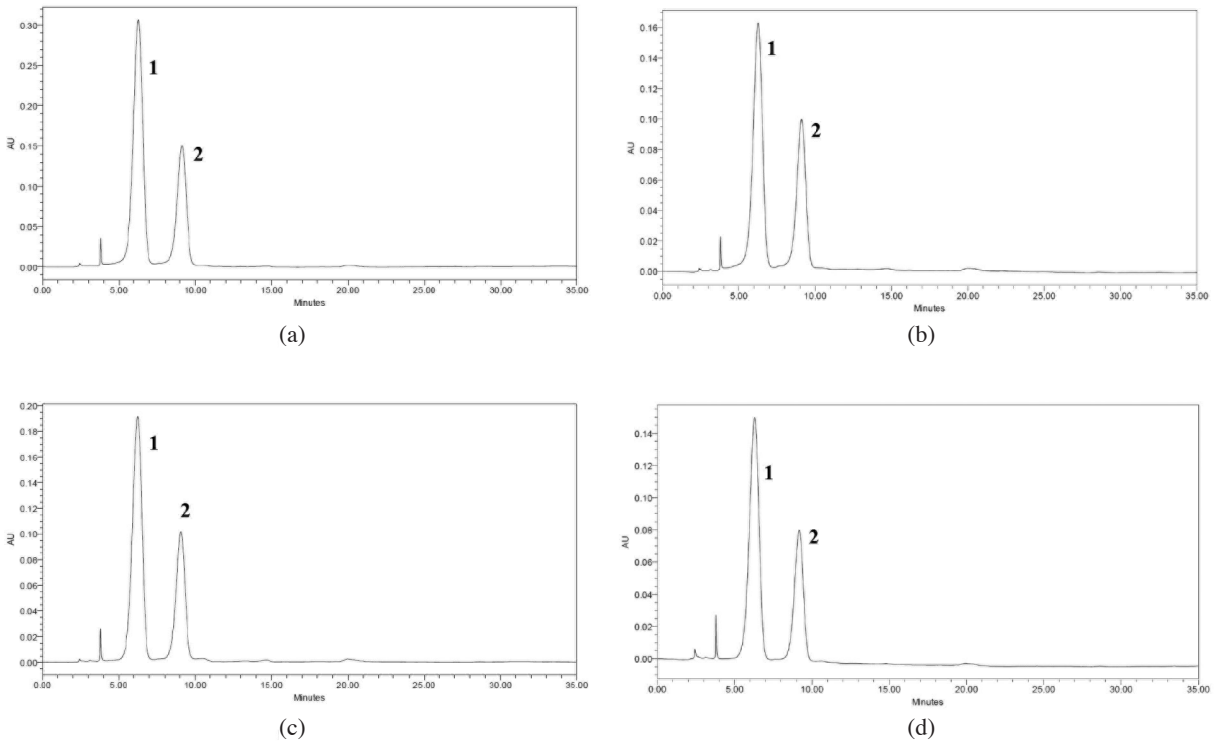
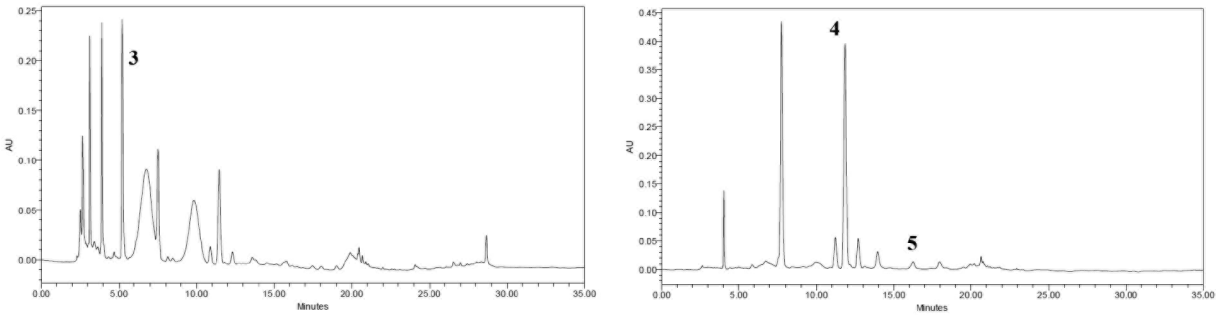


FIGURE 4. HPLC profile of anthocyanin content in Roselle calyx at different stages of development: (a) Stage 1 (7-9 DAA); (b) Stage 2 (14-16 DAA); (c) Stage 3 (21-23 DAA); (d) Stage 4 (28-30 DAA). 1. Delphinidin-3-*O*-sambubioside; 2. Cyanidin-3-*O*-sambubioside



(a) (b)

FIGURE 5. HPLC profile of phenolic content in Roselle calyx at (a) 265 nm and (b) 320 nm. 3. Ascorbic acid; 4. Chlorogenic acid; 5. Caffeic acid

TABLE 1. Changes in phenolic and ascorbic acid contents of calyx development stages for *H. sabdariffa* var. UKMR-2

Compound	(mg/g DW)			
	Stage 1	Stage 2	Stage 3	Stage 4
Delphinidin 3-*O*-sambubioside	2.069 ± 0.481 [a]	2.470 ± 0.847 [a]	2.305 ± 0.606 [a]	2.211 ± 0.724 [a]
Cyanidin 3-*O*-sambubioside	0.554 ± 0.078 [c]	0.732 ± 0.180 [b, c]	0.831 ± 0.134 [a, b]	1.011 ± 0.423 [a]
Ascorbic acid	4.878 ± 3.116 [b]	9.883± 3.903 [a]	4.030 ± 1.862 [b]	3.339 ± 1.826 [b]
Chlorogenic acid	0.495 ± 0.095 [a]	0.654 ± 0.233 [a]	0.640 ± 0.133 [a]	0.601 ± 0.214 [a]
Caffeic acid	0.077 ± 0.004 [a]	0.085 ± 0.014 [a]	0.090 ± 0.016 [a]	0.088 ± 0.018 [a]

The values represent the mean of three replicates ± standard deviation. Different letters (a – c) in the same rows mean statistically significant differences for the individual phenolic compound at different maturity stages by Tukey-Kramer test ($p<0.01$). DW = dry weight

the five compounds are detectable in all stages of the calyx's development.

Hibiscus sabdariffa var. UKMR-2 which was cultivated by Universiti Kebangsaan Malaysia (UKM) in 2009, has special characteristics such as the ability to produce a high yield of calyces per plant, has a shorter life cycle, higher lodging resistance compared to their parent variety 'Arab' and other local varieties (Osman et al. 2011). The colour of UKMR-2's calyces is deep red compared to their parental variety 'Arab' which has a dark red colour. In general, colour is a simple and important indicator to determine fruit maturity and quality. Based on the colour observed by the naked eyes, UKMR-2 calyces have been transformed from bright red to deep red when they reach the ripened stage. It appears that bright red calyces show higher delphinidin 3-*O*-sambubioside, while the deep red ripened calyces contain higher cyanidin 3-*O*-sambubioside. Hibiscus anthocyanins have been identified as having delphinidin-3-sambubioside (70% of the anthocyanins) and cyanidin-3-sambubioside as their major pigments (Amor & Allaf 2009). Delphinidin and cyanidin are known to contribute to the bioactive function because they have higher bioavailability compared to the other anthocyanins (Ichiyanagi et al. 2006).

Kouakou et al. (2015) reported that delphinidin-3-*O*-sambubioside & cyanidin-3-*O*-sambubioside contents in *H. sabdariffa* L. originated from Côte d'Ivoire are 21.38 and 17.11 mg/g DW, respectively, which were 9 - 17 times greater than UKMR-2. These results showed that the content and distribution of the anthocyanins in Roselle are believed to be influenced by the type of cultivation, the degree of fruit maturation, postharvest storage condition, environmental conditions, genetic factors and the variety of the plant (Bureau et al. 2009; Deshmukh et al. 2011). It is well known that the degradation of anthocyanins which is highly reactive and relatively unstable might happen during processing and longer storage time (Alighourchi & Barzegar 2009). Meanwhile, the phenolic acid concentration will decrease during fruit ripening (Manach et al. 2004). The increase in the acid content in the early stages of developing Roselle calyces may be attributed to the increased anabolic activities, while the acid contents decline as they progress to the ripened stages and this might be attributed to the increased rate of catabolism more than anabolism (Hassanein et al. 2005). Nevertheless, Raffo et al. (2002) reported that not all biologically active compounds increased during the ripening process, but the accumulation depended on different biosynthetic pathways and mechanisms of metabolic control.

According to Hassanein et al. (2005), the most suitable harvesting age for *H. sabdariffa* L. calyces originated from Egypt is around 50-60 days after flower, due to the highest number of anthocyanins and acid contents at this age. Similar results were obtained by Fakir et al. (2012) who reported that the size of the capsule, epicalyx, calyx and ovary of *H. sabdariffa* L. var. *sabdariffa* originated from Bangladesh became maximum around 45 DAA, which correlated with good protein contents (9.09%) and calyces deep red colour. In addition, Haryati et al. (2018) stated that the appropriate harvest time for *H. sabdariffa* L. originated from Indonesia was at the age of 33 DAA where

the average weight of calyx and seeds reached 11.9 g and 2.3 g per flower, respectively.

CONCLUSION

This study presents the data on the phenolic compositions and antioxidant activity of *H. sabdariffa* var. UKMR-2 at different maturity stages. It can be concluded that the composition of phenolics and ascorbic acid content of roselle calyces are strongly influenced by the ripening stages. Although the antioxidant activity increases with maturity, it does not differ significantly within the stages ($p>0.05$). Although Stage 2 produces three compounds with a higher concentration, our study suggests that Stage 4 (28-30 DAA) is the most appropriate harvesting time for UKMR-2 due to the high total phenolic and total anthocyanin contents as well as the free radical scavenging activity in calyces, which may provide benefit to human health.

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Siti Aishah Mohd Ali* & Jalifah Latip
Centre for Advanced Materials and Renewable Resources
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 UKM Bangi, Selangor Darul Ehsan
Malaysia

Siti Aishah Mohd Ali*
Faculty of Science and Natural Resources
Universiti Malaysia Sabah, Jalan UMS
88400 Kota Kinabalu, Sabah
Malaysia

Che Radziah Che Mohd Zain
School of Biosciences and Biotechnology
Faculty of Science and Technology
Universiti Kebangsaan Malaysia
43600 UKM Bangi, Selangor Darul Ehsan
Malaysia

*Corresponding author; email: jalifah@ukm.edu.my

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