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QUANTITATIVE ANALYSIS OF PHENOLICS CONTENT IN TWO ROSELLE VARIETIES (*Hibiscus sabdariffa*) BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

(Analisis Kuantitatif bagi Sebatian Fenolik dalam Dua Varieti Roselle (*Hibiscus sabdariffa*) menggunakan Kromatografi Cecair Berprestasi Tinggi)

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Abstract

Phenolics content of two roselle varieties, *H. sabdariffa* var. UKMR-2 and *H. sabdariffa* var. UMKL-1 was determined based on high performance liquid chromatography method using a photodiode array detector (HPLC-PDA) with gradient elution. Roselle calyces were extracted with water *via* sonication (50 °C, 30 minutes). The chromatographic separation was carried out on Purospher STAR RP-18e LichroCART column (250 mm × 4.6 mm × 5 µm) and detection was carried out at three different wavelengths (265, 320 and 520 nm). The developed HPLC method provided high percentage of recovery (95 - 102%), good linearity at $R^2 > 0.99$, low limit of detection (LOD) and low limit of quantitation (LOQ). Two predominant anthocyanins were detected by HPLC-PDA in both *H. sabdariffa* varieties and were identified as delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside. In addition, chlorogenic acid, caffeic acid and ascorbic acid were also detected. High content of phenolics and ascorbic acid were detected in UKMR-2 as compared to UMKL-1, suggested this variety have high potential for commercial cultivation.

Keywords: anthocyanins, *Hibiscus sabdariffa*, HPLC-PDA

Abstrak

Kandungan fenolik bagi dua varieti rosel, *H. sabdariffa* var. UKMR-2 dan *H. sabdariffa* var. UMKL-1 ditentukan berdasarkan kaedah kromatografi cecair berprestasi tinggi menggunakan pengesan foto-diod (KCPT-PFD) dengan elusi kecerunan kekutuban. Kaliks rosel diekstrak dengan air secara sonikasi (50 °C, 30 minit). Pemisahan kromatografi dilakukan menggunakan turus Purospher STAR RP-18e LichroCART (250 mm × 4.6 mm × 5 µm) dan pengesanan dilakukan pada tiga panjang gelombang yang berbeza (265, 320 dan 520 nm). Kaedah KCPT yang dibangunkan mempunyai peratusan dapatan semula yang tinggi (95 - 102%), lineariti yang baik pada $R^2 > 0.99$ serta had pengesanan (LOD) dan kuantifikasi (LOQ) yang rendah. Dua sebatian antosianin dikesan oleh KCPT-PFD dalam kedua-dua varieti *H. sabdariffa* dan dikenal pasti sebagai delfinidin-3-*O*-sambubiosida dan sianidin-3-*O*-sambubiosida. Selain itu, asid klorogenik, asid kafeik dan asid askorbik juga berjaya dikesan. UKMR-2 dikesan mengandungi sebatian fenolik dan asid askorbik yang tinggi berbanding UMKL-1, mencadangkan varieti ini mempunyai potensi yang tinggi sebagai tanaman komersil.

Kata kunci: antosianin, *Hibiscus sabdariffa*, KCPT-PFD

Introduction

The species *Hibiscus sabdariffa*, popularly known as 'Asam Paya' or 'Asam Susur' in Malaysia, is a member of the Malvaceae family. This plant exhibits anti-oxidant, anti-cancer, anti-hypertensive, anti-hyperlipidemic, hepatoprotective and many other properties [1], being commonly consumed as beverages, jams and syrup, and used in folk medicine as diuretic, mild laxative, digestive, antiseptic, sedative, emollient of cardiac and nerve diseases [2, 3]. Roselle is rich in various bioactive compounds such as organic and phenolic acids, flavonoids, tannins and anthocyanins such as cyanidin-3-*O*-sambubioside and delphinidin-3-*O*-sambubioside, which are also responsible for the bright red colouration and antioxidant properties of calyces [4, 5]. *H. sabdariffa* var. UMKL-1 (also known as *Terengganu* variety) was launched in the early 1990s by Universiti Malaya (UM) for commercial cultivation purpose due to its high quality and calyx yield. In 2009, a new Malaysia roselle (mutant) var. UKMR-2 was released by Universiti Kebangsaan Malaysia (UKM). The new variety has special characteristics compared to their parent variety 'Arab' and other local varieties [6]. In general, var. UKMR-2 has a shorter life cycle, higher lodging resistance and produce higher yield of calyces per plant. The colour of roselle var. UKMR-2's calyces has deeper red color compared to var. UMKL-1 [6]. In general, red anthocyanin pigment colour is the reliable indicator to determine the maturity and quality of fruit.

Phenolics content have been widely investigated in various plants using different chromatographic techniques due to their nutritional potential and therapeutic value. Chemical verification using high performance liquid chromatography (HPLC) is the most popular and reliable technique among all chromatographic separation techniques for the separation of bioactive compound. HPLC has been reported in the identification and quantification of anthocyanins in roselle extract [7-8]. Sukwattanasinit et al. [7] and Bernal et al. [9] reported that delphinidin-3-sambubioside and cyanidin-3-sambubioside were two major anthocyanin compounds found in roselle dried calyces. Besides that, other anthocyanins (cyanidin-3-glucoside, delphinidin-3-glucoside), phenolic acid (chlorogenic acid, protocatechuic acid, hydroxycitric acid) and flavonoids (quercetin, kaempferol, gossypetin, hibiscetin, luteolin and its glycoside) have also been previously reported [5, 8-10].

Although the chemical composition of plants is largely determined by the genetic characteristics of the species, several factors can coordinate or change the rate of production such as various environmental factors [11], time collection [12] and the employment of different extraction methods [13]. Despite of the bioactive compounds and increasing demand of *H. sabdariffa* calyxes worldwide, little is known about chemical components such as phenolic constituents in different Malaysia roselle varieties. Hence, this paper aims to develop a simple analytical methodology to simultaneously identify and quantify phenolic content present in the calyx extracts of *H. sabdariffa* var. UKMR-2 and *H. sabdariffa* var. UMKL-1. The compound identification and quantification were carried out using high performance liquid chromatography with photodiode array detection (HPLC-PDA) with gradient elution.

Materials and Methods

Chemicals and standards

Formic acid and acetonitrile HPLC grade used for preparing mobile phases were purchased from Merck (Darmstadt, Germany) and Fisher Scientific (USA), respectively. The standard, ascorbic acid, chlorogenic acid, caffeic acid, delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside were purchased from Merck (Darmstadt, Germany) and Extrasynthese (Genay Cedex, France). Acetonitrile HPLC grade was degassed in an ultrasonic bath (Branson, USA) before using. As for vortex mixer, G560E Vortex-Genie 2 (Scientific Industries, Bohemia, NY, USA) was used. Distilled water was purified and deionized by EVOQua water system (Water Technologies, Fahrenberg, Germany).

Sample collection and preparation

Roselle var. UKMR-2 was collected from *Kompleks Rumah Tumbuhan* greenhouse, Universiti Kebangsaan Malaysia (UKM), Bangi at the end of December 2017, while for roselle var. UMKL-1 was purchased from HERBagus Sdn. Bhd. in Kepala Batas, Penang. The fresh calyces from each variety were washed with water, drained, followed by seed removal then air dried at room temperature. Dry calyces were ground into powder and the extraction was prepared according to the method described by Chumsri et al. [14]. Briefly, the extraction of dried calyces with water in a ratio of 1:10 conducted in a water bath at a constant temperature of 50 °C for 30 minutes.

Each extract was filtered with a piece of clean, sterile coarse cloth then filtrates were dried using Alpha 1-2 LDplus freeze dryer (Martin Christ, Germany). 10 mg of freeze-dried extracts from each variety were dissolved in 1 mL of 0.1% formic acid in water. Samples were sonicated for 5 minutes and filtered through a 0.45 µm PTFE membrane syringe filter (Gema Medical, Spain) and directly injected into the HPLC system.

High performance liquid chromatography with photodiode array detector

HPLC analyses were performed on the HPLC Waters e2695 separation module equipped with a degasser, an auto-sampler automatic injector and 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). HPLC-PDA separation and identification were 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 minutes), 15 - 90% B (15-25 minutes), 90-10 % B (25-30 minutes) and 10% B (30-35 minutes). The chromatograms were monitored at 265, 320 and 520 nm. All compounds were determined by standard reference calibration curves and were expressed as mg per g dried weight. Linear correlation co-efficient were > 0.996 for each compound.

Method validation

The method was validated according to the International Conference on Harmonization, ICH guidelines [15]. The stock solution (1 mg/mL) of each standard (delphinidin-3-*O*-sambubioside, cyanidin-3-*O*-sambubioside, chlorogenic acid, caffeic acid and ascorbic acid) was diluted with 0.1% formic acid in water. Stock dilution was prepared using the same solvent into eight different concentrations ranges between 4.17 and 150 µg/mL. These standard solutions are kept at 4 °C before being analysed. The following validation characteristics were evaluated: Linearity, precision, accuracy and the limits of detection and quantification (LOD and LOQ). Linearity was determined by injecting 30 µL of the standard mixture in eight concentrations ranges from 4.17 to 150 µg/mL in triplicate. The calibration curves were obtained for each individual compound by plotting the peak area versus concentration. Regression analysis was performed in order to determine the linearity (R^2) of the calibration graphs. Precision was performed according to ICH guidelines that included repeatability and intermediate precision.

For repeatability (intra‐day assay precision), three concentrations of the standard compounds (25, 50 and 100 µg/mL) were injected in three replicates during the same day and under the same experimental conditions. The intermediate precision was performed by the same analyst using the same concentrations of marker compounds and injected in three different days in triplicates. The precision method was reported as a percentage of relative standard deviation (% RSD) of peak concentration and retention time. Limit of detection (LOD) and limit of quantification (LOQ) were calculated based on signal to noise (S/N) ratio through the slope and standard deviation (SD) method using the following formula; LOD = (3.3 × σ) / s and LOQ = (10 × σ) / s, where σ = SD of blank response and s = slope of the linear regression equations. The accuracy of an analytical method expresses the nearness between the expected value and the experimental value. The accuracy was analysed by calculating the average percentage of recoveries (% Recovery) for each standard at three different concentrations (25, 50 and 100 µg/mL). All standard solutions were carefully prepared and analysed (n = 3) by the proposed method in three non-consecutive days. The same solutions were used to calculate the precision. The recovery percentage of the experiment were analysed using formula in equation 1:

$$\% \text{ Recovery} = (\text{Recovered concentration} / \text{Injected concentration}) \times 100 \quad (1)$$

Results and Discussion

HPLC validation method

Validation method provides documented evidence, and a high degree of assurance that an analytical method employed for a specific test is suitable for its intended use. The regression equation with correlation equation (R^2), LOD and LOQ were showed in Table 1. All compounds showed good linearity with correlation equation (R^2) range from 0.9951 to 0.9992 and % RSD of < 2.0%. According to Anvisa [16], the minimum acceptable correlation coefficient is 0.990. The LOD was < 0.46 µg/mL and LOQ was < 1.39 µg/mL. LOD and LOQ values in this method showed good sensitivity that can be detected and quantified at a very low concentration (< 1.5 µg/mL).

Table 1. Linearity, correlation coefficient (R^2), LOD and LOQ of compounds

Compounds	Linearity Range (µg/mL)	Regression Equation	R^2 (*n*=3)	LOD (µg/mL)	LOQ (µg/mL)
Delphinidin 3-*O*-sambubioside	6.26 - 150	y = 36342x - 185574	0.9976	0.22	0.67
Cyanidin 3-*O*-sambubioside	6.26 - 150	y = 38423x - 162923	0.9951	0.46	1.39
Chlorogenic acid	4.17 - 100	y = 69202x - 89921	0.9991	0.04	0.13
Caffeic acid	4.17 - 100	y = 41593x - 13799	0.9992	0.08	0.26
Ascorbic acid	4.17 - 100	y = 3483.3x - 5052.3	0.9984	0.15	0.47

y = peak area; x = concentration (µg/mL); LOD = Limit of Detection; LOQ = Limit of Quantification; R^2 = Correlation equation

The RSD values for repeatability (intra‑day precision) were within the range of 0.21 - 1.83% for peak concentration and 0.01 - 0.46% for retention time. The RSD values for inter-day were in the range of 0.21 - 1.75% for peak concentration and 0.04 - 0.49% for retention time. These values for both intra-day and inter-day analyses were within the prescribed limits of the ICH guidelines (RSD < 2%). It is therefore the proposed method is precise and reproducible. Tables 2 and 3 showed the average percentage RSD values and average percentage recovery ± SD of the reference compounds. The results also showed a good accuracy between the experimental and theoretical values, which indicated the method is reliable and reproducible for quantitative determination of five compounds in roselle calyx extracts. Several HPLC quantification methods have been reported in roselle extracts [7-9, 17-18]. However, most of these methods are not properly validated. Moreover, the methods are either time consuming or require complex mobile phase, which will limit their application. Therefore, we herein presented an improved method for qualitative and quantitative evaluation of phenolic compounds and ascorbic acid with shorter run time, enhanced in reliability, efficiency and reproducibility.

Table 2. Repeatability analysis (intra‑day precision) of compounds

Compounds	Concentration (µg/mL)	Repeatability (Intra-Day Precision) (*n* = 3)			
		Mean ± SD (µg/mL)	% RSD	RT ± SD	% RSD
Delphinidin 3-*O*-sambubioside	25	23.98 ± 0.11	0.46	6.17 ± 0.03	0.42
	50	48.11 ± 0.20	0.42	6.13 ± 0.03	0.45
	100	100.46 ± 0.27	0.27	6.06 ± 0.02	0.34
Cyanidin 3-*O*-sambubioside	25	24.96 ± 0.46	1.83	9.10 ± 0.04	0.46
	50	47.65 ± 0.10	0.21	9.06 ± 0.04	0.46
	100	101.00 ± 1.27	1.26	9.00 ± 0.03	0.29
Chlorogenic acid	25	25.16 ± 0.06	0.25	11.57 ± 0.02	0.15
	50	48.81 ± 0.11	0.23	11.57 ± 0.04	0.33
	100	100.98 ± 0.22	0.22	11.57 ± 0.01	0.05
Caffeic acid	25	25.94 ± 0.14	0.53	15.55 ± 0.02	0.12
	50	47.17 ± 0.28	0.60	15.56 ± 0.04	0.28
	100	100.68 ± 0.22	0.22	15.56 ± 0.01	0.04
Ascorbic acid	25	24.09 ± 0.42	1.75	5.09 ± 0.00	0.05
	50	50.61 ± 0.68	1.35	5.08 ± 0.00	0.05
	100	101.76 ± 0.96	0.95	5.08 ± 0.00	0.01

SD=Standard deviation; % RSD=Percentage of relative standard deviation; RT=Retention time

Table 3. Accuracy and intermediate precision (inter‑day precision) data for the developed method

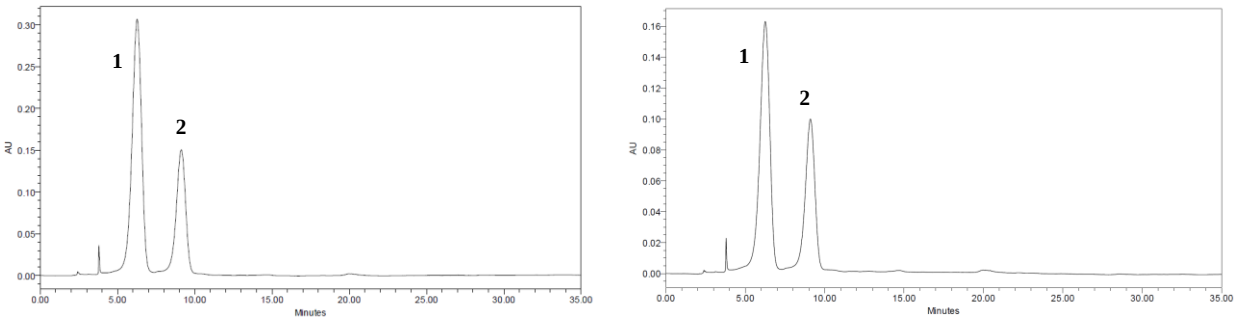
Compounds	Concentration (µg/mL)	Repeatability (Inter-Day Precision) (*n* = 3)				% Mean Recovery
		Mean ± SD (µg/mL)	% RSD	RT ± SD	% RSD	
Delphinidin 3-*O*-sambubioside	25	23.81 ± 0.17	0.70	6.32 ± 0.02	0.26	95.3
	50	48.08 ± 0.24	0.51	6.30 ± 0.03	0.49	96.2
	100	102.21 ± 1.38	1.35	6.29 ± 0.02	0.33	102.0
Cyanidin 3-*O*-sambubioside	25	25.22 ± 0.36	1.43	9.19 ± 0.02	0.20	100.9
	50	47.50 ± 0.26	0.54	9.17 ± 0.03	0.37	95.0
	100	101.67 ± 0.82	0.81	9.14 ± 0.03	0.30	101.7
Chlorogenic acid	25	25.37 ± 0.12	0.46	11.65 ± 0.02	0.19	101.5
	50	48.87 ± 0.11	0.23	11.65 ± 0.04	0.33	97.7
	100	101.02 ± 0.25	0.25	11.63 ± 0.02	0.19	101.0
Caffeic acid	25	26.01 ± 0.11	0.44	15.62 ± 0.02	0.11	104.0
	50	47.23 ± 0.17	0.36	15.61 ± 0.04	0.24	94.5
	100	100.72 ± 0.21	0.21	15.60 ± 0.02	0.13	100.7
Ascorbic acid	25	23.80 ± 0.12	0.51	5.10 ± 0.00	0.06	95.2
	50	50.04 ± 0.70	1.40	5.10 ± 0.01	0.14	100.1
	100	99.78 ± 1.74	1.75	5.09 ± 0.00	0.04	99.8

SD=Standard deviation; % RSD=Percentage of relative standard deviation; RT=Retention time

Identification and quantification of phenolic contents

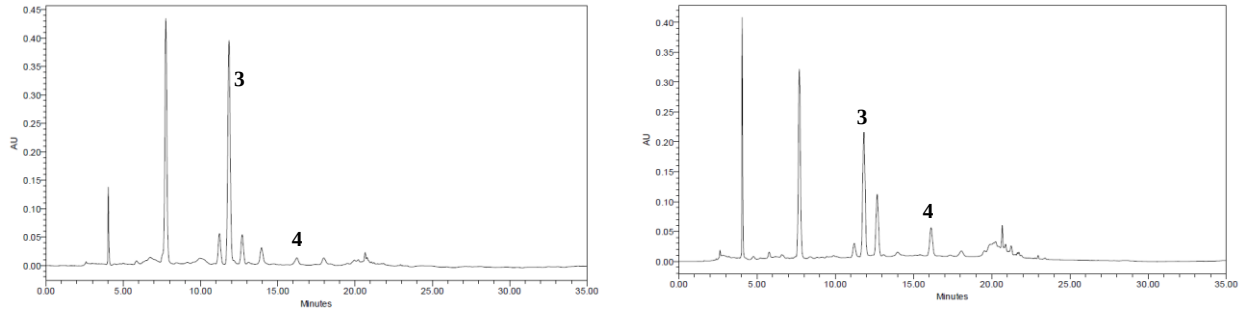
Measurements of phenolic content, especially on anthocyanins have long been utilized as an indicator to determine the quality and processing of roselle products. The methodology employed to determine the phenolic constituents by HPLC-PDA enables the separation and identification of five compounds as shown in Figure 1 - 3. In this study, phenolic compounds were identified and quantified based on their retention times, which were compared with the standard reference materials. Peaks were identified as; **1**. Delphinidin-3-*O*-sambubioside; **2**. Cyanidin-3-*O*-sambubioside; **3**. Chlorogenic acid; **4**. Caffeic acid; and **5**. Ascorbic acid. The phenolic and ascorbic acid content in the calyces of two roselle varieties is shown in Table 4. From the result, there are significant differences between compounds ($p < 0.05$) in different roselle varieties.

There are two main anthocyanins detected at 520 nm in both varieties of *H. sabdariffa* calyces with a retention time of 6.27 minutes and 7.81 minutes. The peaks were identified as delphinidin-3-*O*-sambubioside (**1**) and cyanidin-3-*O*-sambubioside (**2**). Anthocyanin production (based on the total amounts of anthocyanins) in UKMR-2 is 27.78 mg/g FDE, which almost three times higher than in the calyx of UMKL-1 (10.01 mg/g FDE). However, both varieties indicated delphinidin-3-*O*-sambubioside concentration was higher compared to cyanidin-3-*O*-sambubioside (Table 4). These findings agree with Bernal et al. [9] on Colombian *H. sabdariffa* L. and Kouakou et al. [8] on Côte d'Ivoire *H. sabdariffa* L. Delphinidin-3-sambubioside and cyanidin-3-sambubioside were identified as the major pigments with almost 70% of the anthocyanins in *H. sabdariffa* [19]. Delphinidin and cyanidin are known to contribute to the bioactive function because they have higher bioavailability as compared to the other anthocyanins [20].



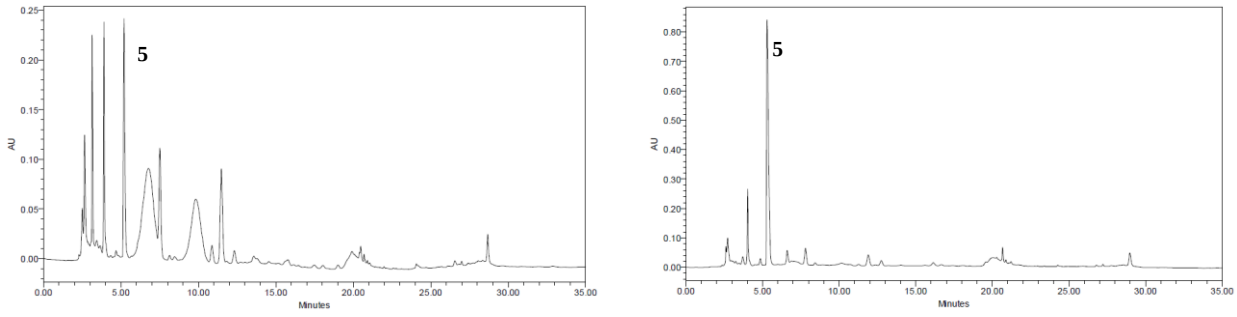
(a) (b)

Figure 1. HPLC-PDA profiles of phenolics in roselle calyces at 520 nm; (a) UKMR-2 and (b) UMKL-1. 1. Delphinidin-3-*O*-sambubioside; 2. Cyanidin-3-*O*-sambubioside



(a) (b)

Figure 2. HPLC-PDA profiles of phenolics in roselle calyces at 320 nm; (a) UKMR-2 and (b) UMKL-1. 3. Chlorogenic acid; 4. Caffeic acid



(a) (b)

Figure 3. HPLC-PDA profiles of ascorbic acid in roselle calyces at 265 nm; (a) UKMR-2 and (b) UMKL-1. 5. Ascorbic acid

Table 4. Concentrations of phenolics and ascorbic acid in two roselle varieties (UKMR-2 and UMKL-1)

Compound (peak)	Minutes	(mg/g FDE)		
		UKMR-2	UMKL-1	References
Delphinidin 3-O-sambubioside (**1**)	6.13 – 6.17	18.98 ± 5.11[a]	7.10 ± 0.02[b]	21.28 ± 0.05 [8]; 21.20 ± 2.16 [18]
Cyanidin 3-O-sambubioside (**2**)	9.05 – 9.10	8.80 ± 3.67[a]	2.91 ± 0.04[b]	17.11 ± 0.10 [8]; 5.17 ± 4.20 [18]
Chlorogenic acid (**3**)	11.54 – 11.57	5.11 ± 1.23[a]	4.06 ± 0.03[a]	2.70 ± 0.20 [23]
Caffeic acid (**4**)	15.52 – 15.56	0.76 ± 0.07[a]	1.58 ± 0.01[b]	2.98 ± 0.45 [18]
Ascorbic acid (**5**)	5.05 – 5.09	34.15 ± 13.23[a]	13.12 ± 1.07[a]	3.62 ± 2.14 [24]

Values represent the mean of three replicates ± standard deviation. Mean denoted by different letters in the same row indicate significant differences ($p < 0.05$). FDE, freeze-dried extract.

The results presented showed that the content of anthocyanins in both varieties is lower than previous findings. Kouakou et al. [8] reported that delphinidin-3-*O*-sambubioside and cyanidin-3-*O*-sambubioside contents in *H. sabdariffa* L. originated from Côte d'Ivoire is 21.38 mg/g DW and 17.11 mg/g DW respectively. The content and distribution of the anthocyanins in roselle are believed to be influenced by the fruit source, type of cultivation, the degree of fruit maturation, post-harvest storage condition, environmental conditions, genetic factors and the variety of the plant [13, 21, 22].

On the other hand, the analysis showed that only three non-anthocyanin compounds can be identified based on standard reference materials. At 320 nm, two peaks were identified as chlorogenic acid (**3**) and caffeic acid (**4**) with a retention time 11.91 and 16.29 min, respectively. Meanwhile, at 265 nm, only ascorbic acid (**5**) has been identified with a retention time of 5.39 min. The presence of all compounds is in agreement with previous reports on other *H. sabdariffa* varieties worldwide [8, 18, 23, 24]. UKMR-2 presented a higher diversity of phenolic compounds and ascorbic acid compared to UMKL-1. Figure 4 shows the structures of the identified compounds.

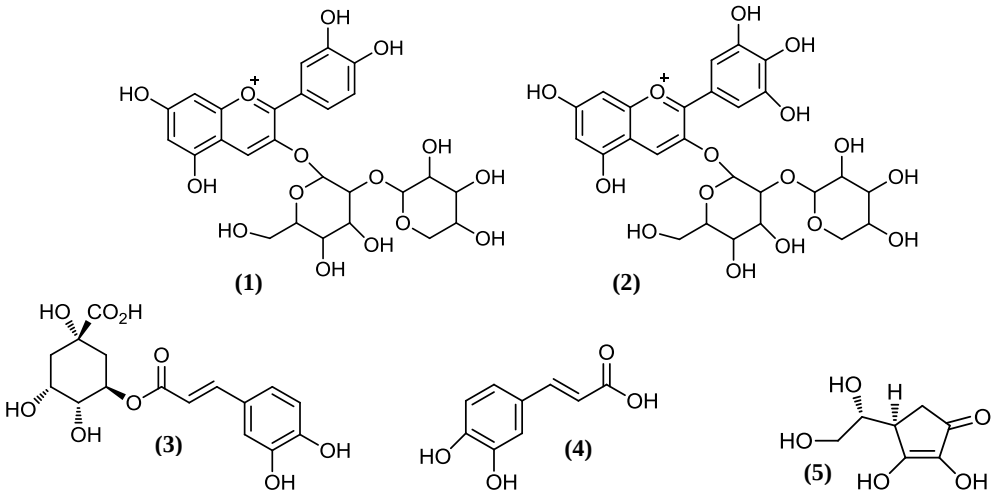


Figure 4. Proposed structures for the identified compounds in the *H. sabdariffa* water extract

A remarkable amount of ascorbic acid was found in UKMR-2 with 2.5-fold greater than in the calyces of UMKL-1. Ascorbic acid, a water-soluble vitamin is an essential nutrient in human diets and ubiquitous in fruits and vegetables. Due to the remarkable antioxidant and other biological properties of this compound, it is widely applied

in pharmaceutical and cosmetic industry [25]. The wide variation of ascorbic acid content in fruits and vegetables can be influenced by various factors such as genotype differences, pre-harvest climatic conditions, cultural practices, maturity and harvesting methods and post-harvest handling procedures [26, 27]. Due to its relatively high concentration especially in UKMR-2, ascorbic acid may contribute significantly to the antioxidant activity of roselle calyces. In addition, chlorogenic acid is reported to have potential protective effect on human health, in part by acting as an antioxidant through radical scavenging and metal chelation [28, 29]. The present study revealed a low amount of caffeic acid found in both calyces of *H. sabdariffa* varieties. Caffeic acid is one of the major hydroxycinnamic acid component and a well-known antioxidant, which boosts immunity, controls lipid levels in blood and anti-mutagenic. Caffeic acid is found mainly in the form of its ester (an immediate precursor of chlorogenic acid) in fruits, vegetables and herbs [30].

These results showed that the distribution of the phenolic and ascorbic acid compounds in both roselle varieties may depend on the genetic variety and growing techniques. This is likely due to different cultivation methods where UMKL-1 is grown at the farm in Kepala Batas, Penang, while UKMR-2 is grown in a nursery polyethene bag at the *Kompleks Rumah Tumbuhan* greenhouse, UKM. The two different cultivation methods (in terms of soil type, irrigation, fertilizer, weeding and spacing) can affect the production of secondary metabolites in roselle. Anthocyanin and phenolic contents are also directly related to the maturity degree as well as to the seasonal changes [31]. According to Khafaga and Koch [32], the acid content of the calyces increases during growth, but decreases when it reaches maturity or ripens. The acid content changes during maturity may be attributed to the anabolism and catabolism activities in calyx [33]. These results agree with several studies which report reductions of phenolic compounds during ripening [34-36]. Nevertheless, Raffo et al. [36] reported that the accumulation of some biologically active compounds was depended on different biosynthetic pathways and mechanisms of metabolic control.

Conclusion

A simple HPLC method was developed for quantitative analysis of major phenolics and ascorbic acid content in *H. sabdariffa* var. UKMR-2 and *H. sabdariffa* var. UMKL-1. The proposed HPLC method were validated and proved to be reliable. The HPLC-PDA profiles of the roselle water extract showed the presence of delphinidin-3-*O*-sambubioside, cyanidin-3-*O*-sambubioside, ascorbic acid, caffeic acid and chlorogenic acid. High content of phenolics and ascorbic acid detected in *H. sabdariffa* var. UKMR-2 suggested this variety may replace the current UMKL-1 variety for commercial cultivation.

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