Research Article

Characteristics and antioxidant activities of anthocyanin fraction in red dragon fruit peels (*Hylocereus polyrhizus*) extract

Imam Prabowo[1]*, Edi Priyo Utomo[2], Agung Nurfaizy[2], Aris Widodo[3], Edi Widjajanto[4], Puji Rahadju[5]

ABSTRACT

Background: Dragon fruit has been reported for its high antiradical activities with the presence of phenolic compounds. Some significant characteristics of plant pigment are betacyanin, anthocyanins, and other flavonoids. This study aimed to determine the anthocyanin components, antioxidant activity, and Total phenolic content (TPC) of anthocyanin from the extract of red dragon fruit peels. **Materials and Methods:** The research was used methanol extraction, followed by other analyses, and thin-layer chromatography (TLC) to obtain the fractionated components. Anthocyanins were analyzed with a liquid chromatography-mass spectrometry (LC-MS) instrument to obtain cyanidin, malvidin, and delphinidin. Total phenolic content of anthocyanin was measured with Folin-Ciocaltau. **Results:** Antioxidant activity revealed 56.68% (crude extract), 36.91% (F3), and 36.24% (F9). This study used TLC followed by column chromatography for better separation of phenolics. Among the three samples, the highest radical scavenging activity demonstrated by lethal concentration$_{50}$ was the crude extract, followed by F3 and F9. The LC-MS assay identified the single types of phenolic compounds such as cyaniding (31.63%), malvidin (7.37%), and delphinidin (7.53%). **Conclusions:** Red dragon fruit peel extract (*Hylocereus polyrhizus*) containing phytochemical compounds is an effective antioxidant from natural plant sources, with anticarcinogenic and anti-inflammation properties, and may help with other degenerative disorders.

KEY WORDS: Anthocyanins, Antioxidant activities, Column chromatography, Fraction red dragon fruit (*Hylocereus polyrhizus*), Phenolic compound

INTRODUCTION

The red pitaya is known as a red dragon fruit (*Hylocereus polyrhizus*). It is one of the tropical fruits in the cactus family, Cactaceae. Dragon fruit plants were first discovered in Mexico and then have been developed mostly in tropical countries such as Malaysia, the Philippines, and Indonesia. In general, there are four types commonly found in Indonesian markets, i.e., red dragon fruit (*H. polyrhizus*) and white dragon fruit (*Hylocereus undatus*). They are available in various sizes and shapes, and the average weight is around 350 g.[1] Our study used samples from local farmers of red dragon fruit from Sragen, in Central Java, which has been certified by the Indonesian Institute of Science (LIPI) Purwodadi East Java, according to the originality and specific characteristics of plant and soil in Indonesia. Red dragon fruit (*H. polyrhizus*) recently has drawn much attention from growers worldwide, due to its powerful antioxidant activities.[1,2] Furthermore, dragon fruit has been reported for its high antiradical activities with the presence of phenolic compounds. Some significant characteristics of plant pigment are betacyanin, anthocyanins, and other flavonoids. Betacyanin and anthocyanin are both polar anthocyanins, but betacyanin is more hydrophilic. They can dissolve in three common polar solvents such as water, methanol,

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and ethanol.[3] The polar solvent we chose was methanol because it is practical and easy to find.

This present study aimed to determine the anthocyanin components, antioxidant activity, and a Total phenolic content (TPC) of anthocyanin from the extract of red dragon fruit peels. Thin-layer chromatography (TLC) and column chromatography (CC) were used due to their beneficial laboratory assay and easy to obtain fractionated phenolic compounds. CC based on TLC can fractionate components from pulps of red dragon fruit peels. Antioxidant activity assay was measured with 1,1-diphenyl-2-picryl-hydrazine (DPPH). Liquid chromatography-mass spectrometry (LC-MS) assay was able to identify types of phenolic compounds.

To the best of our knowledge, there have been limited published reports on the characteristics of anthocyanins with two methods of separation of phenolic compounds, TLC, and CC, and analysis of the type of phenolic with LC-MS. Meanwhile, this study is essential to determine the anthocyanin from the peels of dragon fruit.

MATERIALS AND METHODS

Sample Collection

The species of dragon fruits used for the present study was red dragon fruit (*H. polyrhizus*). Dragon fruits were purchased from local commercial farm plantations in Sragen (central Java), which has been certified by the Indonesian Institute of Science Purwodadi East of Java, Indonesia.

Sample Preparation and Extraction

Red dragon fruits were washed and peeled to separate the peels from the pulps for further tests. For each container, 500 g of peels were weighed and put into four sample containers. The peels were crushed to a paste-like consistency using a blender for 1 min. Each sample was then poured into 250 mL volumetric flasks, methanol was added to the volume of 1500 mL. Then, 15 ml of HCl 1% was added; after that, it was shaken using the mechanical shaker until a carmine color was obtained. The mixture was dried for 24 h and every 60 min over 6 h it was mixed. Next, the solution was separated from the mixture. This drying procedure was repeated for 3 times before filtering it with Whatman No.4 filter paper. Afterward, the filtrate was rotary evaporated for 4 h at 56–60°C to remove the solvent and added with extract material. The extract material sample was stored at 4°C and protected from lights until further tests.

TLC Assay

Silica gel TLC plate 60 F_{24} from Merck was added to each eluent with the components of: n-Butanol:acetic acid glacial:water (5:1:4); n-Butanol: HCl 0.001 N (4:1); HCl 0.001 N:ethyl acetate:methanol (1:2.5:1.5); HCl 0.001 N:ethyl acetate:methanol (0.5:2.5:2); HCl 0.001 N:methanol (0.5:2:1.5); and HCl 0.001 N:ethyl acetate:methanol (1:2: 1). The separation of components used a TLC plate with the size of 10 cm × 2 cm for each eluent. The extract sample was dripped for 20 ml in the tip spotter of TLC and the solution rose in the capillary mechanism. The results were observed with chromatography using ultraviolet (UV) light.[4]

CC

CC preparation was made by weighing 60 GF_{254} silica gel in 20 g, then mixed with 50 ml of eluent of 1-Buthanol:acetic acid glacial:aqua (5:1:4) in the stationary phase. After that, the column was filled by subtly tapping it gently until it turned to solid. Then, it was left alone for 24 h. Next, 0.5 mg crude extract was added, repeating the procedure until all components were eluted and collected every 10 ml. Part of the eluent was analyzed with TLC to ensure the separation of the components. This CC separation obtained two active components, F3 and F9.

DPPH Assay

The radical scavenging activity of each sample was measured using a modified method, Scherer and Godoy.[5] The standard solution used Vitamin C (ascorbic acid) with a series concentration (5, 10, 15, 20, 25, 30, 35, and 40 mg/L) and dissolved in methanol. Separation of each fraction from TLC and pulps of the red dragon peel was diluted in 1% methanol. A volume of 0.1 ml of standard solution was mixed with 3.90 ml of 0.2 mM DPPH reagent in methanol and mixed thoroughly. From each of them was taken 0.1 ml: 3.90 ml then mixed with 0.5 ml: 3.90 ml DPPH solution (0.2 mM in methanol). Then, each of the fractions from CC and crude extract was made in 1% methanol followed by stratification from 0.1 ml to 0.5 ml: 3.9 ml (DPHH sample). The blank sample was defined as 0.10 ml methanol +3.90 ml 0.2 nM DPPH. All of these solutions were incubated in a dark room at room temperature for 90 min; absorbance solution was measured with a spectrophotometer at 517 nm. Antioxidant activity index (AAI) was calculated as follows: AAI = (final concentration of DPPH in the reaction)/half maximal inhibitory concentration [IC_{50}], whereas the final concentration of the reaction was 30.75 µg/mL. The concentration of 50% inhibition of lethal concentration$_{50}$ (LC_{50}) (IC_{50}) was calculated using computation probit analysis. The scavenging effect was calculated as I% = ([Abs0 − Abs1]/Abs0) × 100, in which Abs0 indicated absorbance of the negative control, and Abs1 is the absorbance with the tested extract at different concentrations. Scherer and Godoy established the following criteria of AAI values for plant extracts:

Poor activity < 0.05 < moderate < 1.0 < strong < 2.0 < very strong.[6] In the terms of LC_{50} (analysis with probit analyzer supported by Windows SPSS ver. 17), the lowest value was shown by positive control with 95% confidence limits of concentration (mg/ml) of standard solutions (Vitamin C) and followed by a crude extract of *H. polyrhizus* and each fractioning concentration.

Total Phenolic Content Assay

The total phenolic content (TPC) in the extracts was determined by a modified Folin-Ciocalteu method.[7] Extracts and fractionated chromatography agent were diluted in 10% distilled water. The standard solutions were a gallic acid with the concentrations of 50, 100, 150, 200, and 250 mg/L in distilled water. 1 mL was taken from each concentration, diluted in 70 mL distilled water (blank solution). Each solution was added to 5 mL Folin-Ciocalteu reagent, shaken gently for 1–8 min in the dark at room temperature, 15 mL of aqueous Na_2CO_3 solution (75 g/L) was added to each. The mixtures were homogenized and then incubated for 120 min in the dark at room temperature. Sample absorbances were measured at 765 nm. TPC was expressed in milligrams of gallic acid equivalents per gram of dry extract as in the calibration curve, $y = 0.0009 \times -0.0112$ ($r^2 = 0.9778$), whereas y denotes absorbance and × denotes gallic acid concentration in mg/L

LC-MS Assay

Shimadzu instrument of LC-MS with C 18 column BEH (100 mm × 2.1 mm, inner diameter 1.7 Im, Waters Milford, MA, USA) and spectrophotometer UV-visible Shimadzu 1700 double beam was used to separate the fractions (F3 and F9). We compared and analyzed each sample to identify specific phenolic compounds.

RESULTS

TLC Assay

The maceration of dragon fruit peel produced a red extract in 0.001 NHCl. The addition of thin HCl solution was aimed to prevent anthocyanin degradation. Storage in a dark bottle with a temperature of 4°C was needed for maintaining stability for 3 months. The results of the TLC assay revealed the comparison eluent of 1-butanol:acetic acid glacial 0.01 N:eEthanol ac:methanol and 1-butanol:acetic acid glacial:aqua distilled. The best result contained the composition of 1-butanol: Acetic acid glacial: Aqua distilled (5:1:4). The visual observation using UV light with the wavelength of 165 and 275 nm showed three optimally separated spots with an Rf difference of >0.3 and tailing. To ensure that this was anthocyanin compound, the solutions were passed through the acetic acid and condensed ammonia vapor, revealing the change of color from brownish red to yellow. The next separation step used CC with the volume of 10 ml for each fraction (F3 and F9). To ensure a single compound of anthocyanin, visual observation was performed. It demonstrated a similar color to that of TLC [Figure 1].

DPPH Assay

The DPPH free radical scavenging assay has been widely used to evaluate the antioxidant capacity as it has stable resonance and special blockade of benzene rings. The purple chromogen radical DPPH is reduced by the antioxidant compound to the corresponding pale yellow hydrazine.[8] The results of the scavenging effect of DPPH assay were illustrated in Figure 2.

In our study, peels of *H. polyrhizus* appeared faded purple. All study samples were dissolved in

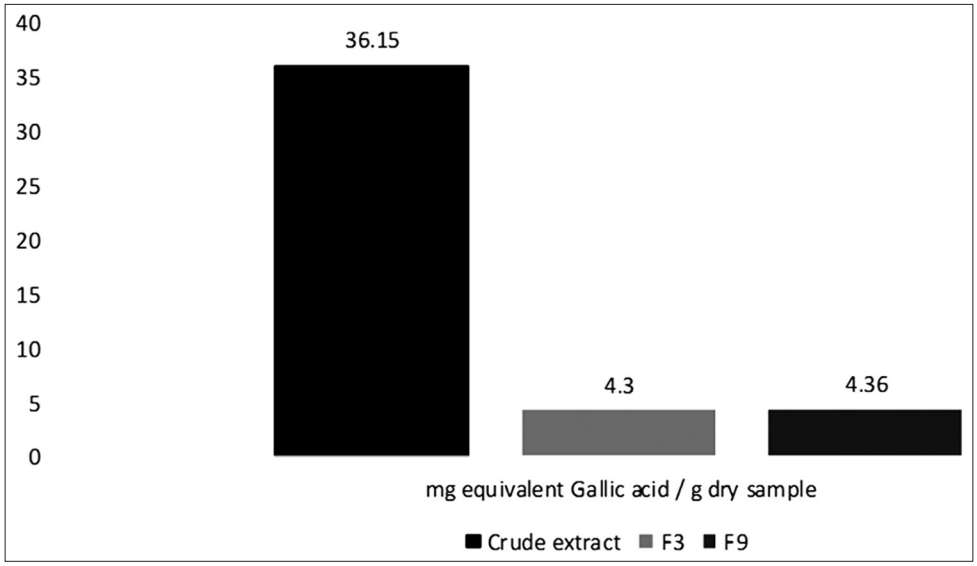


Figure 1: Level of total phenolic content crude extract, F3, and F9

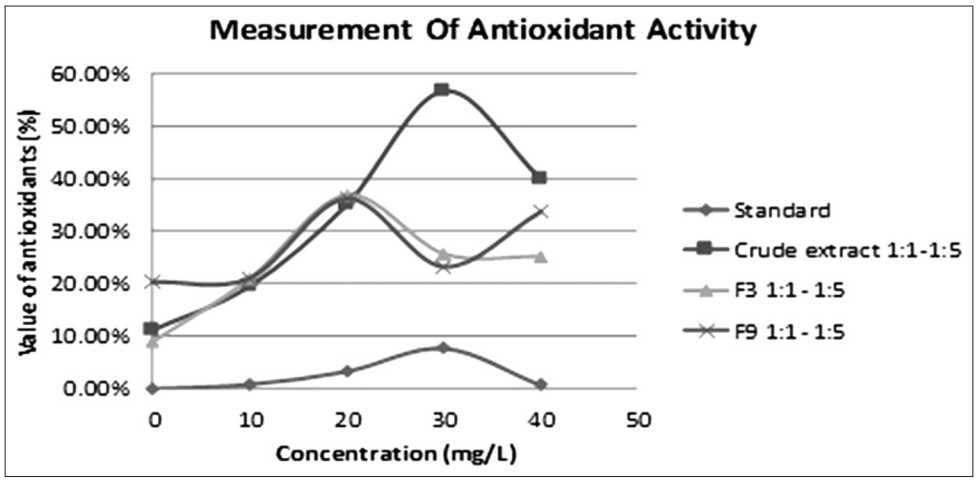


Figure 2: Comparison of scavenging effect from standard solution with series concentration (5, 10, 15, 20, 30, and 40 mg/l) and crude extract very high activity 56.68% (very strong), with ratio of 1:4 solutions, F3 36.91% with ratio 1:3 of solution and F9 36.24% with ratio1:3

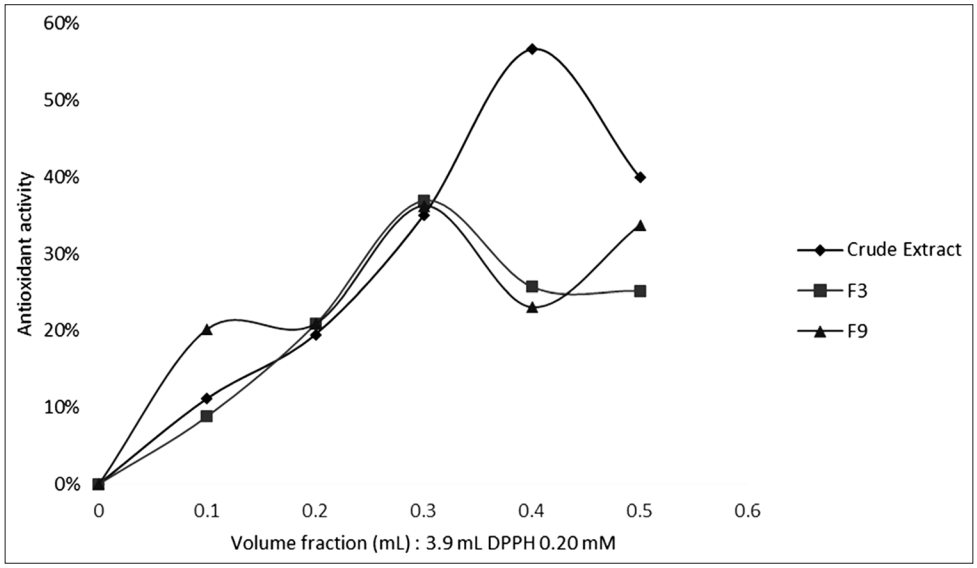


Figure 3: Antioxidant activity from crude extract, F3, and F9

methanol for 5 times. At different concentration levels, the radical activity of the crude extract, with a ratio of Vitamin C used as positive control for standard solutions, and methanol at a concentration of 0.3 mg/ml was 34.99% and increased to 56.68% at the concentration level of 0.4 mg/ml. The highest F3 ratio, at a concentration level of 0.3 mg/ml, was 36.91% and decreased at a concentration level of 0.4–0.5 mg/ml. F9 increased at the concentration level of 0.1–0.2 mg/ml and the highest antioxidant activity was observed at a concentration level of 0.3 mg/ml and was 36.24% [Figure 3].

In the terms of LC_{50}, the lowest value was shown by the positive control with 95% confidence interval of concentration (mg/ml), standard solutions of Vitamin C (13,528.651 mg/ml) and followed by crude extract of *H. polyrhizus* (10,012.494 mg/ml), F3 (30,963.638 mg/ml), and for F9 (73,614.328 mg/ml). However, LC_{50} for crude extract and both fractions (F3 and F9) could not be directly determined from the graph, due to their high percentage of radical scavenging activities over the measured extract concentration [Table 1].

According to Figure 4, the pattern from F3 showed readings of molecular weights of 287.5 (cyanidin), 302.5 (delphinidin), and 330.4 (malvidin). On the reading and analysis with LC-MS for fraction 9 (F9), we observed molecular weights of 288.5 (cyanidin), 302.5 (delphinidin), and 330.5 (malvidin).

LC-MS Assay

MS has been described as the smallest scale in the world because its microanalytical technique can be used selectively to detect and determine the quantity of a given analysis. The tools of MS are mass spectrometers, and the data are called mass spectra

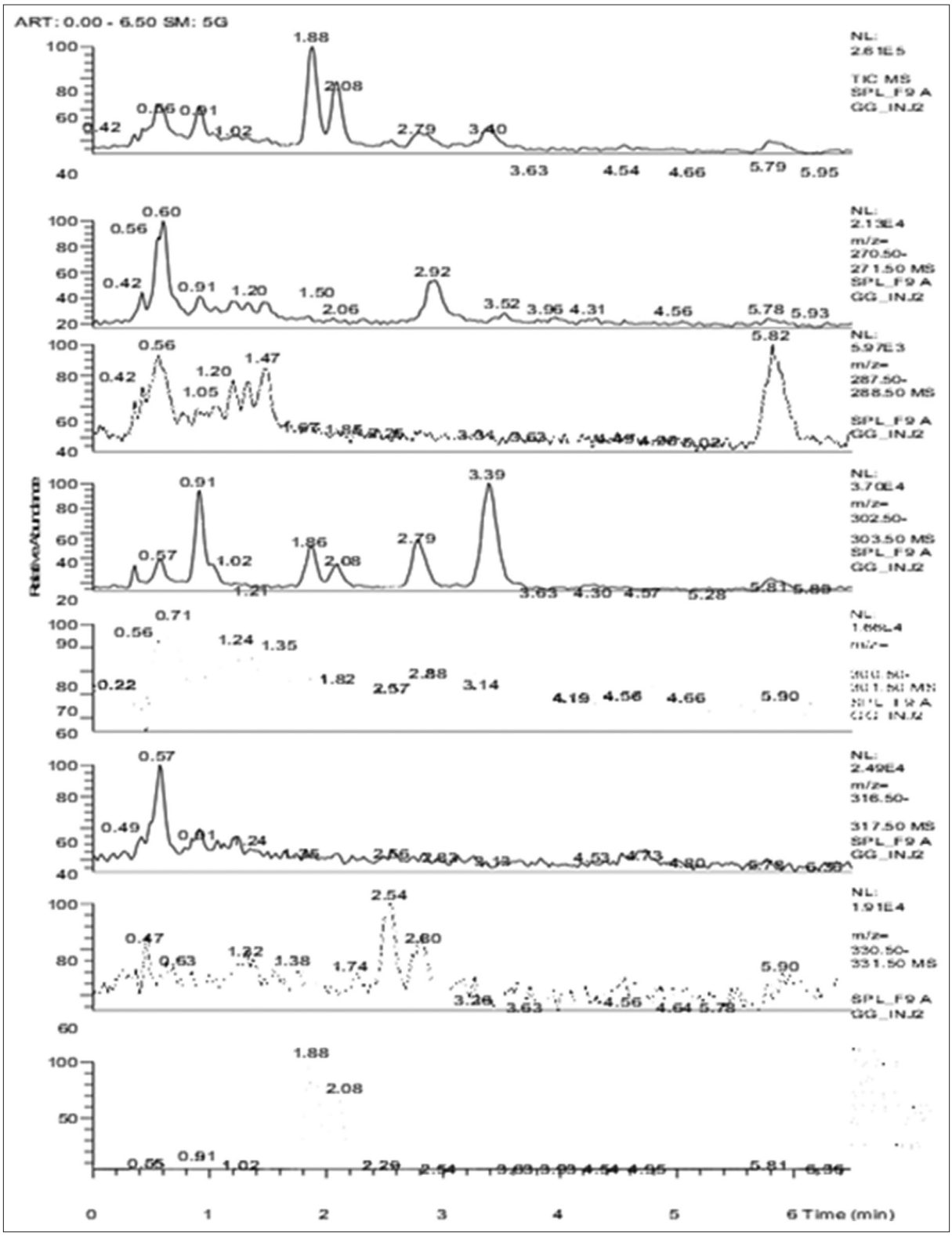


Figure 4: Liquid chromatography-mass spectrometry reading to identify a molecule from crude extract, F3, and F9

which can be displayed in many different ways, which allow the desired information of the analyte to be easily extracted.[9]

The benefit and cost-effective part of LC/tandem MS (MS/MS) are particularly evident when multiple analyses are measured simultaneously in the same sample. It can be applied from one test for a single disorder to one test for many disorders. However, despite the advantages of LC/MS/MS over traditional biochemical and immunoassays, in routine diagnostic laboratories, the traditional methodologies are still

Table 1: LC_{50} of crude extract, F3, and F9 by probit analysis

Probability (%)	95% confidence limits for concentration (mg/L)			
	Standard (Vitamin C)	Crude extract	Fraction (F3)	Fraction (F9)
5	60.139	1195.662	604.444	68.364
10	198.916	1911.871	1441.902	319.550
15	445.844	2624.191	2592.310	904.465
20	846.757	3375.245	4131.951	2067.808
25	1468.089	4188.740	6163.890	4203.412
30	2406.445	5085.095	8827.600	7948.365
35	3804.142	6086.077	12,313.698	14,343.634
40	5874.556	7217.523	16,886.840	25,115.397
45	8944.626	8512.031	22,921.982	43,183.428
50	13,528.651	10,012.494	30,963.638	73,614.328
55	20,461.941	11,777.452	41,826.526	125,489.559
60	31,155.447	13,889.810	56,774.796	215,766.814
65	48,111.878	16,472.028	77,860.192	377,803.101
70	76,055.916	19,714.485	108,607.881	681,784.164
75	124,668.444	23,933.218	155,542.493	1,289,207.257
80	216,147.403	29,701.558	232,032.477	2,620,682.452
85	410,512.245	38,202.264	369,842.669	5,991,462.663
90	920,107.252	52,435.555	664,918.306	1.696E7
95	3,043,340.451	83,844.791	1,586,163.880	7.927E7

LC_{50}: Lethal concentration$_{50}$

used more regularly. This has limited the routine use of LC/MS/MS in the clinical diagnostics laboratory.

As reviewed, high-performance LC (HPLC) has been the method of choice for the qualitative and quantitative analysis of anthocyanins. The extent of HPLC and its various detection methods will be discussed in depth, as well as the recent advances and future directions regarding the analytical methodologies of anthocyanin.[9] The type of anthocyanin from LC-MS is shown in Table 2.

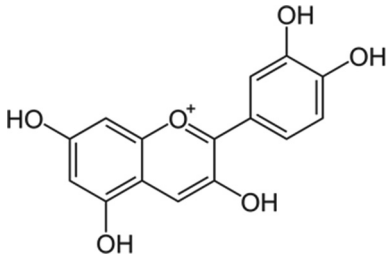
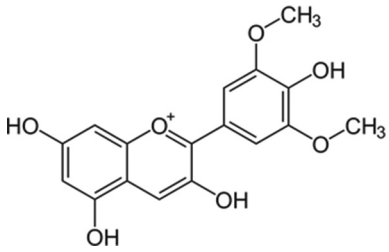
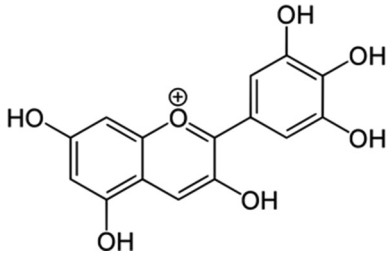
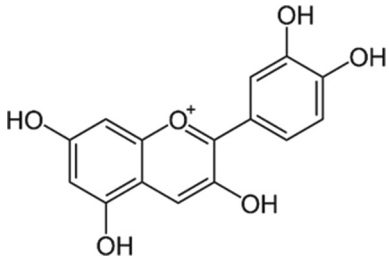
DISCUSSION

Malvidin, cyanidin, and delphinidin are anthocyanin compounds found in peels of the red dragon fruit, which are correlated with antioxidant activity. Anthocyanins from red dragon fruit peels occur ubiquitously in plants and confer bright red, blue, and purple colors to fruits. Their potential importance to human health is the relatively high concentration of anthocyanins in the diet. Epidemiologic studies suggest that the consumption of anthocyanins lowers the risk of cardiovascular disease, diabetes, arthritis, and cancer, due to their antioxidant and anti-inflammatory activities.[10]

Anthocyanin, as a phenolic compound, has been extensively exploited because of its multiple biological activities such as anti-mutagenic, anticarcinogenic, antiaging, and also antioxidant.[1] According to Bertoncelj *et al*.,[11] there were several studies showing that antioxidant activity was strongly connected with the content of total phenolic compounds. In our study, the Folin-Ciocalteu assay was performed to measure the antioxidant level. The assay depends on the basic mechanism of the oxidation and reduction reaction and refers to redox properties of compounds present in the sample, called antioxidants. These compounds react with the Folin-Ciocalteu reagent, thus allowing the measurement of phenolic compounds. In the present study, the dark blue color produced in the reaction mixture indicated that peels of red dragon fruit contained high levels of phenolic compounds. The higher level of the phenol content in peels of *H. polyrhizus* in our study may be due to the structure of the fruit and presence of different kinds of antioxidant compounds than previously observed in a study by Nurliyana *et al*.,[1] which compared peels of *H. polyrhizus* to peels of *H. undatus* (white dragon fruit). Anthocyanin has never occurred simultaneously with betalains and its distribution is restricted to 13 families within the plant kingdom. Some basidiomycetes have identical absorption spectra that contribute to the deep purple colored pulp.[2]

The extraction method used for peels of red dragon fruit must be one that allows the recovery of the greatest possible amounts of anthocyanins with minimum losses due to enzymatic and non-enzymatic changes. Anthocyanins are generally isolated in acidic media. Among the methods that have been tested for extracting anthocyanins from various natural sources, it was found that using methanol acidified with 1% acetic acid (HCl) as an extraction solvent, the amount of pigment extracted increased greatly, at the same time it maintained the stability of the anthocyanins. Based on a modified technique by Vargas *et al*.,[12] the efficiency of extraction can use trifluoroacetic acid which is attributed to the low pH in the system leading to the higher solubility of anthocyanins in methanol. The powdered peels of dragon fruit are subjected to extraction in a dark room for 12 h, with overnight stirring in the extraction solvent, to allow for the

Table 2: LC-MS from peels of red dragon fruit in F3 and F9

Number	Start RT	Area	%area	Compound	Molecular structure
F9					
1	0.3	577,767.6	18.32	–	–
2	0.82	265,481.3	8.36	–	v
3	1.76	100,3947	31.63	Cyanidin	
4	1.99	634,268.1	19.98	Cyanidin	
5	2.46	37,717.35	1.19	Malvidin	
6	2.68	233,867.6	7.37	Malvidin	
7	3.25	239,059.8	7.53	Delphinidin	
8	5.7	182,105.4	5.74	–	–
F3					
1	0.34	2,835,597.3	44.17	–	–
2	0.78	134,515.8	20.95	–	–
3	1.77	171,637.4	26.73	Cyanidin	
4	2.08	52,284.66	8.14	Cyanidin	

LC-MS: Liquid chromatography-mass spectrometry

diffusion of anthocyanins through the cell membranes. After the first extraction with a high concentration of anthocyanins, an additional extraction solvent was required to wash the residue of anthocyanins. CC in this study aimed to identify the separated polarity of anthocyanin from peels red dragon fruit, usually extracted with organic solvent and most commonly methanol.[13] The results of the solvent extraction can be explained by the relationship between the polarity of solvents and the structure of pigments and polyphenols (colorants). Methanolic extraction gave the highest yield because pigments and polyphenol compounds such as flavonoids (anthocyanins and flavonoids) and non-flavonoids (phenolic acid) are soluble in methanol. Khanavi *et al*.[13] compared the effect of different solvent extraction methods on antioxidant activity and content of total phenolic content and reported significant difference among solvents used; methanolic extraction gave the highest antioxidant activity and TPC.

The procedure using TLC helped to determine the number of components in a mixture and to identify the purity of compounds. This procedure used a thin glass plate coated with either aluminum oxide or silica gel as the solid phase. The principle of TLC is the distribution of a compound between a solid fixed phase applied to a glass or plastic plate and a liquid mobile phase, which moves over the solid phase.

This procedure was followed by CC to separate the phenolic compound, as it is convenient, practical, and a low laboratory expense. CC is one of the most useful methods for separation and purification of both solids and liquids. This is a solid-liquid technique in which the stationary phase is stable, and mobile phase is liquid. CC is nearly the same as TLC which separate the mixture of compounds carried out by mobility through stationary phases. The stationary phase in CC is a glass column filled with alumina or silica gel. The mobile phase as the solvent, which is pushed down through the column and the solvent is collected as it stays in the bottom and separated compounds can be obtained. CC in this study aimed to identify the separated polarity of anthocyanin from peels red dragon fruit, usually extracted with organic solvent and most commonly methanol.

The antioxidant activity of the peels red dragon fruit such as crude extract, F3, and F9 was identified by DPPH scavenging activity. The scavenging activity (36.24–56.68%) is similar to those reported by Fidrianny *et al*.[14] Manihuruk *et al*.[15] reported that, from a procedure modified by Lourith and Kanlayavattanakul,[16] the red dragon fruit peel extract with solvent water had better antioxidant activity than in ethanol, while our study used methanol and showed a better outcome using a modified technique by Khanavi *et al*.[13]

The total phenolic compounds (TPC) of the red dragon fruit peel, from crude extract, were the highest of all, 36.15 mg followed by F9 (4.36 mg) and F3 (4.30 mg). This result indicates a very high concentration of TPC in the crude extract from the peels red dragon fruit. The DPPH assay also indicated that in 1% of crude extract, antioxidant activities were 56.68%, 36.91% (F3), and 36.24% (F9). The activity of antioxidant levels of all samples was high, but the highest of all was from the crude extract. This potent antioxidant activity of the crude extract demonstrates the mechanism of several parts of the phenolic compounds and the synergetic reaction of intermolecular antioxidant compounds was better than the single action of antioxidant measured in F3 and F9. The result of LC_{50} indicates the strong values of each sample (crude extract, F3, and F9) and toxicity levels of crude extract, F3, and F9 but they were less than the standard solution of Vitamin C.

Although most of the protective effects of anthocyanins are attributed to their ability to scavenge reactive oxygen species, they also function by chelating metals and by direct binding to proteins. The radical scavenging (antioxidant) activity of anthocyanins is primarily due to the presence of hydroxyl groups in position three of ring C, and also in the 3′, 4′, and 5′ positions in ring B of the molecule. This was demonstrated in cyanidin, malvidin, and delphinidin with the LC-MS assay that presented the molecular structure of hydroxyl with molecular weights of cyanidin (288.5), malvidin (330.5), and delphinidin (302.5).

Measuring the absorbance at different wavelengths provides an absorption spectrum for the pigments and colorants in the extracts. In this study, the peels of red dragon fruit absorbed the wavelengths of violet, reflecting the yellow (passing through above ammonia condensed). From the previous study, anthocyanin absorbs mainly in the green and yellow regions of the spectrum. The results of our analysis are not much different from the study by Karageorgou and Manetas.[17] Spectrophotometric analysis of the various fractions of the plant extract had a similar major absorption peak at wavelengths in the UV areas, and maximum absorption changed with each fraction. The UV spectrum of the same derivatives, such as flavonoids aglycones, anthocyanin, phenolic acid, and their derivatives, has a similar spectrum for each group that can be used for spectrum analysis for qualitative and selective evaluation of classification of plant phenolic.

The technique of coupling HPLC and MS instrumentation has had a significant effect on the quantitative and qualitative analytical methodology of anthocyanin in the past decade. This combination, LC-MS, offers the separation advantages of LC combined with the identification advantages of MS. MS is a susceptible method of molecular analysis, and due to its separation by mass, good selectivity can be obtained, and the identification of individual compounds in a mixture of compounds is permitted. Electrospray ionization has been an efficient ionization technique for LC-MS and, along with atmospheric pressure chemical ionization, they are the two most common ionization techniques used in clinical laboratories. Another likely reason for the increased interest in LC-MS/MS assays over the past 10 years has been due to the unexpected withdrawal of immunoassay equipment, kits, or assays, leaving laboratories struggling to provide a service.[18]

As for the edible portion of the *Hylocereus* species, pulps of *H. polyrhizus* show higher phenolic content; color appears from pulps of the peels red dragon fruit (*H. polyrhizus*). The red color pulps may indicate the presence of Higher phenolic content and betalains.[19] Esquivel *et al*. found that betalains were responsible for the major antioxidant capacity of purple *Hylocereus* juices evaluated, while non-betalains phenolic compounds contributed only to a minor extent.[20]

HPLC analyzed the purified extracts containing anthocyanin; the chromatogram of the standard mixture, where the first peak from F3 corresponded

to cyanidin, with a retention time of 1.77 min; the second peak from F9 corresponded to cyanidin with a retention time of 1.76 min, the second was malvidin with retention time 2.46 min and the third was delphinidin with retention time 3.25 min. A large number of peaks were observed in the chromatograms from the extracts of dragon fruit skin that could not be identified. According to the study by Vargas *et al*.,[12] three anthocyanins could be identified by commercial standards: 3.5 O diglucoside, pelargonidin 3.5 O-di glucoside, and cyanidin 3 oglucoside; they presented retention times of 6.12, 11.09, and 16.22 min, respectively, coinciding with the standards used for identification. Differentiating the retention time from the specific kind of anthocyanin can require different methods, preparation, and equipment.

CONCLUSIONS

Red dragon fruit peel extract (*H. polyrhizus*) containing phytochemical compounds is an effective antioxidant from natural plant sources, with anticarcinogenic and anti-inflammation properties, and may help with other degenerative disorders. In determining phenolic compounds, TLC and CC have reliable value, due to separation compound and efficiencies. The highest TPC was found in the crude extract, followed by F3 and F9. Among the three samples, the highest radical scavenging activity demonstrated by LC_{50} was the crude extract, followed by F3 and F9. The LC-MS assay identified the single types of phenolic compounds such as cyanidin, malvidin, and delphinidin. The majority of single types of phenolic compounds was dominated by cyanidin, but there were different weights of molecular atoms. Of the three samples, the antioxidant level of crude extract was the highest, followed by F3, then F9. Thus, the crude extract of red dragon fruit peel may be helpful for health promotion and prevention studies.

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