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Antioxidant properties of two species of *Hylocereus* fruits

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ABSTRACT

The antioxidant properties of fruits (peels and pulps) and pulps of two species of Hylocereus, Hylocereus polyrhizus (red dragon fruit) and Hylocereus undatus (white dragon fruit) were investigated. The ascorbic acid and total phenolic contents of pulps were higher than those of fruits (peels and pulps) of the two species of Hylocereus. The anti-radical power measured using 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical scavenging activity ranked in the following order: pulp of H. undatus ≈ pulp of H. polyrhizus > fruit (peel and pulp) of H. polyrhizus > fruit (peel and pulp) of H. undatus. The ferrous ion chelating activity ranked in the following order: pulp of H. undatus > fruit (peel and pulp) of H. polyrhizus > fruit (peel and pulp) of H. undatus > pulp of H. polyrhizus. Both phenolics and ascorbic acids contributed to the anti-radical power of the two species of Hylocereus fruits but the latter contributed more. By taking into account the free radical scavenging activity and ferrous ion chelating activity, pulp of H. undatus had the highest antioxidant properties.

Keywords: dragon fruit, pitaya fruit, anti-radical power, chelating activity.

INTRODUCTION

Hylocereus polyrhizus (red dragon fruit) and *Hylocereus undatus* (white dragon fruit) are fruits belong to the vine cacti from the subfamily Cactoideae of the tribe Cacteae [1,2]. There are about 16 species of *Hylocereus* in Central America and Mexico [3]. Varieties of *Hylocereus guatemalensis*, *Hylocereus polyrhizus* and *Hylocereus undatus* as well as hybrids of these three species are grown commercially worldwide. *H. undatus* has white pulp with pink skin, *H. polyrhizus* has red pulp with pink skin while *Selenicereus megalanthus* has white pulp with yellow skin [4]. The pulp is delicate and juicy and contains numerous small soft seeds. The fruits are covered with bracts or "scales', hence the name dragon fruit [5]. These edible fruits are also mostly known as pitaya or pitahaya, which means the "the scaly fruit" in Latin America [1]. Red dragon fruit weighs up to 1 kg and is a rich source of nutrients and minerals such as vitamin B1, vitamin B2, vitamin B3 and vitamin C, protein, fat, carbohydrate, crude fiber, flavonoid,

thiamin, niacin, pyridoxine, kobalamin, glucose, phenolic, betacyanins, polyphenol, carotene, phosphorus, iron and phytoalbumin [6,7]. It is also rich in phytoalbumins which are highly valued for their antioxidant properties [8]. The most important fruit pigments in red dragon fruit are the betalains such as the betacyanins and betaxanthins [9,10]. Betalains are used as natural food colorants in various areas of the food industry [11].

Dragon fruit plants are grown in the open in tropical areas but must be protected from intense solar radiation and subfreezing temperatures when cultivated under subtropical conditions [12]. This fruit is being cultivated on a large scale in Malaysia [13] and grown commercially in Nicaragua, Columbia, Vietnam, Israel, Australia and U.S. [14]. The pulp of *H. polyrhizus* is used for the production of red-violet colored ice cream in Israel [1]. In Malaysia, the fruit of *H. polyrhizus* (peel and pulp) is sometimes used to produce juice but when eaten as a fruit, the consumers usually discard the peel.

H. polyrhizus fetches higher price than *H. undatus* in Malaysia due to consumers' demand for *H. polyrhizus* and its perceived higher antioxidant properties. There is no report on the comparison of antioxidant properties of *H. polyrhizus* and *H. undatus* grown in Malaysia. Mahattanatawee et al. [8] investigated the antioxidant activity of selected tropical fruits grown in South Florida, U.S.A. and reported that the antioxidant activity of red pitaya was higher than that of white pitaya. Recently, Kim et al. [15] investigated the antioxidant and antiproliferative activities of red and white pitaya grown in Jeju Island, Korea and reported that the peel extract and pulp extract of white pitaya had higher bioactivity index values that those of red pitaya.

The objective of this study was to investigate the antioxidant properties of fruit (peel and pulp) and pulp of *H. polyrhizus* and *H. undatus*. Antioxidant contents of the two species of *Hylocereus* fruits were measured using ascorbic acid and total phenolic contents. Antioxidant activities of the two species of *Hylocereus* fruits were investigated using free radical scavenging activity and ferrous ion chelating activity.

MATERIALS AND METHODS

Samples

H. polyrhizus (red dragon fruit) and *H. undatus* (white dragon fruit) were obtained from the local markets. All fruits were analyzed within one week after acquisition. Triplicate analysis from different fruit samples were carried out.

Chemicals and solvents

Folin-Ciocalteu's reagent (2N), gallic acid, L-ascorbic acid and 2,2-diphenyl-1-picryl hydrazyl (DPPH) radical were purchased from Sigma-Aldrich (U.S.A.). Ethanol, iodine 0.1N, sulfuric acid and sodium carbonate were purchased from Merck (Germany). Potato starch was purchased from Bendosen Laboratory Chemicals (Norway). Iron (II) sulphate 7-hydrate and ferrozine iron hydrate were purchased from Acros Organics (Belgium).

Sample preparation

The sample preparation was based on the method of Lim et al. [16] with slight modification. Sample of fruits (peels and pulps) and pulps for *H. polyrhizus* and *H. undatus* were prepared. The fruits were washed and wiped dry. All fruits were cut into a few portions. The portions (20 g) were crushed to a paste-like state for approximately 2 min (with intermittent stops to minimize heating) using a Waring blender. The homogenized sample was transferred into a 100-mL

volumetric flask and 50% ethanol was added up to the mark. The mixture was shaken manually for 10 min, followed by centrifugation at 1500 × g. The extracts were stored at -20ºC. All tests were performed within a week.

Determination of ascorbic acid content

The ascorbic acid content was determined using the iodine titration method [17] with slight modification. Starch indicator solution was prepared by mixing 1g of starch with 200mL of boiling water. The solution must immediately removed from heat and left for cool.

Each 25mL of fresh juice sample was transferred into a 250mL Erlenmeyer flask. Twenty-five milliliter of 2N sulfuric acid was added, mixed, diluted with water (50 mL) and starch indicator solution (3 mL) was added. The solution was directly titrated with 0.001N standardized iodine solution. A blank titration was performed prior to titration of each sample. One mL of 0.001N iodine was equivalent to 88.06 µg ascorbic acid.

Determination of total phenolic content

Total phenolic content was determined according to the method of Lim et al. [16]. Samples (0.3 mL) were measured into test tubes followed by 1.0 mL of Folin-Ciocalteu's reagent (diluted 10 times with water) and 1.2mL of sodium carbonate (7.5% w/v). The tubes were vortexed, covered with parafilm and allowed to stand for 30 min. Absorbance at 765 nm was measured against a reagent blank. If the sample absorbance exceeded 1, the sample was appropriately diluted to give reading less than 1. A standard calibration curve was prepared using gallic acid. Total phenolic contents were expressed in gallic acid equivalents, GAE, in mg per 100 g fresh fruit. Since ascorbic acid was responsible to the formation of the blue molybdenum – tungsten complex, the absorbance originating from it was corrected by preparing ascorbic calibration curve. An ascorbic calibration curve was therefore prepared. The total phenolic content reported was corrected for ascorbic acid.

Free radical scavenging activity assay

Free radical scavenging activity was determined according to the method of Suja et al. [18] with slight modification. Fruit extract (1 mL) was added to 2 mL DPPH solution (2mL of 0.02g/L DPPH) in ethanol. The reduction of DPPH was measured at 517 nm against a blank assay at 30 min. The percentage of remaining radical in medium is calculated as the absorbance of the sample divided by that of DPPH control at the same time multiplied by 100. The amount of sample needed to decrease the initial DPPH concentration by 50%, EC_{50}, was calculated graphically. The anti-radical power (ARP) of extract was calculated as

$ARP = 1 / EC_{50}$

Ferrous ion chelating activity

Ferrous ion chelating activity was determined according to the method of Lim et al. [19]. Iron sulphate (2 mM) and ferrozine (5 mM) were prepared and diluted 20 times. Fruit extract (1mL) were mixed with diluted $FeSO_4$ (1 mL), followed by diluted ferrozine (1 mL). The tubes were mixed well and allowed to stand for 10 min at room temperature. Absorbance of each extract was measured against blank at 562 nm. The ability of the sample to chelate ferrous ions was calculated and expressed as:

Chelating effect (%) = (Acontrol – Asample) / Acontrol x 100

Statistical analysis

Results were expressed as the means ± standard deviation of three replicates. Data were interpreted by one-way analysis of variance (ANOVA) with Duncan's multiple-range test using SAS software package (SAS Institute Inc., Cary, NC, USA). The statistical significant was evaluated at $p < 0.05$ level.

RESULTS AND DISCUSSION

Ascorbic acid content

The two species of *Hylocereus* fruits showed higher ascorbic acid contents in pulps than in fruits (peels and pulps) [Table 1]. This indicates that more ascorbic acids were found in the pulps than in the peels of the two species of *Hylocereus*. There were no significant differences ($p < 0.05$) in the ascorbic acid contents of the pulps of *H. polyrhizus* and *H. undatus*. However, the ascorbic acid contents of fruits (peels and pulps) of *H. polyrhizus* were higher than those of *H. undatus*. These results are not in accordance with the study of Mahattanatawee et al. [8] where the ascorbic acid content of pulp of *H. undatus* (55.8 ± 2.0 mg/100g puree) was found to be higher than that of *H. polyrhizus* (13.0 ± 1.5 mg/100g puree). This difference was most likely due to different sample preparation method and different experimental method to determine ascorbic acid content. Mahattanatawee et al. [8] extracted the fruit puree using 100% methanol and concentrated it under partial vacuum at 40°C. In addition, Mahattanatawee et al. [8] determined the ascorbic acid content of the fruit puree using HPLC.

Table 1: Ascorbic acid contents of fruits (peels and pulps) and pulps of *H. polyrhizus* and *H. undatus.*

Dragon fruit	Fruit part	Ascorbic acid content (mg/100g)
H. polyrhizus	Fruit (peel and pulp)	18.94 ± 2.51^{b}
	Pulp	32.65 ± 1.59^{a}
H. undatus	Fruit (peel and pulp)	11.56 ± 1.25^{c}
	Pulp	31.05 ± 1.22^{a}

Values are means ± standard deviations.

^{abc} *Different superscript letters within a column indicate significant difference at $p < 0.05$.*

The ascorbic acid content of the pulp of *H. polyrhizus* in this study was relatively higher than that (8.0 ± 1.6 mg/100g) of Lim et al. [16]. This difference in ascorbic acid content was most likely due to the differences in the maturation stage and environmental growth variation of the fruits. The ascorbic acid contents of the two species of *Hylocereus* fruits in this study were higher than those of star fruit (5.2 ± 1.9 mg/100g) and banana (4.9 ± 0.6 mg/100g) but were lower than those of orange (67 ± 9 mg/100g), guava (144 ± 60 mg/100g) and papaya (108 ± 16 mg/100g) [16]. To the authors' best knowledge, there is no literature reporting on the ascorbic acid content of fruits (peels and pulps) of *H. polyrhizus* and *H. undatus*.

Total phenolic content

The two species of *Hylocereus* fruits showed higher total phenolic contents in pulps than in fruits (peels and pulps) [Table 2]. This indicates that the pulps of the two species of *Hylocereus* contained higher phenolic compounds than the peels. This result is not in accordance with the study of Wu et al. (2006) where it was reported the amount of total phenolics per 100g of pulp or peel of *H. polyrhizus* grown in Taiwan was similar. The total phenolic content of pulp of *H. undatus* was significantly higher ($p < 0.05$) than that of *H. polyrhizus*. However, Mahattanatawee et al. [8] reported that the total phenolic content of *H. undatus* (523.4 ± 33.6 µg

gallic acid/g puree) was almost double the amount of *H. polyrhizus* (1075.8 ± 71.7 µg gallic acid/g puree). This difference was most likely due to different sample preparation method (fruit puree was extracted using 100% methanol and concentrated under partial vacuum at 40°C), environmental growth variation and/or different maturation stage of the fruits.

Table 2: Total phenolic contents of fruits (peels and pulps) and pulps of *H. polyrhizus* and *H. undatus*

Dragon fruit	Fruit part	Total phenolic content (mg gallic acid equivalent/100g)
H. polyrhizus	Fruit (peel and pulp)	15.92 ± 1.28[d]
	Pulp	24.22 ± 0.95[b]
H. undatus	Fruit (peel and pulp)	20.14 ± 1.15[c]
	Pulp	28.65 ± 1.79[a]

Values are means ± standard deviations.

[abcd] *Different superscript letters within a column indicate significant difference at $p < 0.05$.*

According to Lim et al. [16], the total phenolic content in pulp of *H. polyrhizus* was found at 21 ± 6 mg gallic acid equivalent/100g, which is relatively lower than the results obtained in this study. Wu et al. [7] reported higher phenolic content at 42.4 ± 0.04 mg gallic acid equivalent/100g for pulp of *H. polyrhizus*. This difference in total phenolic content was most likely due to environmental growth variation and/or difference in the maturation stage of the fruits. The total phenolic contents of the two species of *Hylocereus* fruits in this study were found to be lower than orange (75 ± 10 mg gallic acid equivalent/100g) and tropical fruits such as guava (seeded) (138 ± 31 mg gallic acid equivalent/100g), star fruit (131 ± 54 mg gallic acid equivalent/100g) and banana (51 ± 7 mg gallic acid equivalent/100g) [16]. To the authors' best knowledge, there is no literature reporting on the total phenolic content of fruits (peels and pulps) of *H. polyrhizus* and *H. undatus*.

Free radical scavenging activity

The main mechanism of antioxidant action in foods is radical scavenging activity. Therefore many methods had been developed in which the antioxidant activity was evaluated by the scavenging of synthetic radicals in polar organic solvents such as ethanol [20]. In this study, the antioxidant activities of fruits (peels and pulps) and pulps of *H. polyrhizus* and *H. undatus* were determined using free radical scavenging activity and ferrous ion chelating activity.

Table 3: Efficient concentration, EC_{50} and anti-radical power of fruits (peels and pulps) and pulps of *H. polyrhizus* and *H. undatus*

Dragon fruit	Fruit part	EC_{50} (mg/mL)	Anti-radical power
H. polyrhizus	Fruit (peel and pulp)	11.34±0.22[b]	0.0882±0.0017[b]
	Pulp	9.93±0.47[c]	0.1008±0.0048[a]
H. undatus	Fruit (peel and pulp)	14.61±0.82[a]	0.0686±0.0039[c]
	Pulp	9.91±0.42[c]	0.1011±0.0042[a]

Values are means ± standard deviations.

[abc] *Different superscript letters within a column indicate significant difference at $p < 0.05$.*

DPPH radical scavenging activity assay is a widely used method to evaluate antioxidant activities in a relatively short time compared with other methods. This assay measures the ability of a sample to donate hydrogen to DPPH radical. The increased amount of antioxidant in a given volume of fruit extract is responsible for the increased reduction of the DPPH solution. The efficient concentration of fruit required to scavenge 50% of DPPH, EC_{50}, is shown in Table 3. The lower the EC_{50}, the better it is able to scavenge radicals, particularly peroxy radicals which

are propagators of the oxidation of lipid molecules and thus halt the free radical chain reaction [16].

For fruits (peels and pulps) and pulps of the same species of *Hylocereus*, the higher the total phenolic content, the higher the anti-radical power is (Table 3). This is in accordance with Wu & Ng [21] who reported that the scavenging ability of antioxidants was proportional theoretically to polyphenolic compounds. There was no significant difference in anti-radical power of pulps of the two species of *Hylocereus* species. This result is not in accordance with the study of Mahattanatawee et al. [8] and Kim et al. [15] where the DPPH radical scavenging activity of *H. polyrhizus* was found to be higher than that of *H. undatus*. These differences may be due to environmental growth variation and/or difference in the maturation stage of the fruits. The anti-radical power of pulps of the two species of *Hylocereus* species was not proportional to the total phenolic content. The total phenolic content of pulp of *H. undatus* was higher than that of *H. polyrhizus* (Table 2) but there was no significant difference in the ascorbic acid content of the two species of *Hylocereus* (Table 1). These results indicate that ascorbic acid contributed significantly to anti-radical power and ascorbic acid may act synergistically with the phenolics in the radical scavenging activity.

The anti-radical power of fruits (peels and pulps) of *H. polyrhizus* was higher than those of *H. undatus* (Table 3) but the total phenolic content of fruits (peels and pulps) [Table 2] of *H. polyrhizus* was lower than that of *H. undatus* (Table 1). The ascorbic acid content of fruits (peels leand pulps) of *H. polyrhizus* was, however, higher than that of *H. undatus*. These results indicate that ascorbic acids played important role in free radical scavenging activity of the fruits. According to Zhang and Hamauzu [22] and Scalzo [23], ascorbic acid acts as a free radical scavenger and its reaction was faster compared to other scavenging molecules such as polyphenols.

Ferrous ion chelating activity

Free ferrous iron is quite sensitive to oxygen and gives rise to ferric iron and superoxide and generates hydrogen peroxide [24]. Reaction of ferrous ion with hydrogen peroxide generates the hydroxyl radical, which oxidizes the surrounding bio-molecules. In this process, known as the Fenton reaction, hydroxyl radical production is directly related to the concentration of copper or iron [25,26]. Chelating power measures the effectiveness of compounds to compete with ferrozine for ferrous ion. A high chelating power extract reduces the free ferrous ion concentration by forming a stable iron (II) chelate and thus decreasing the extent of Fenton reaction which is implicated in many diseases [27]. The pulp of *H. undatus* displayed the highest ferrous ion chelating activity at all concentrations (Fig. 1), followed by the fruit (peel and pulp) of *H. polyrhizus*, fruit (peel and pulp) of *H. polyrhizus* and pulp of *H. polyrhizus*. It was observed that a gradual increment of chelating activity as the concentration of the fruit extract increased. The chelating activity of pulp of *H. polyrhizus* in this study was almost similar to that of Lim et al. [16]. So far, there are no studies on ferrous ion chelating activity of fruits (peels and pulps) of the two species of *Hylocereus* and the pulps of *H. undatus*.

Ascorbic acid is known for its complex multi-functional effects, as an antioxidant, a prooxidant, a metal chelator, reducing agent or an oxygen scavenger [28]. There was no correlation between ferrous ion chelating activity and ascorbic acid content of the fruits in this study. There was no correlation between ferrous ion chelating activity and total phenolic content of the fruits in this study as well. This is in accordance with the study of Lim et al. [19] where ferrous ion chelating activity did not correlate with the total phenolic content.

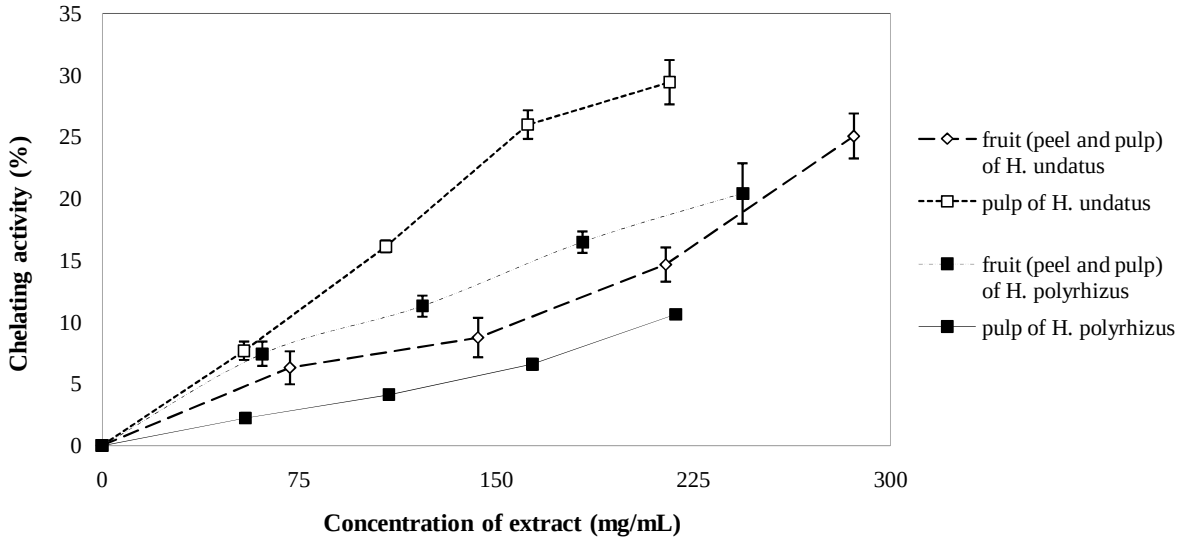

Fig. 1. Ferrous ion chelating activity of fruits (peels and pulps) and pulps of *H. polyrhizus* and *H. undatus*.

CONCLUSION

The free radical scavenging activity exists in the order of pulp of *H. undatus* ≈ pulp of *H. polyrhizus* > fruit (peel and pulp) of *H. polyrhizus* > fruit (peel and pulp) of *H. undatus*. Similarly, the same trend was also observed for the order of ascorbic acid content. In terms of function as a primary antioxidant or chain-breaking antioxidant, the pulps of the two species *Hylocereus* showed equal anti-radical power. By taking the function as secondary antioxidant or metal chelator, the pulp of *H. undatus* was much better than the pulp of *H. polyrhizus* and may offer benefit as inhibitor of the Fenton reaction *in vivo*. The contribution of phenolics and ascorbic acids to each sample's antioxidant activity varied markedly from one to another, depending on their levels in each sample. This study also showed that the peel of both *H. polyrhizus* and *H. undatus* have antioxidant potential.

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