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Full Length Research Paper

Pigment identification and antioxidant properties of red dragon fruit (*Hylocereus polyrhizus*)

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The aim of this study was to identify pigments present in the red dragon fruit (*Hylocereus polyrhizus*) and to further investigate the antioxidant properties in this fruit. Pigment was identified using the High Performance Liquid Chromatography (HPLC) and results confirmed the presence of betanin in sample at a retention time of 11.5 min which corresponded to the retention time of the betanin standard used. In the antioxidant properties determination, there were 86.10 mg of total polyphenolic compound in 0.50 g of dried dragon fruit extract using the total polyphenol assay which expresses gallic acid as equivalent. The reducing power assay further confirmed the antioxidant activity present in dragon fruit where the reducing capability increased from 0.18 to 2.37 with the increase of dry weight sample from 0.03 to 0.5 g. The Vanillin-HCl assay which measures the amount of condensed tannin showed that the dried dragon fruit sample had an equivalent of 2.30 mg catechin/g. The DPPH• radical scavenging activity determination showed that the effective concentration (EC_{50}) for dragon fruit was 2.90 mM vitamin C equivalents/g dried extract.

Key words: Antioxidant, betacyanin, dragon fruit, *Hylocereus polyrhizus*, natural dye.

INTRODUCTION

Hylocereus polyrhizus which originated from Latin America is a member in the Cactaceae family (Stintzing et al., 2002). Members of the Cactaceae family are mainly appreciated for their ornamental qualities but there are at least 250 cultivated species of fruit-bearing and industrial crop in this drought resistant family (Le Bellec et al., 2006).

The exotic aesthetic characteristics of dragon fruit with its attractive deep purple coloured pulp make it highly appealing in the European and United States market (Rebecca et al., 2008a) and widely cultivated in Vietnam, Malaysia, Taiwan, China, Okinawa, Israel and Southern China. The deep purple colour of the pulp is contributed by a set of pigments known as the betalains which are nitrogen-containing pigments (Wyler and Dreiding, 1957; Harivaindaran et al., 2008), made up of the red-violet betacyanins and yellow betaxanthins with maximum absorptivity at 535 and 480 nm, respectively (Herbach et al., 2006b). Betalains have never been found occurring together with anthocyanins and its distribution is restricted to 13 families within the plant kingdom and in some Basidiomycetes (Stintzing and Carle, 2004). According to Stintzing et al. (2002) and Wybraniec and Mizrahi (2002), dragon fruits are totally devoid of betaxanthins and there are at least seven identified betacyanins in the *Hylocereus* genus namely: betanin, isobetanin, lhyllocactin, isophyllocactin, betanidin, isobetanidin and bougainvillein-R-I where all have identical absorption spectra that contribute to the deep purple coloured pulp. Studies have shown that a mature dragon fruit contains considerable amount of total soluble solids, rich in organic acids (Stintzing et al., 2003), protein (Le Bellec et al., 2006) and other minerals like potassium, magnesium, calcium and vitamin C. Cai et al. (2003) reported that the structure-activity relationships of various betacyanins and betaxanthins exhibited free radical scavenging capacities which further contribute to the elevation of interest in *H. polyrhizus* to be a source of antioxidant. Furthermore, Vaillant et al. (2005) established that the dragon fruit exhibited high antiradical activities with the presence of

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Abbreviations: **HPLC,** High performance liquid chromatography; **EC_{50},** effective concentration; **DPPH•,** 2,2'-diphenyl-1-picrylhydrazyl radical.

other phenolic compounds but characterization has yet to be reported. The objectives of this study are to identify the pigment present in the dragon fruit and also to determine the antioxidant capabilities using selected antioxidant assays.

MATERIALS AND METHODS

Sample preparation

Samples for HPLC analysis and all antioxidant activity determination were prepared according to Herbach et al. (2006a) with minor modifications. Fruits from the five weeks after anthesis stage were obtained from a local farm situated 20 km from the laboratory. Fruits were halved and peeled manually. Fruit pulp was squeezed manually through a commercial sieve and the resulting juice was filtered using mira cloth. The pectic substances in the filtered juice were precipitated with 96% ethanol at a ratio of 2 ml of ethanol to 1 ml of juice.

Precipitates were removed using mira cloth and the filtrate was rotary evaporated at 30°C for 90 min. The extract was freeze-dried and frozen at -20°C prior to analysis. Samples for HPLC were purified on Sephadex G-25 in a glass column before analysis. All experiments were carried out in triplicates.

Qualification of betacyanins using HPLC method

The part of the study is aimed at qualifying the main peak observed in *H. polyrhizus* using HPLC by comparing it with the one and only currently commercially available betalain standard which is the betanin standard. HPLC was carried out with modifications according to Wybraniec et al. (2001) by using a Shimadzu Class VP series (LC-10AT-VP HPLC System) with a UV/VIS detector (SPD-10A-VP) equipped with a LiChroCart Purospher Star RP-18 column (id. 250 mm x 4.6 mm x 5 µm) (MERCK). An aqueous trifluoroacetic acid (0.5% TFA) in acetonitrile was used as the mobile phase in an isocratic mode. The detection was set at 537 nm and 10 µL of sample was allowed to elute through the system for 25 min at a flow rate of 1.0 ml/min and column temperature was set to 30°C. The betanin standard was purchased from ABCR GmbH and Co. KG (Karlsruhe, Germany).

Determination of total phenolic contents and reducing power assay

The total phenolic contents of dragon fruit pulp were determined using the Folin-Ciocalteu method according to Bae and Suh (2007) by calculating the polyphenol concentration from a calibration curve (r^2 = 0.9794) using gallic acid as standard with sample detection at 750 nm. The reducing power assay was also carried out according to Bae and Suh (2007) with sample detection set at 700 nm.

Determination of flavonoid content (Vanillin-HCl assay)

The vanillin-HCl assay which measures the amount of condensed tannins was carried out with modification according to Nakamura et al. (2003) from a calibration curve (r^2 = 0.9792). 1 ml of sample was dispensed into a test tube and 5 ml of Vanillin reagent (8% HCl in methanol/1% vanillin in methanol, 1:1, v/v) was added to the sample and incubated in water bath for 20 min at 30°C. Samples were measured at 500 nm and the condensed tannins content was expressed as catechin equivalents in mg. Absorbance of samples were calculated according to the following formula:

$$A = (A_s - A_b) - (A_c - A_o)$$

A_s = sample (1 ml) + vanillin reagent (5 ml).
A_b = methanol (1 ml) + vanillin reagent (5 ml).
A_c = sample (1 ml) + methanol (2.5 ml) + 8% HCl (2.5 ml).
A_o = methanol (1 ml) + methanol (2.5 ml) + 8% HCl (2.5 ml).

2,2 -Diphenyl- 1 -picrylhydrazyl (DPPH$^{\bullet}$) radical scavenging activity

The DPPH$^{\bullet}$ radical scavenging assay was carried out according to Cai et al. (2003) with minor modifications. 0.1 ml of sample was reacted with 3.9 ml of 80% ethanolic 0.1 mM DPPH$^{\bullet}$ solution in a test tube. The test tube was vortexed for 15 s and solution was allowed to stand at room temperature (25 ± 2°C) for 180 min. Absorbance was measured at 515 nm. Antioxidant activity was expressed by (i) calculating the radical scavenging activity: Median effective concentration (EC_{50}) = concentration of sample required to decrease 50% in absorbance of DPPH$^{\bullet}$ radicals and (ii) inhibition (%) of DPPH$^{\bullet}$ absorbance = $(A_{control} - A_{test}) \times 100/A_{control}$. Ethanol (80%) was used as blank and DPPH$^{\bullet}$ solution without test sample was used as control. A dose-response curve (% inhibition of DPPH$^{\bullet}$ versus concentration of sample) was established and the EC_{50} was determined (r^2 = 0.9809) using vitamin C as a standard. Results were expressed as vitamin C equivalents.

RESULTS

Qualification of betacyanins using HPLC method

In the pigment qualification, only one peak could be confidently identified in the dragon fruit pulp sample by comparing the results with the available betanin standard. The sample peak was observed at 11.5 min with a peak area of 215703.3µV*s (Figure 1). This observation corresponds with the results from the betanin standard which gave a similar peak at 11.6 min with a peak area of 78167 µV*s (Figure 2).

Antioxidant properties

The total polyphenol assay which expresses gallic acid as equivalent showed that there was 86.13 ± 17.02 mg of total polyphenolic compound in 0.50 g of dry dragon fruit extract (Table 1). The reducing power assay showed that the reducing capability of antioxidants in the dragon fruit extract increased from 0.18 ± 0.02 in 0.03 g extract to 2.37 ± 0.18 in 0.50 g extract (Figure 3). The Vanillin-HCl assay showed that the sample had an equivalent of 2.3 ± 0.2 mg catechin/g dried extract (Table 1). The DPPH• radical scavenging activity determination showed that the effective concentration (EC_{50}) for dragon fruit was 2.9 ± 0.4 mM vitamin C equivalents/g dried extract (Table 1).

DISCUSSION

Qualification of betacyanin using HPLC method

Betanin was the first described betacyanin in red beetroot

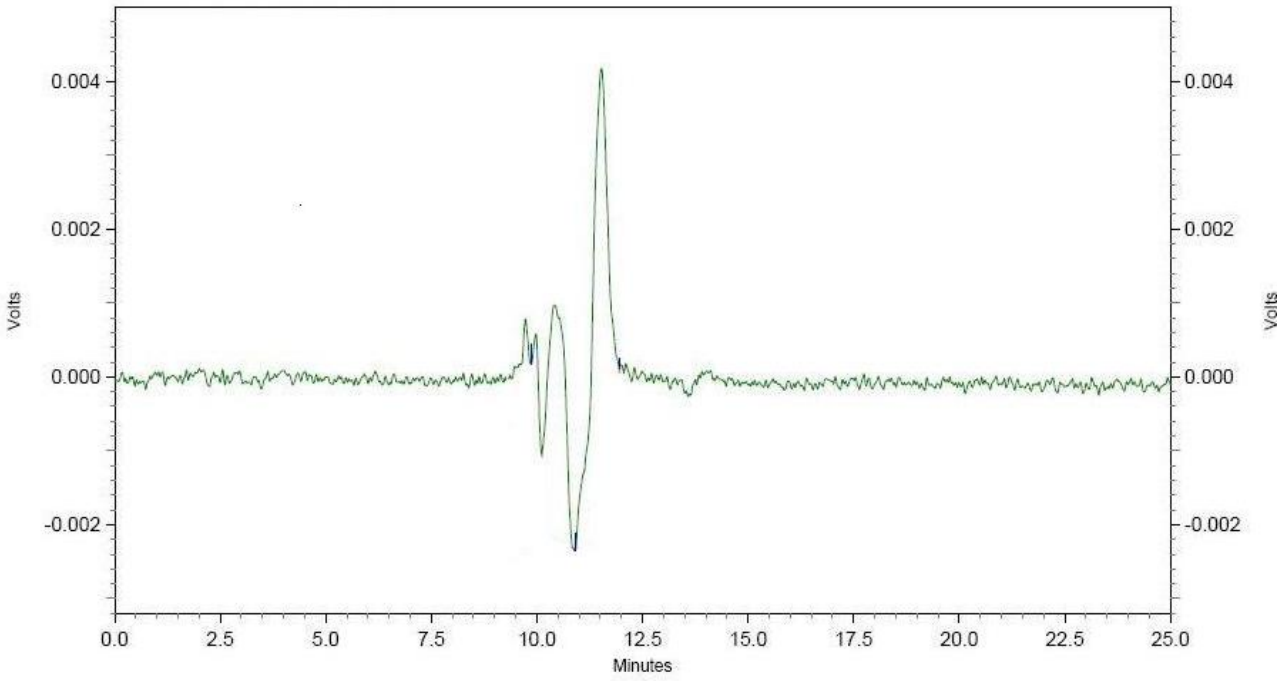

Figure 1. Peak and retention time of *H. polyrhizus* sample using HPLC observed at 11.5 min.

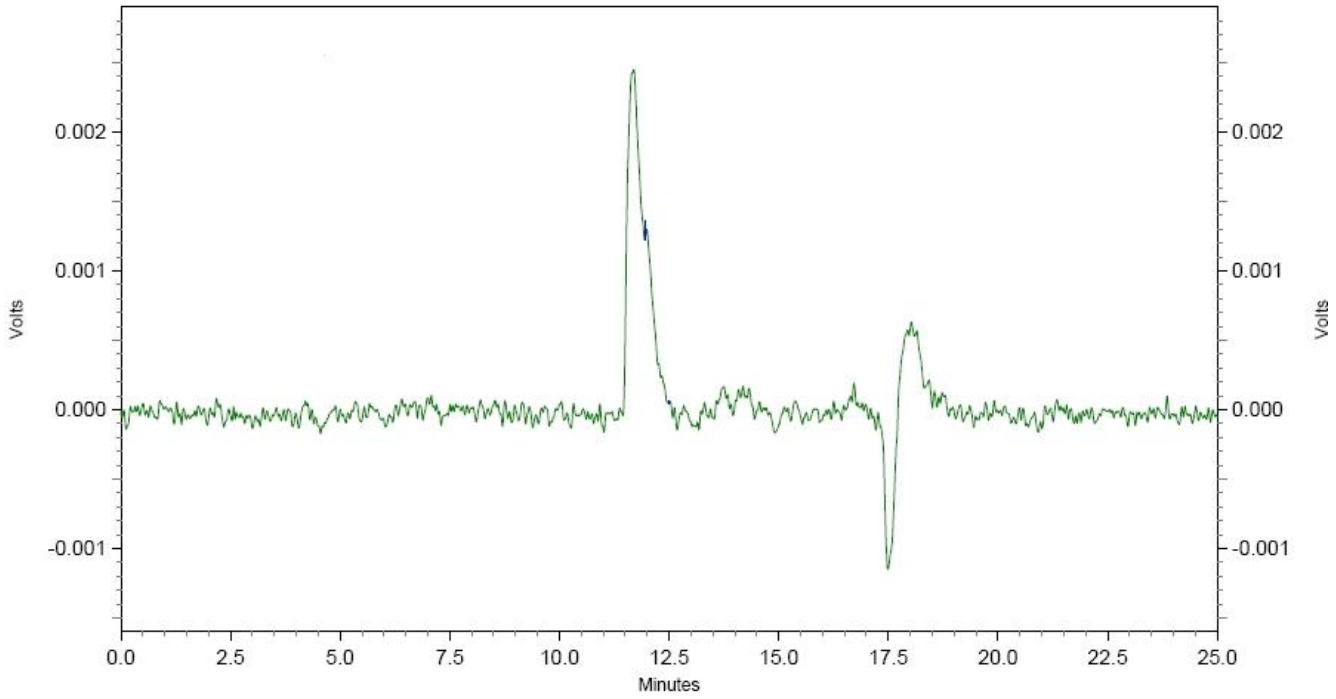

Figure 2. Peak and retention time of betanin standard using HPLC observed at 11.6 min.

(Gandia-Herrero et al., 2005; Wyler and Dreiding, 1957) and is one of the main pigments present in the *Hylocereus* genus. Looking at the results, the single peak from the purified dragon fruit sample corresponded to the peak eluted from the betanin standard. To date, betacyanin identification employs a two-way strategy which is first, using the HPLC to establish the retention time of sample and then secondly, using the electrospray MS-MS and ^{1}H

Table 1. Total polyphenolic content, flavonoid content and DPPH• radical scavenging activity.

Sample	Total polyphenolic content (mg/0.5 g gallic acid)	Total flavonoid content (mg/g catechin)	DPPH• radical scavenging activity (EC50) (mM vitamin C)
Dragon fruit	86.129 ± 17.016	2.3 ± 0.20	2.9 ± 0.40

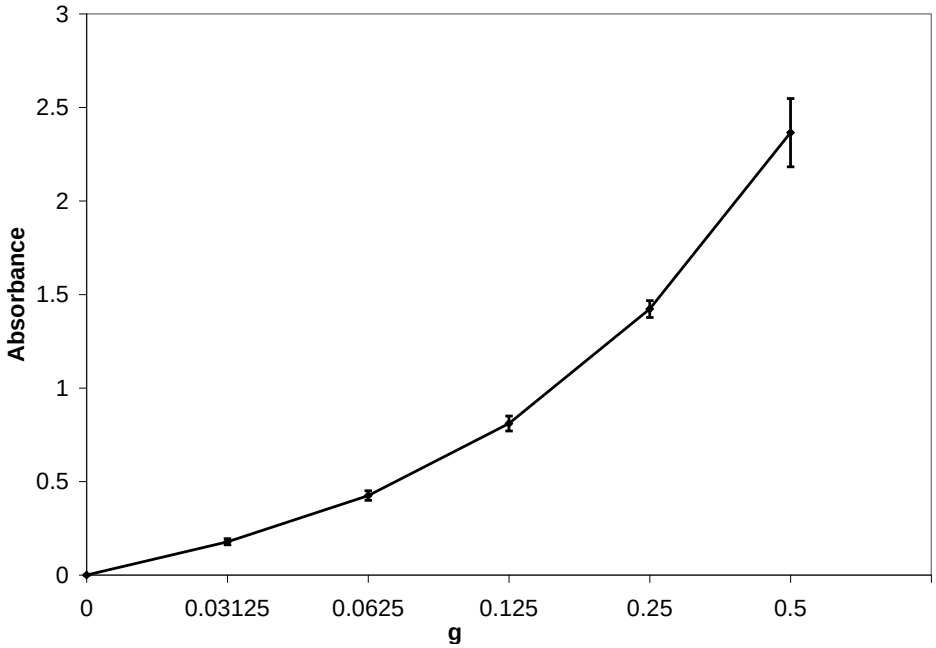

Figure 3. Reducing power in dragon fruit pulp extract.

NMR techniques to further elucidate their structure (Cai et al., 2006; Wybraniec and Mizrahi, 2002). Wu et al. (2006) and Cai et al. (2003) have succeeded in identifying three and more peaks in *H. polyrhizus* using a comparison analysis of pre-determined HPLC retention time and available literatures from many other betalain producing plant like *Amaranthus* sp. and *Beta vulgaris*. Results obtained from this study identified the presence of betanin in *H. polyrhizus* and as the main contributing pigment in the deep purple coloured pulp.

Antioxidant properties

Bae and Suh (2007) reported that active oxygen species such as hydroxyl (OH•) are thought to be agents that cause oxidative damage and much attention has been focused on active oxygen scavenging agents such as natural phenolics to prevent cell damage. One of the important constituents in dragon fruit is the betacyanins which are strong antioxidants (Wu et al., 2006; Vaillant et al., 2005; Stintzing et al., 2003). According to Somers and Evans (1977), there are no methods to precisely quantify total phenolics because of its diverse chemical structures. In this study, the Folin-Ciocalteu method was used because it is a generally preferred analytical method for determination of total polyphenol using gallic acid as an arbitrary standard (Lako et al., 2008; Rebecca et al., 2008b; Wu et al., 2006; Cai et al., 2004). According to Lako et al. (2007, 2008), common fruits with significant content of total polyphenol include: *Musa* sp. (Banana) with 110 µg/g total polyphenol; *Ananas comosus* (Pineapple) with 150 µg/g; *Carica papaya* (Papaya) with 260 µg/g; tomatoes with 350 µg/g; cherries with 670 µg/g and blueberries with 3180 µg/g. In this study, result shows a total phenolic content of 86.13 ± 17.02 mg in 0.50 g of dry *H. polyrhizus* extract. This amount is highly significant if compared to the aforementioned common fruits and it is definitely a good source of polyphenol to be integrated into the human diet. The reducing power method employs ferric chloride ($FeCl_3$) as an oxidant and ferrous ions are produced from the redox reaction which forms a coloured complex with trichloroacetic acid. According to Kumaran and Karunakaran (2006), reducing capabilities of materials are usually associated with the presence of reductones which exhibit antioxidant action by stopping the free radical chain reaction by donating a hydrogen atom. In the reducing power assay results, samples showed reduction capability even at low concentrations, indicating that antioxidant activities were present

in *H. polyrhizus* and the reducing capability increases with increasing sample concentration. The Vanillin-HCl assay which is based on the metal-complexing properties and the high affinity of tannins to form protein-tannin complexes is used to detect the presence of condensed tannins and is preferred because of its sensitivity and simplicity. Condensed tannins consist of two or more flavan- 3 -ol like catechin, epicatechin or gallocatechin. In the Vanillin-HCl assay, vanillin is protonated in an acidic solution which gives a weak electrophilic carbocation that will react with available flavonoid ring (Nakamura et al., 2003). The DPPH• radical scavenging activity is determined by the decrease in absorbance induced by antioxidant, reducing the purple colour of DPPH• radical to a yellow diphenylpicryhydrazine. In both Vanillin-HCl and DPPH• assay, the results indicated that the dragon fruit extract contained phenolic contents comparable to standard anti-oxidant agents like Vitamin C and catechin. The assays selected in this study to determine the antioxidant properties of *H. polyrhizus* were based on the viability of each assay. This is because the *H. polyrhizus* natural red coloured pigment easily interferes with many antioxidant assays which are highly dependent on the reduction or formation of colour from the antioxidant reactions. Hence, the results from this study may vary from previous studies on *H. polyrhizus* or other choice of material because there were modifications carried out as mentioned in the methodology.

Conclusion

The HPLC analysis using a single betanin standard successfully qualified one peak from the dragon fruit sample. In the antioxidant analysis, the dragon fruit represent a significant source of antioxidants which is a value added characteristic to any food crop. Further analysis and work on *H. polyrhizus* should continue as it is a new valuable crop with significant amount of antioxidants which could be beneficial for consumers and the pharmaceuticals industry.

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