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Determination of radical scavenging activity and Vitamin A, C and E in organically grown Red Pitaya (*Hylocereus* sp.)

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Abstract: This study was conducted to determine radical scavenging activity and vitamin antioxidant composition in red pitaya from organic plantation. For antioxidant vitamins analysis, a reverse-phase high performance liquid chromatography was used and radical scavenging activity of methanolic and water extract were determined using 1,1-diphenyl-2-pircrylhydrazyl assay. Results for radical scavenging activity, red pitaya methanolic extract achieved the highest percentage 70.13% compared with water extract (47.13%). Antioxidant vitamins composition in red pitaya showed that the concentration of vitamin A is 120.13 ± 0.69 µg/100 g freeze-dried sample, vitamin C is 540.27 ± 0.59 µg/100 g fresh samples and vitamin E is 105.67 ± 0.56 µg/100 g freeze-dried samples. This shows that red pitaya may become an alternative and potential source of natural antioxidant.

Keywords: Red pitaya, antioxidant vitamin, radical scavenging activity

Introduction

The red pitaya (*Hylocereus sp*.) have recently drawn much attention of growers worldwide, not only because of their red-purple colour and economic value as food products but also for their nutritional content (Wybraniec *et al.*, 2001). Red pitaya is believed native to Southern Mexico, the pacific side of Guatemala and Costa Rica and El- Salvador derive from climbing epiphytes belonging to the Cactaceaca family (Stintzing, 2002). Red pitaya possesses large scales instead of spines and its pulp only contains small digestible seeds (Raveh *et al.,* 1998). They are currently being grown commercially in Taiwan, Nicaragua, Colombia, Vietnam, Israel, Australia, USA and now in Malaysia. Efforts have been made to study the nutritional value of white pitaya (Lee, 2002); however there is little information available on antioxidant vitamins in red pitaya.

Plants and fruits are potential sources of natural antioxidants. They produce various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive (Huda-Faujan *et al.,* 2007; Lu and Foo, 1995) Antioxidant vitamins in fruits are some of the important nutrients besides other vitamins, mineral, flavanoids and phytochemicals which have been reported to contribute to health. In recent years, great interest has been focused on antioxidant vitamins (A, C and E) particularly because of their likely role in prevention of coronary heart disease and cancers (Bauman, 2004; Parillo and Ricardi, 2004). The level of the essential antioxidant vitamins in contrast to other antioxidative defenses, are determined mainly by their dietary supply. Fruits and vegetables are the main source of antioxidant vitamins, making these foods essential to human health (Abdulnabi *et al*., 1998). The objectives of this study were to evaluate the antioxidant vitamins composition and radical scavenging activity in red pitaya.

Materials and Methods

Extraction for free radical scavenging activity

For determination of free radical scavenging activity red pitaya fruit were obtained from plantation in Lembah Bidong, Terengganu, Malaysia. The fruits were carefully washed under running tap water, dried with a soft cloth and the skin peeled; the fresh flesh was then cut into small pieces (1.5 cm x 1.5 cm x 1.5 cm) and macerated in 80% (v/v) methanol for 5 days. Sample was extracted twice. The methanol residue

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was removed from the extract by using a rotary evaporator (Buchi Rotavor R-200, Switzerland) under reduced pressure for 20 minutes at 700C and the sample was then lyophilised and store at -200C until further analysis.

Extraction for vitamins analysis

For vitamins analysis, upon arrival at the Department of Nutrition and Health Sciences laboratory, the red pitaya immediately washed under tap water. Edible portion (100 g) of the red pitaya were cut into small pieces and homogenized using blender (National, model MX-291N) for 2 minutes. The homogenized sample was transfer into air-tight container and kept at -80°C for 2 days before freeze dried using freeze dryer (BEWHAY/SB4/United Kingdom) at 45°C, pressure 12 militor for 3 days. After freeze-dried, sample was kept at 20°C before analysis. All procedures were carried out carefully without much exposure to light.

Measurement of radical scavenging activity

The free radical scavenging activity of the samples was measured in accordance to the method of Brand-Williams *et al.* (1995) with slightly modification. A total of 10 g extracts were dissolved in 1.0 mL methanol and the solution added to a 1.0 mL $DPPH^{\cdot}$ solution at room temperature. The absorbance at 515 nm was measured utilizing UV-1601 Shimadzu spectrophotometer. The results were expressed as percentage of reduction of the initial $DPPH^{\cdot}$ absorption by test samples as follows:

$$\text{DPPH scavenging effect (\%)} = [(A_0-A_t) / A_0] \times 100$$

Where is the absorbance of the control at t = 0 min, and A_t is the absorbance of the antioxidant at t = 15 minutes. The IC_{50} is defined as the concentration of antioxidant necessary to decrease the initial DPPH concentration plot and is expressed as mg/mL.

Extraction of vitamin A

Vitamin A, was extracted accordingly to the method described by Amin and Cheah (2003) and Tee *et al.* (1996) with slightly modification. 5 g sample was added with 40 mL of 95% ethanol and 10 mL of 100% (w/v) potassium hydroxide and homogenized for 3 minute. The mixture was saponified by means of a refluxing apparatus and heated using an electric heating mantle for 30 minute and then cooled to room temperature. The mixture was frequently agitated to avoid any aggregation. For the extraction step, the mixture was transferred into separation funnel and 50 mL n-hexane was added. The funnel was inverted, vented and then shaken vigorously for a few seconds, and the layer was allowed to separate. The upper layer (hexane extract) was pipette out and the aqueous layer was re-extracted twice, each time with 50 mL of n-hexane. Then, the upper layer was pooled and washed with distilled water until free of alkali. The extract was then filtered through anhydrous sodium sulphate to remove any water residue. The hexane residues were removed under reduced pressure at 45°C using rotary evaporator (Laboratory 4000, Heidoplh Instruments GmbH and Co. KG, Germany). The resulting extract was diluted to 10 mL with HPLC grade-hexane. Samples were carried out in five-replicates.

Extraction of vitamin C

Vitamin C was extracted accordingly to the combination of method of Amin and Cheah (2003) and Abdulnabi *et al.* (1996) with slightly modification. 5 g sample was added with 50 mL of 2% metaphosphoric acid. The mixture was placed in a conical flask (wrapped with aluminium foil) and agitated at 100 rpm with the aid of an orbital shaker for 15 min at room temperature. The mixture was then filtered through a Whatman No.4 filter paper to obtain a clear extract. The ratio of the sample to extraction solution was 1 to 1. Sample was extracted in triplicates.

Extraction of vitamin E

Vitamin E was extracted accordingly to the method describe by Amin and Cheah (2003) and Abdulnabi *et al.* (1996) with slightly modification. 5 g sample was added with 20 mL methanol. The mixture was mixed with 60 mL CCl_4 methanol (3:1) and agitated at 100 rpm with the aid of an orbital shaker for 20 minutes. The CCl_4 fraction was separated from the aqueous phase in a separatory funnel and dried over Na_2SO_4. The filtrate was evaporated to dryness under pressure 45°C using rotary evaporator (Laboratory 4000, Heidoplh Instruments GmbH and Co. KG, Germany). The extract lipid fraction was saponified by refluxing with 4 mL of 30% methanolic KOH for 30 minute at the boiling point of methanol in the presence of 0.5 g ascorbic acid. After cooling the flask at room temperature, 15 mL of salted water added and the analogues of tocopherol were extract twice with 40 mL petroleum ether in a separator funnel. The ether fractions were colleted, washed twice with distilled water and dry over anhydrous sodium sulphate. The solvent was evaporated at 45°C using rotary evaporator. The residues were re-dissolved in 5 mL of HPLC-grade hexane. All samples were carried out in triplicates.

Table 1. HPLC conditions for separation and identification of vitamin A, C and E

Parameters	Conditions		
	Vitamin A	Vitamin C	Vitamin E
Mobile phases	Acetonitrile-methanol-ethyl acetate (88:10:2)	i) 0.1M potassium acetate pH 4.9 ii) Acetonitrile water (50:50)	Acetonitrile-methanol-ethyl acetate (88:10:2)
Flow rate	1.0 mL/min	1.5 mL/min	1.0 mL/min
Detection	250 nm	254 nm	250 nm

Table 2. Radical scavenging activity and IC_{50} value of red pitaya

Sample	Antioxidant Activity (%)	IC_{50} (mg/mL)
Methanolic extract		
0.1 mg/mL	50.21 ± 1.34[a]	1.32 ± 0.18[a]
0.2 mg/mL	54.32 ± 1.04[b]	1.57 ± 0.29[b]
0.3 mg/mL	59.21 ± 1.32[b]	1.67 ± 0.13[b]
0.4 mg/mL	65.78 ± 1.14[c]	1.78 ± 0.73[b]
0.5 mg/mL	71.20 ± 1.34[d]	1.96 ± 0.23[c]
Water extract		
0.1 mg/mL	38.19 ± 1.14[a]	1.12 ± 0.12[a]
0.2 mg/mL	40.72 ± 1.54[a]	1.27 ± 0.32[b]
0.3 mg/mL	42.31 ± 1.12[b]	1.37 ± 0.14[b]
0.4 mg/mL	45.68 ± 1.21[c]	1.39 ± 0.25[b]
0.5 mg/mL	47.54 ± 1.64[d]	1.45 ± 0.19[c]

Each mean represents analyses of five independent samples
abc Variation in the following letters between samples indicates significant of difference by Duncan's test at 5% level ($p<0.05$)

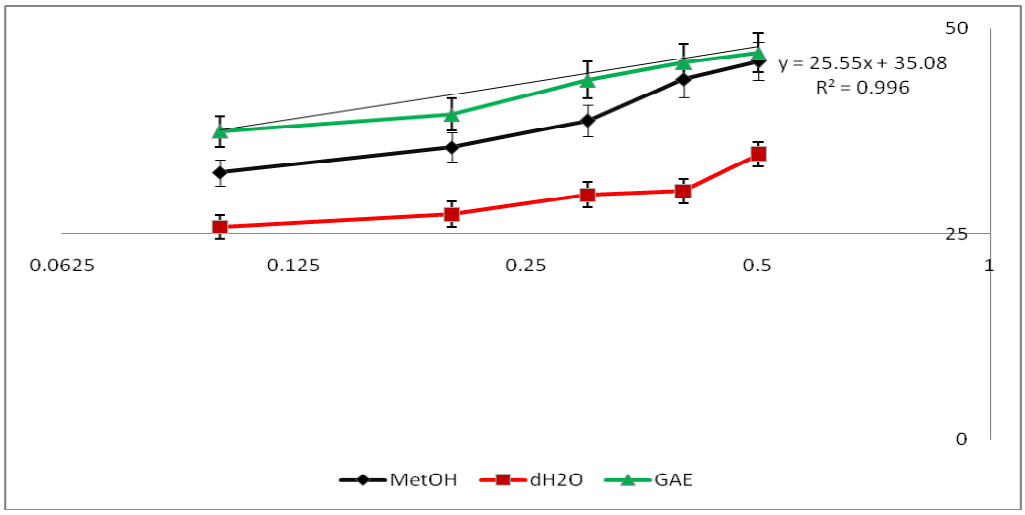

Figure 1. Radical scavenging activity and IC_{50} value of methanolic and water extract of red pitaya

Each mean represents analyses of five independent samples
Variation in the following letters between samples indicates significant of difference by Duncan's test at 5% level ($p<0.05$)

The vitamins were determined by a reverse-phase HPLC technique. A Hewlett Packard HPLC series 1100, USA equipped with degasser, quaternary pump, auto sampler and diode array detector was used. A Utrasphere octadecylsilyl (ODS) Hypersil C18, 5 mm particle size, in a 250 nm length x 4.0 nm I.D stainless steel column (Hewlett Packard) was used to determine the vitamins. The separation conditions for the antioxidant vitamins are tabulated in Table 1

Identification and quatification of vitamins by HPLC

For identification and quantification of vitamins by HPLC, the peak of vitamin A was identified used on two techniques: comparing the retention time and spiking test with that of trans-β-carotene (Sigma, Co. Chemical, St Louis, USA). 10 mg of trans-β-carotene was weighed and dissolved in pure n-hexane to give stock solution of 100 µg/mL. The solution was stored in a brown bottle and kept as stock in the fridge (45°C). Peak for vitamin C was identified on the chromatogram by comparing the retention time and spiking test what that of L-ascorbic acid (Sigma, Co. Chemical, St Louis, USA). Ascorbic acid standard was prepared by dissolving 100 mg of L-ascorbic acid in a metaphosphoric acid (0.3M)- acetic acid (1.4 M) solution at the final concentration of 1 mg/mL. The peak for vitamin E also identified based on comparing the retention time and spiking test what that of α-tocopherol (Sigma, Co. Chemical, St Louis, USA). 10 mg of α-tocopherol was weighed and dissolved in pure n-hexane to give stock solution 100 µg/mL. The solution was stored in a brown bottle and kept as stock in the fridge (4-5°C).

Statistical analysis

All data was reported as mean ± standard deviation (SD) of triplicate determination and analyzed using one-way analysis of variance (ANOVA) with significant differences between means determined at $p<0.05$, measured with Duncan's multiple range tests using Statistical Package for Social Science Research Version 14.0 (SPSS).

Results and Discussion

Many plants are consumed not only as fruits or used in food preparations but there are also utilized for medicinal purposes. Fruits contain significant levels of biologically active components that impart health benefits beyond basic nutrition (Oomah and Mazza, 2000). DPPH, a pragmatic compound with an odd electron, shows strong absorption band at 517 nm in methanol. The absorbance decreases as the result of color change from purple to yellow due to the scavenging of free radical by antioxidants through donation of hydrogen to form the stable DPPH-H molecule (Azizah *et al.,* 2009; Chandrasekar *et al.,* 2006).

The methanolic and water extract of red pitaya (Table 2) show the ability to reduce the stable of purple colour radical, DPPH, into the yellow-coloured DPPH-H reaching 50% of reduction. For methanolic extract shows the highest value of radical scavenging activity (71.20%) and IC_{50} value is 1.96 mg/mL at the higher concentration (0.5 mg/mL) compared to water extract shows value only at 47.54% and IC_{50} value is 1.45 mg/mL at the same concentration. Data show that the sample increased their reducing ability when the concentration of extracts was increased, from here we can conclude that the highest values of radical scavenging activity show the higher antioxidant capacity in red pitaya. This result is similar to that reported by Gulcin *et al.* (2003) and Noriham *et al.* (2004), who demonstrated antioxidative activity on *Pimpinella anisum* seeds extracts and four types of Malaysia plants. For vitamin quantification was calculated from the curve generated by plotting the peak area of each authentic standard versus concentration.

Antioxidant vitamins composition in red pitaya was quantification as follow, vitamin A value was 120.13 ± 0.69 µg/100 g freeze-dried weights. The red pitaya had the highest content of vitamin C of 540.27 ± 0.59 µg/100 g fresh weight compared with the other vitamins. The vitamin E content was 105.67 ± 0.56 µg/100 g freeze-dried weights. From the results, red pitaya has been shown to have high antioxidant vitamins content. This antioxidant vitamins work both or singly and synergistically to prevent or delay oxidative reactions that lead to degenerative disease, including cancers, cardiovascular disease, cataracts and other disease (Elliot, 1999). From these results, it can be concluded that red pitaya content high in antioxidant vitamins. In this study factors such as environment and post harvest practices that could influence vitamin content were not controlled. Nevertheless, this study is useful as a step towards further work on the generation of vitamin content database in fruits.

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