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Antioxidant and antiproliferative activities of red pitaya

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Abstract

The red pitaya, rich in micronutrients, has recently generated a great deal of consumer interest, therefore, this paper was designed to study the total phenolic content, antioxidant activity and antiproliferative activity of red pitaya on melanoma cells and to determine if it is a valuable source of antioxidants and an anticancer agent. The total phenolic contents of flesh (42.4 ± 0.04 mg of gallic acid equivalents (GAE)/100 g of flesh fresh weight) and peel (39.7 ± 5.39 mg of GAE/100 g of peel fresh weight) were similar. The flavonoid contents of flesh and peel did not vary much (7.21 ± 0.02 mg vs. 8.33 ± 0.11 mg of catechin equivalents/100 g of flesh and peel matters). The concentrations of betacyanins expressed as betanin equivalents per 100 g of fresh flesh and peel were 10.3 ± 0.22 and 13.8 ± 0.85 mg, respectively. The antioxidant activity, measured by the DPPH· method at EC_{50}, was 22.4 ± 0.29 and 118 ± 4.12 μmol vitamin C equivalents/g of flesh and peel dried extract; the values of EC_{50}, determined by the $ABTS^{\cdot+}$ approach, were 28.3 ± 0.83 and 175 ± 15.7 μmol of trolox equivalents antioxidant capacity (TEAC)/g of flesh and peel dried extract, respectively. The antiproliferative study on B16F10 melanoma cells revealed that the peel (EC_{50} 25.0 μg of peel matter) component was a stronger inhibitor of the growth of B16F10 melanoma cancer cells than the flesh. The results indicated that the flesh and peel were both rich in polyphenols and were good sources of antioxidants. The red pitaya peel fulfilled its promise to inhibit the growth of melanoma cells.

Keywords: Phenolics; Red pitaya; Polyphenols; Antioxidant activity; DPPH·, $ABTS^{\cdot+}$; B16F10 melanoma cells

1. Introduction

The leading causes of death in the United States are cardiovascular diseases and cancers. Similarly, in Taiwan, around 27% of deaths are from cancer and 18% of deaths are from cardiovascular and heart diseases (Department of Health Web, 2004). It was estimated by Willet (1994) that roughly 32% (range of 20%–42%) of deaths from cancer could be avoided by dietary modification. Epidemiological studies have strongly suggested that diet plays an important role in the prevention of chronic diseases (Bauman, 2004; Parillo & Riccardi, 2004; Willet, 1995). Polyphenolics, thiols, carotenoids, tocopherols, and glucosinolates commonly found in fruits, vegetables and grains, provide chemoprotective effects to combat oxidative stress in the body and maintain balance between oxidants and antioxidants to improve human health (Adom & Liu, 2002; Dragsted, Strube, & Larsen, 1993; Jia, Tang, & Wu, 1999; Wolfe, Wu, & Liu, 2003). An imbalance caused by excess oxidants leads to oxidative stress, resulting in damage to DNA and protein and increased risk of

Abbreviations: GAE, Gallic acid equivalents; TEAC, Trolox equivalent antioxidant capacity; CHD, Coronary heart disease; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

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degenerative diseases such as cancer (Farombi, Hansen, Ravn-Haren, Moller, & Dragsted, 2004; Llopiz et al., 2004; Moller & Loft, 2004; Sander, Chang, Hamm, Elsner, & Thiele, 2004; van Meeteren, Hendriks, Dijkstra, & van Tol, 2004).

Consumption of fresh fruits and vegetables, as well as grains, has been associated with reduced risk of coronary heart disease (CHD) (Bazzano, Serdula, & Liu, 2003; Joshipura et al., 2001; Polidori, 2003; Srinath Reddy & Katan, 2004), stroke (Gillman et al., 1995; Voko, Hollander, Hofman, Koudstaal, & Breteler, 2003), symptoms of chronic obstructive pulmonary disease (Fabricius & Lange, 2003; Liu, Sobue, Otani, & Tsugane, 2004), and different types of cancer, including breast and ovarian cancer (Duncan, 2004; Hakimuddin, Paliyath, & Meckling, 2004; Khanzode, Muddeshwar, Khanzode, & Dakhale, 2004; Larsson, Holmberg, & Wolk, 2004) and colon cancer (Frydoonfar, McGrath, & Spigelman, 2003; McCullough et al., 2003). Polyphenolic compounds, widely distributed in higher plants, have been found to have potential health benefits that are believed to arise mainly from their antioxidant activity (Liu, 2003). There is considerable scientific and public interest in the important role that antioxidants may play in health care, such as by acting as cancer chemopreventive and anti-inflammatory agents and by reducing risk of cardiovascular mortality (Cos et al., 2004; Siddiqui, Afaq, Adhami, Ahmad, & Mukhtar, 2004). Numerous studies have reported the antioxidant and antiproliferative activities of different fresh produces, such as apples (Wolfe et al., 2003), grape seeds (Calliste, Trouillas, Allais, Simon, & Duroux, 2001; Chidambara Murthy, Singh, & Jayaprakasha, 2002), strawberries (Meyers, Watkins, Pritts, & Liu, 2003), common vegetables (Chu, Sun, Wu, & Liu, 2002), cranberry fruits (Gunes, Liu, & Watkins, 2002), and raspberries (Liu et al., 2002). However, the antioxidant activity of some plant foods has not been well studied. Investigation of their antiradical characteristics is therefore of interest, primarily to discover promising new sources of natural antioxidants, functional foods, and neutraceuticals.

The fruits of *Hylocereus* cacti, known as red pitaya or pitahaya, 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 antioxidative activity from the betacyanin contents (Wybraniec & Mizrahi, 2002). The *Hylocereus* genus consists of climbing or epiphytic tropical American cacti with angular stems and mostly white, fragrant, night-blooming flowers. The pitaya, the fruit of some strains of this cactus family, which is native to the tropical forest regions of Mexico and Central and South America (Mizrahi, Nerd, & Nobel, 1997) and is mentioned as a popular Aztec fruit in historical documents, has a long lineage in the western hemisphere. The skin is covered with bracts, or "scales" – hence the name dragon fruit. They are currently being grown commercially in Taiwan, Nicaragua, Colombia, Vietnam, Israel, Australia and the USA.

In cacti, the most important fruit pigments are the betacyanins and betaxanthins (Wybraniec et al., 2001). Betalains, composed of red-violet betacyanin and yellow betaxanthins, are water-soluble pigments that provide colours in flowers and fruits. The known betacyanin pigments of *Hylocereus polyrhizus* flesh are betanin, phyllocactin (6′-*O*-malonylbetanin), and a recently discovered betacyanin, hylocerenin (5-*O*-[6′-*O*-(3″-hydroxyl-3″-methyl-glutaryl)-β-D-glucopyranoside) (Wybraniec & Mizrahi, 2002; Wybraniec et al., 2001). Betacyanin, with 5-*O*-glycosides (betanin) or 6-*O*-glycosides, is commonly detected in plants. More-complicated esterification of 5-*O*-glycosides with hydrocinnamic acids such as ferulic or *p*-coumaric acids (Strack, Steglich, & Betalains, 1993) and malonic acid (Kobayashi, Schmidt, Nimtz, Wray, & Schliemann, 2000; Minale, Piattelli, De Stefano, & Nicolaus, 1996) have also been studied.

It has been reported that betalains from *Amaranthus* (Cai, Sun, & Corke, 2003) and beet roots (Escribano, Pedreòo, Garcia-Carmona, & Munoz, 1998) demonstrated antiradical and antioxidant activity. Betanin, from red beet, effectively inhibited lipid peroxidation and heme decomposition, suggesting that these pigments may provide protection against certain oxidative stress-related disorders (Kanner, Harel, & Granit, 2001). Polyphenolics, on the other hand, play an important role in antioxidant activity (Wu, Chen, Ho, & Yang, 2003). They have evidently shown antiproliferative activity or cytotoxicity to human oral cancer cells (Seeram, Adams, Hardy, & Heber, 2004), melanoma cells (Rodriguez et al., 2002), and lung metastasis induced by B16F10 melanoma cells (Menon, Kuttan, & Kuttan, 1995).

Efforts have been made to study the chemistry of betalains in *H. polyrhizus* (Stintzing, Conrad, Klaiber, Beifuss, & Carle, 2004; Wybraniec et al., 2001; Wybraniec & Mizrahi, 2002); however, there is little information available on its phenolic content, antioxidant activity, and antitumor activity. The objectives of this study were to evaluate the nutritional quality of red pitaya flesh component and peel. Total phenolic content, antioxidant capacity, and antiproliferative effect on B16F10 melanoma cells were quantified and compared to those characteristics of betanin in relation to the same cancer cell. In addition, it could also be determined whether the peel of red pitaya, the waste product from juice manufacture, could be utilized as a potential alternative for various sources of nutrients or antioxidants to improve human health.

2. Materials and methods

2.1. Chemicals

Folin–Ciocalteu reagent, sodium nitrite, catechin, gallic acid, Sephadex G-25, MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide), and all other chemicals and organic solvents were purchased from Sigma Chemical Co. (St. Louis, MO). LH-20 was obtained from Amersham Biosciences (Uppsala, Sweden). Whatman No.1 filter paper was bought from Fisher Scientific (Fair Lawn, NY). HPLC-grade acetonitrile and acetic acid came from Merck (Darmstadt, Germany). The B16F10 melanoma cells were obtained from the American Type Culture Collection (ATCC) (Rockville, MD). Fetal bovine serum (FBS) was purchased from Gibco Life Technologies (Grand Island, NY), and betanin standard was obtained from TCI (Tokyo, Japan).

2.2. Sample preparation

Red pitaya, *H. polyrhizus*, used in this study was obtained locally from four lots of fruits (Puli, Nantou, Taiwan), which were washed and stored at −20 °C before analysis. The fruit was peeled prior to analysis. The phenolic content, of different components of red pitaya, was determined by extracting 50 g of the flesh and peel components with 200 g of chilled 80% acetone solution in a Waring blender for 10 min (Wolfe et al., 2003). The slurry was filtered through Whatman No. 1 filter paper in a Buchner funnel under vacuum. The solids were re-extracted with 150 g of 80% acetone and homogenized again for an additional 5 min before refiltering. The filtrate was then evaporated using a rotorary evaporator at 45 °C until less than 10% of the initial volume remained. The extract was freeze-dried and frozen at −70 °C prior to analysis.

2.3. Determination of total phenolic contents

The total phenolic contents of flesh and peel samples of red pitaya were determined using a modified Folin-Ciocalteu method (Wolfe et al., 2003). A 125 μl aliquot of a known dilution of the extract was added to the test tube and combined with 0.5 ml of Folin–Ciocalteu's reagent. The tubes were vortexed for 15 s and then allowed to stand for 6 min at 20 °C. About 1.25 ml of 7% sodium carbonate solution was then added to the test tubes, and the mixture was diluted to 3 ml with distilled, de-ionized water. Colour developed for 90 min, and absorbance was measured at 760 nm using the Hewlett Packard UV–Vis spectrophotometer (Palo Alto, CA). The measurement was compared to a standard curve of prepared gallic acid solutions and expressed as gallic acid equivalents in milligrams. Triplicate determinations were performed on each sample; data shown later represent the means of three measurements.

2.4. Determination of flavonoid content (vanillin-HCl assay)

The amount of flavonoid (condensed tannins) was measured by vanillin assay (Price, van Scoyoc, & Butler, 1978). One millilitre aliquots of peel and flesh extracts were dispensed into test tubes. Tubes were incubated in the water bath at 30 °C. Five millilitres of the vanillin reagent (1% vanillin in methanol) were added at 1.0 min intervals to one set of samples, and 5.0 ml of the 4% HCl solution were added at 1.0 min intervals to the second set of samples. After 20 min of incubation, the samples were removed and the absorbance at 500 nm was read. The condensed tannins content (flavonoid) was expressed as catechin equivalents in milligrammes. Triplicate determinations were performed on each sample; data shown later represent the means of three measurements.

2.5. Quantitation of betacyanins

Red pitaya pigment content was measured in a similar way to that described by Wybraniec and Mizrahi (Wybraniec & Mizrahi, 2002). The betacyanin content of dried extracts, determined by the spectrophotometric method, were calculated and expressed as betanin equivalents (mg/100 g of fresh materials) by the following formulas: concentration of betacyanins (mg/g of dried extracts weight) = A_{538} (MW)V (DF) × 100/εLW. A_{538} is the absorbance at 538 nm (λ_{max}); L (path length) = 1.0 cm; DF is the dilution factor; V is the pigment solution volume (ml); W is the dried pigment weight (g). For betanin, ε (molar extinction coefficient) = 65,000 and MW = 550. Individual pigment content was expressed as the percentage of peak area determined by HPLC.

2.6. Determination of anthocyanin contents

The monomeric anthocyanin content of red pitaya flesh and peel components was measured using a spectrophotometric pH differential protocol (Boyles & Wrolstad, 1993). The red pitaya peel and flesh extracts were thoroughly mixed with 0.025 M potassium chloride (pH 1.0) in a known dilution. The absorbance of the mixture was measured at 515 and 700 nm using distilled water to zero the spectrophotometer. The red pitaya peel and flesh extracts were then combined with 0.4 M sodium acetate buffer (pH 4.5), and the absorbances were measured at the same wavelengths. The absorbance of the diluted sample (A) was as follows: $A = (A_{515} - A_{700})\text{pH}\ \ 1.0 - (A_{515} - A_{700})\text{pH}\ \ 4.5$. The anthocyanin content was calculated as the total of

monomeric anthocyanin pigment (mg/litre) = ($A \times MW \times DF \times 1000$)/($\varepsilon \times 1$), where A is the absorbance of the diluted sample and DF is the dilution factor. MW and ε in this formula correspond to the predominant anthocyanin in the sample. Since the sample composition was unknown, pigment content was calculated as cyanidin-3-glucoside, where MW = 449.2 and ε = 26,900.

2.7. Chromatographic determination of betanin

Standard betanin, containing betanin and isobetanin, was obtained commercially and prepared in a two-step procedure prior to HPLC analysis (Wybraniec & Mizrahi, 2002). Briefly, the extracts were first separated by gel filtration on a Sephadex G-25 column (40 × 2.2 cm) pre-equilibrated with 1% acetic acid. The 1% acetic acid fractions, eluted at a flow rate of 1.0 ml/min, were collected and examined spectrophotometrically at 538 nm for the presence of betanin. Fractions with 538 nm absorbance were collected and lyophilized for further purification. The residue was redissolved in water and subsequently purified on an LH-20 column (40 × 2.2 cm) with water as the eluent. Fractions showing 538 nm absorbance were collected and lyophilized for HPLC analysis.

A high-performance liquid chromatographic method was employed to analyze the betanin content for red pitaya peel and flesh components (Wybraniec & Mizrahi, 2002). An Agilent 1100 liquid chromatographic system (Palo Alto, CA), equipped with a photodiode array detector and a Rheodyne (Cotati, CA) model 7125 injector with a sample loop of 20 μl, was used, along with an Agilent ChemStation for LC. Final signal integration was performed using the Agilent ChemStation for LC running on a Pentium-4 computer. A Hypersil ODS (Supelco Inc., Bellefonte, PA), 5 μm, 250 × 4.6 mm I.D. column was used to determine the betanin content, and the elution programme consisted of a 35 min isocratic elution of 90% solvent A (0.5% aqueous TFA in water) with 10% solvent B (acetonitrile) with a flow of 0.5 ml/min. For each analysis, 15 μl of the filtered extracts was directly injected into the chromatographic column. The identities of the different chromatographic peaks were confirmed by their visible spectral characteristics in comparison to standards and retention times.

2.8. DPPH· radical scavenging activity

A 0.1 ml aliquot of the acetone extract prepared above was mixed with 3.9 ml of an 80% ethanolic 0.6 mM DPPH· solution. The tubes were vortexed for 15 s and allowed to stand for 180 min, as described by Cai et al. (2003), after which the absorbance of the mixture was measured at 515 nm using the Hewlett Packard UV–Vis spectrophotometer (Palo Alto, CA). Most tested compounds should be reacted completely within 180 min in this condition (Cai et al., 2003) and reaction time for vitamin C is less than 1 min due to its fast oxidation. Ethanol (80%) was used as a blank solution and DPPH· solution without test samples (3.9 ml of DPPH· + 0.1 ml of 80% ethanol) served as the control. All tests were performed in triplicate. The antioxidant activity of the test samples was expressed as (i) the median effective concentration for radical-scavenging activity (EC_{50}): total phenolics (mg) of antioxidant (test sample) required for a 50% decrease in absorbance of DPPH· radicals, and (ii) inhibition (%) of DPPH· absorbance = $(A_{control} - A_{test}) \times 100/A_{control}$. A plot of absorbance of DPPH· vs.concentration of antioxidant was made to establish the standard curves (dose–response curves) and to calculate EC_{50}. $A_{control}$ is the absorbance of the control (DPPH· solution without the test sample), and A_{test} is the absorbance of the test sample (DPPH· solution plus 0.1 ml of 5 μM test compound). Vitamin C served as a standard, and the results of the assay were expressed relative to vitamin C equivalent.

2.9. $ABTS^{\cdot+}$ assay

For $ABTS^{\cdot+}$ generation from ABTS salt, 2.45 mM of potassium persulfate ($K_2S_2O_8$) was reacted with 7 mM ABTS salt in 0.01 M phosphate-buffered saline, pH 7.4, for 16 h at room temperature in the dark. The resultant $ABTS^{\cdot+}$ radical cation was diluted with 0.01 M phosphate-buffered saline, pH 7.4, to give an absorbance of around 0.70 at 734 nm. The standard and sample red pitaya peel and flesh extracts were diluted 100 X with the $ABTS^{\cdot+}$ solution to a total volume of 1 ml and allowed to react for 3 min (Re et al., 1999). Absorbance was measured spectrophotometrically at different time intervals. Control (without a standard or sample) was used as blank and 990 μl of PBS were added to these control samples instead. Trolox, the water-soluble α-tocopherol (Vitamin E) analog, served as a standard, and the results of the assay were expressed relative to trolox in terms of TEAC (trolox equivalent antioxidant capacity).

2.10. Determination of inhibition of B16F10 melanoma cell proliferation

The extracts of the flesh and peel samples of red pitaya were used to measure their ability to inhibit B16F10 melanoma cell proliferation (Nakano et al., 1998). The cell cultures were exposed to various concentrations of the extracts during a 48 h growth period. Cell proliferation was measured by the ability of viable cells to reduce 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) to formazan, whose absorbance can be analyzed spectrophotometrically at 570 nm. All measurements were performed in triplicate.

The absorbance was measured using the Hewlett Packard UV–Vis spectrophotometer (Palo Alto, CA). The effective median dose (EC_{50}) was determined and expressed as milligrammes of red pitaya flesh and peel samples ± SD.

2.11. Statistical analysis

Results are expressed as the means ± standard deviation of three replicates. The Student's *t* test was used for statistical analysis of the difference noted.

3. Results and discussion

3.1. Content of phenolic compounds

The total phenolic contents of the flesh and peel of the red pitaya were measured according to the Folin–Ciocalteu method. The Folin–Ciocalteu reagent determines total phenols (and other easily oxidized substances), producing a blue colour by reducing yellow heteropolyphosphomolybdate-tungstate anions. This method gives a general measure of phenolic content, as it is not completely specific for phenolic compounds and not all phenolic compounds exhibit the same level of activity in the assay. Phenolics were extracted from red pitaya flesh or peel with 80% acetone. The total extractable phenolic contents were 42.4 ± 0.04 and 39.7 ± 5.39 mg of GAE/100 g of flesh and peel, respectively. The amounts of total phenolics per 100 g of flesh or peel were similar, whereas the amounts of total phenolics per gramme of extract of flesh and peel were 4.55 ± 0.03 and 25.4 ± 2.10 mg of GAE, respectively. Although the total phenolic content per gramme of extract in peel was about six times more than that in flesh, the yield of the extracts from the same weight of raw materials (9.91 and 1.56 g from 100 g of raw materials) diminished the differences in total phenolic compounds. It is likely that some of the non-phenolic compounds were co-extracted from flesh, which bulked up the extract.

Betacyanins, the pigments found in *Hylocereus* cacti, also contributed to the total phenolics, due to a phenol structure in the molecule. The concentrations of betacyanins, expressed as betanin equivalents in flesh and peel, were 10.3 ± 0.22 and 13.8 ± 0.85 mg/ 100 g of flesh and peel, respectively. The HPLC chromatogram of betacyanins from fruit flesh and peel after purification was set at 538 nm in Fig. 1. At least four different kinds of betacyanins were separated by the C_{18} column and detected by the photodiode array detector of the HPLC system. By comparing with commercially obtained betanin standards, peaks 1 and 1′ were betanin and isobetanin with retention times of 6.5 and 8.3 min, respectively. It was likely that peak 2 was contributed by phyllocactin

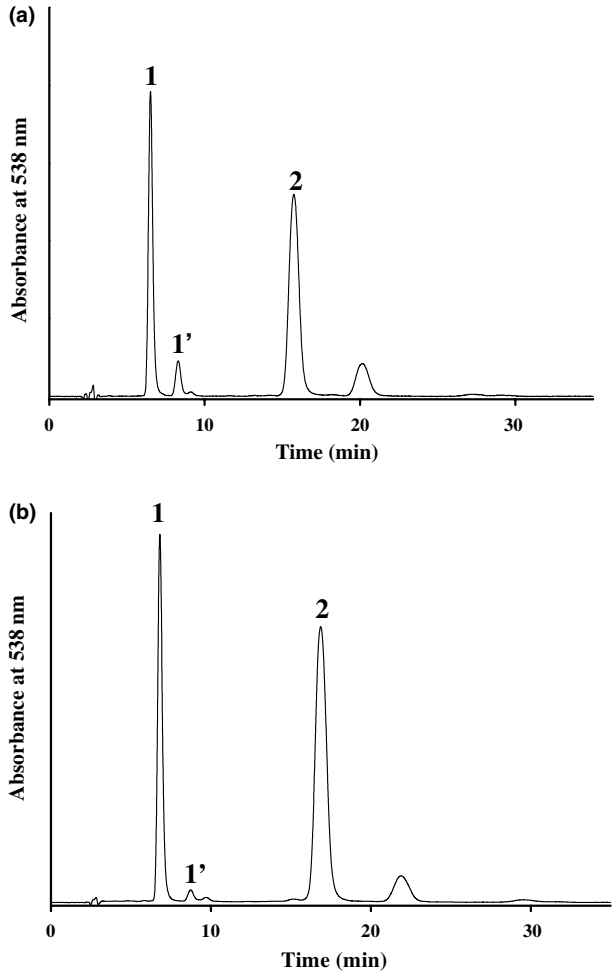

Fig. 1. HPLC chromatographs of betacyanins from flesh of *H. polyrhizus* (a) and peel (b). Peak 1 represents betanin (retention time: 6.5 min); Peak 1′ is isobetanin (retention time: 8.3 min).

(t_R = 15.7 min) if compared with the results of Wybraniec and Mizrahi (Wybraniec & Mizrahi, 2002), which represented the most abundant kind of betacyanin in flesh and peel (51% and 58%, respectively). The percentage of isobetanins (1.3%) in peel was less than that in flesh (4.4%).

The flavonoid contents of flesh and peel were 7.21 ± 0.02 and 8.33 ± 0.11 mg of catechin equivalents/ 100 g of flesh and peel, respectively. The flavonoid contents per gramme of extract in peel were about five times greater than those in flesh (5.33 ± 0.31 vs. 0.77 ± 0.02 mg catechin equivalents), which was similar to the results of measuring total phenolic content. However, no anthocyanins were detected. The non-detectable level of anthocyanins in red pitaya could be due to the absence of monomeric anthocyanin in red pitaya. The red-violet colour of red pitaya might be attributed to betacyanins, but not to anthrocyanin. Betacyanins are a class of water-soluble pigments that provide the colours in a wide variety of flowers and fruits (Strack,

Vogt, & Schliemann, 2003). They cannot be found in plants containing anthocyanin pigments, and structurally they are unrelated. Little is known about the role of betacyanin, and the gain and loss of anthocyanins and betalains during plant evolution remain a mystery (Clement & Mabry, 1996).

3.2. Assessment of antioxidant activity

Of the several approaches to the measurement of antioxidative activity that could have been used, such as DPPH· (Cai et al., 2003), TEAC (Miller, Rice-Evans, Davies, Gopinathan, & Milner, 1993), TOSC (Winston, Regoli, Dugas, Fong, & Blanchard, 1998), ORAC (Cao, Alessio, & Cutler, 1993), FRAP (Pulido, Bravo, & Saura-Calixto, 2000), and TRAP (Ghiselli, Serafini, Maiani, Azzini, & Ferroluzzi, 1995), the DPPH· approach and $ABTS^{\cdot+}$ radical-scavenging assay were chosen for this study. The DPPH· approach is simple and widely applied for the measurement of antioxidant activity of polyphenolics and colourants (Cai et al., 2003; Shi & Le Maguer, 2000). The $ABTS^{\cdot+}$ radical-scavenging assay, which employs a specific absorbance at a wavelength remote from the visible region and requires a short reaction time (Lee, Kim, Lee, & Lee, 2003), can be used in both organic and aqueous solvent systems. It is suggested that the $ABTS^{\cdot+}$ approach is better for evaluating the antioxidant activity of phenolic phytochemicals than the DPPH· radical-scavenging assay (Awika, Rooney, Wu, Prior, & Cisneros-Zevallos, 2003).

The relationship between phenolic content and antioxidant capacity of the pitaya flesh and peel is shown in Fig. 2. Similar patterns were found in the antioxidant activity measured by $ABTS^{\cdot+}$ assay. The effective concentrations (EC_{50}) determined by DPPH radical-scavenging activity for flesh and peel were 22.4 ± 0.29 and 118 ± 4.12 μmol vitamin C equivalents/g dried extract, respectively, and the values of EC_{50} for the ABTS approach were 28.3 ± 0.83 and 175 ± 15.7 μmol TEAC/g dried extract, respectively. Peel extract was a better antioxidant than flesh due to its slightly higher polyphenolic content. The increase in phenolic content of the two extracts from flesh and peel was found to be linearly correlated with antioxidant capacity. In the DPPH· assay, as the total phenolic compounds increased from 0.7 to 3.5 mg gallic acid equivalents of flesh and from 0.12 to 0.6 mg gallic acid equivalents of peel, the percentage of radical-scavenging activity of both increased linearly about 4.8-fold ($R^2 = 0.99$).

Phenolics also influence antioxidant-activity measurement. They interfere with the oxidation process by reacting with free radicals, chelating catalytic metals, and scavenging oxygen. The effect of flesh and peel on antioxidant activity could be a result of the types of polyphenolics they contained. An increase in the number

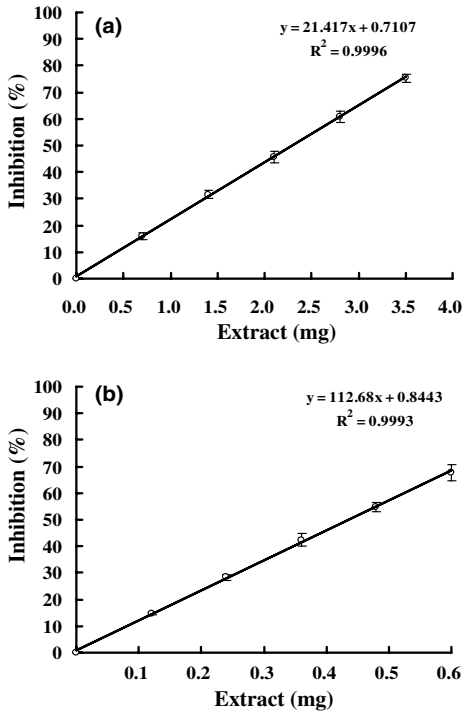

Fig. 2. Antioxidant activity of *Hylocereus* cacti determined by DPPH·. (a) flesh; (b) peel.

of hydroxyl groups (–OH) or other hydrogen-donating groups (=NH, –SH) in the molecular structure led to higher antioxidant activity (Cai et al., 2003). Betanins contain imino groups and hydroxyl groups and would contribute antioxidant activity, which could explain, in part, that peel is a better antioxidant due to the higher level of betanin in the peel.

3.3. Inhibitory effect of extracts on B16F10 melanoma cell proliferation

MTT assay was used to study the antiproliferative activity of red pitaya. MTT is reduced to an insoluble purple formazan by mitochondrial dehydrogenase. Cell proliferative activity was measured by comparison of the purple colour formation. Dead cells, on the other hand, did not form the purple formazan due to their lack of the enzyme. The effect of red pitaya flesh and peel on the growth of B16F10 cells in vitro is shown in Fig. 3(a). Cells were cultured for 48 h with several different levels of flesh or peel extracts. The curves showed that the growth of melanoma cancer cells was inhibited in a dose-dependent manner. The value of EC_{50} of the antiproliferative activity of the peel extract was 10.0 μg of GAE (25.0 mg of peel matter), whereas the EC_{50} value of flesh could not be calculated from the dose–response curve. The difference could be attributed to the different kinds of polyphenolics present in the samples. Evidence indicated that hydroxyl-

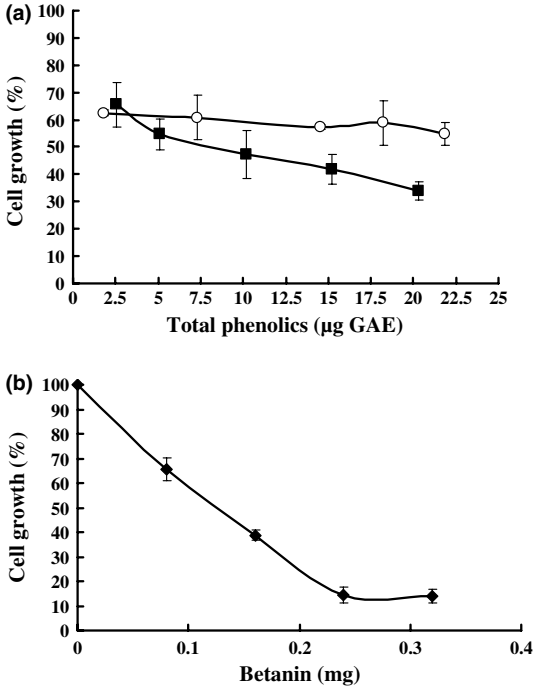

Fig. 3. Inhibition of B16F10 melanoma cell proliferation by phytochemical extracts of *Hylocereus* cacti flesh (○) and peel (■) (a) and betanin (b). The cell growth of treated groups were standardized with untreated control group as A/A_0 (%), where A is the value of A_{570} generated at a given concentration of extracts or betanin by MTT assay, and A_0 is from untreated control group.

ated flavonoids without the C2–C3 double bond could not inhibit the growth of melanoma cell lines, such as B16F10, whereas the presence of at least three adjacent methoxyl groups would confer a more potent antiproliferative effect (Rodriguez et al., 2002). Moreover, flavonoids such as myricetin, baicalein, and gallic acid significantly inhibited the growth of B16F10 after 72 h of exposure. It has been suggested that the presence of a C2–C3 double bond and three adjacent hydroxyl groups in the flavonoid A- or B-rings confer greater antiproliferative activity to the flavonoid (Martinez et al., 2003). Betanin would also have an influence on the antiproliferative effect. As Fig. 3(b) indicates, with increase of betanin concentration, greater inhibitory effect was observed. This could be due to the molecular structural effects similar to those observed in flavonoids.

In summary, considerable amounts of phenolic compounds were found in red pitaya flesh and peel. To our knowledge, this is the first time that the total phenolic content and antioxidant activity of red pitaya flesh and peel have been measured. Our results showed that, in addition to the grape seeds, the peel of red pitaya, an inedible waste product of juice manufacture, might be another good source of antioxidants and an antimelanoma agent. Waste pitaya peel should be regarded as a valuable product and has potential as an economic value-added ingredient that may assist in the prevention of chronic disease.

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