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The protective effect of *Opuntia dillenii* Haw fruit against low-density lipoprotein peroxidation and its active compounds

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Abstract

The antioxidant activity and inhibitory effect of extracts from *Opuntia dillenii* Haw fruit (ODHF) and its active compounds on low-density lipoprotein (LDL) peroxidation were investigated. The results indicated that the antioxidant activity of methanolic extracts of ODHF in Trolox equivalent antioxidant capacity and oxygen-radical absorbance capacity assays were in the order of seed > peel > pulp. The lag time of conjugated diene formation in Cu^{2+}-induced LDL oxidation was increased by incubation of LDL with various methanolic extracts of ODHF. The methanolic extracts from seed, peel and pulp prolonged the lag time compared to control (154.1 min) to 514.8, 163.9 and 190.2 min, respectively, at a concentration of 10 μg/ml. Among the extracts, seed extracts of ODHF (10 μg/ml) possessed the highest inhibitory effect on the formation of thiobarbituric acid reactive substances and relative electrophoretic mobility. The results also demonstrated that seeds of ODHF contained the highest amounts of polyphenols and flavonoids (212.8 and 144.1 mg/100 g fresh seed, respectively), such as gallic acid, catechin, sinapinic acid, epicatechin, *p*-coumaric acid, quercetin and ferulic acid, but no betanin, isobetanin and ascorbic acid as determined by HPLC. However, the peel and pulp contained high amounts of betanin, isobetanin and ascorbic acid, but with lower contents of phenolics and flavonoids as compared to the seed. These findings suggest that phenolics and flavonoids may directly contribute to the antioxidant activity of the seeds of ODHF.

Keywords: *Opuntia dillenii* Haw fruit; Antioxidant activity; Low-density lipoprotein; Phenolics; Betanin

1. Introduction

Reactive oxygen species (ROS) and free radicals are constantly formed in the human body by normal metabolic action, and have been implicated in the pathogenesis of certain human diseases, including cancer, aging, diabetes and atherosclerosis (Moskovitz, Yim, & Chock, 2002). Researchers have shown that atherosclerosis has an intimate relationship with the oxidative modification of LDL cholosterol (Steinberg, Parthasarathy, Carew, Khoo, & Witztum, 1989). The oxidative modification of LDL cholesterol may be suppressed by the increased presence of antioxidants. The main mechanism of this suppression effect is that antioxidants may inhibit LDL cholesterol peroxidation by scavenging free radicals and chelating metal ions. Therefore, much attention has been paid on the use of natural antioxidants to scavenge free radicals and inhibit lipid peroxidation, or to prevent damage from free radicals. Epidemiological studies have shown that a higher intake of fresh fruits, vegetables, tea, and wine is associated with reduced ROS-induced diseases (Kooncumchoo, Sharma, Porter, Govitrapong, & Ebadi, 2006). Phenols, flavonoids and other non-nutrient compounds of fruits and vegetables have been recognized as potential factors that can be beneficial to human health through their antioxidant activities, antimutagenic and/or anticarcinogenic activities, and anti-inflammatory actions.

Opuntia dillenii (Ker-Gawl) Haw is a cactus belonging to the family *Opuntia* which usually grows in semi-desert

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regions in the tropics and subtropics including the Penghu islands belonging to Taiwan and are called Xian Ren Zhang in Chinese. It has yellow flowers and red fruits. The red fruit contains high amounts of ascorbic acid. Its stems are thick, flat and stiff. The stem and fruit of this plant are used in a folk medicine for reducing cholesterol levels, treatment of gastric ulcers, inflammation (Loro, del Rio, & Pérez-Santana, 1999), diabetes (Tao, Zeng, Lu, & Zhang, 2005) and several other diseases. Its reddish fruit is commonly used as a colouring agent for foods, drinks, and ice cream in Taiwan.

Butera et al. (2002) reported that prickly pear (*Opuntia ficus*) white fruit extracts showed the highest protective effects of all models of lipid oxidation due to its high content of betalain, which contributes to the antioxidant activity of prickly pear fruit. Kanner, Harel, and Granit (2001) also specified betalain as a new class of dietary cationized antioxidant. The nutraceutical benefits of *O. ficus* fruit are believed to their antiulcerogenic activity and antioxidant properties related to ascorbic acid, phenolics, and a mixture of betaxanthin and betacyanin pigments (Tesoriere et al., 2003). A number of byproducts have previously been studied as potential sources of antioxidants such as potato peel (Rodrı'guez de Sotillo, Hadley, & Wolf-Hall, 1998), olive oil waste waters (Visioli et al., 1999), grape seeds (Simonetti, Ciappellano, Gardana, Bramati, & Pietta, 2002), and grape pomace peels (Larrauri, Sa'nchez Moreno, & Saura-Calixto, 1998). In fact, an interesting approach to enhance the value of byproducts is their use as sources of natural antioxidant compounds. To the best of our knowledge, studies on the antioxidant capacity and chemical composition of ODHF and its byproducts have not yet been reported. Therefore, the objectives of the present study were to investigate the antioxidant activity of methanolic extracts of different parts of ODHF including peel, pulp, and seed as well as related active compounds.

2. Materials and methods

2.1. Chemicals

6-Hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid (Trolox) was purchased from Aldrich Chemicals Co. (Milwaukee, WI, USA). Fluorescein (FL), 2,2′-azinobis-3-ethylbenzthiazoline-6-sulphonate (ABTS), sulphonic acid, gallic acid, protocatechuic acid, gentisic acid, catechin, syringic acid, epicatechin (EC), *p*-coumaric acid, ferulic acid, sinapinic acid, rutin, quercetin, ascorbic acid, butylated hydroxytoluene (BHT), phosphotungstic acid, sodium dodecyl sulphate (SDS) and 1,1,3,3-tetramethoxypropane were purchased from Sigma Chemicals Co. (St Louis, MO, USA). 2,2-Azobis (2-amidinopropane) dihydrochloride (AAPH) and betanin (red-beet extract) were obtained from Wako Pure Chemicals Co. (Tokyo, Japan). Lipoprotein electrophoresis kit was purchased from Beckman Instruments (Fullerton, CA, USA).

2.2. Opuntia dillenii Haw fruit (ODHF) and extraction

O. dillenii Haw fruits (ODHF), collected during April–May 2004 in Penghu (Taiwan), were plucked along the coast of Maggac town, Penghu and were extracted within 2 days after collection. The fruits were peeled, and the pulp was separated from seeds and weighed; 100 g samples were homogenized with 100 ml of methanol. The mixtures were allowed to stand for 60 min at 4 °C before centrifugation (10 min at 14000*g*). The supernatant was collected and the residue was re-extracted 2 times with the same volume of methanol. The combined extracts were subjected to rotary evaporation to remove organic solvents. The yields of extracts from peel, pulp, and seed of ODHF were 3.93%, 6.25% and 5.61%, respectively. All samples were stored at −80 °C until use.

2.3. Trolox equivalent antioxidant capacity (TEAC)

The total antioxidant activity of the methanolic extracts of the three parts of ODHF was measured using the TEAC assay as described by Miller, Rice-Evans, Davies, Gopinathan, and Milner (1993) with minor modifications. $ABTS^{+}$ was generated by the interaction of ABTS (75 μM), H_2O_2 (50 μM), and peroxidase (4.4 units/ml). To measure antioxidant activity, 0.25 ml of extracts was mixed with an equal volume of ABTS, H_2O_2, peroxidase, and 1.5 ml of deionized water. Absorbance was measured at 734 nm after the interaction of the sample solution for 10 min. The decrease in absorption at 734 nm after the addition of reactant was used to calculate the TEAC value. The TEAC value is expressed as the millimolar concentration of Trolox solution having the antioxidant equivalent to a 1 mg/ml solution of the sample under investigation. The higher the TEAC value of a sample, the stronger the antioxidant activity.

2.4. Oxygen-radical absorbance capacity assay (ORAC)

The ORAC procedure was based on the report by Cao, Sofic, and Prior (1997) with a slight modification, in which the decrease in fluorescence of fluorescein (FL) is measured in the presence of AAPH. The FL fluorescence decay was monitored at 5 min intervals for 800 min (excitation, 485 nm; emission, 520 nm). Briefly, in the final assay mixture, FL (16.7 nM), 75 mM phosphate buffer (pH 7.0), 4 mM AAPH in the presence or absence of methanolic extracts of ODHF were combined and incubated at 37 °C in a Fluostar Galaxy plate reader (BMG LabTechologies, GmbH, Offenburg, Germany). Final fluorescence measurements were expressed relative to the initial reading. Results were calculated based on differences in areas under the fluorescence decay curve between the blank, samples and standards. The standard curve was obtained by plotting the four concentrations of Trolox against the net area under the curve (AUC) of each standard. Final ORAC values were calculated using the regression equation between

Trolox concentration and AUC and expressed as μmoles of Trolox equivalents per gram of dry weight.

2.5. Preparation and oxidation of human LDL

Fasting plasma for LDL isolation was collected from normal human volunteers in tubes containing ethylenediaminetetraacetic acid (EDTA; 1 mg/ml). LDL cholesterol (d 1.019–1.063 g/ml) was isolated by sequential ultracentrifugation using a Hitachi ultracentrifuge (Himac CS 120GX, Hitachi, Tokyo, Japan) described by Yen and Hsieh (2002). Protein was measured by using a Bio-Red kit with bovine serum albumin as a standard. Dialyzed LDL (100 μg protein/ml) was diluted in 10 mM PBS and incubated at 37 °C in the presence or absence of 10 μM $CuSO_4$. The oxidation was performed in the presence or absence of methanolic extracts of ODHF. After the incubation, lipid peroxidation of the LDL was measured as described below.

LDL cholesterol oxidation was followed by continuously monitoring the formation at 37 °C of conjugated dienes (CD) lipid hydroperoxides at 234 nm. The absorbance was measured every 15 min for 195 min using a Hitachi U-2000 spectrophotometer (Hitachi Ltd., Tokyo, Japan). The duration of the lag phase was calculated by extrapolating the propagation phase. TBARS were measured by the method described by Yagi (1989) at 555 nm (excitation wavelength at 515 nm) using a Hitachi F-3010 spectorfluorometer. In these studies, malondialdehyde (MDA) formation from 1,1,3,3,-tetraethoxypropane was used as a reference standard and the results were expressed as nmol MDA equivalents. TBARS were expressed as malonaldehyde equivalents and calculated using the molar extinction coefficient for MDA. LDL electrophoresis was performed at pH 8.6 in 0.05 M barbital buffer on Lipoprotein LIPO gels. The gel was stained with Sudan B black. The increased electrophoretic mobility of LDL was expressed relative to the mobility (REM) of the native LDL cholesterol.

2.6. Determination of total phenolics

Total phenolic compounds in ODHF extracts were determined with the Folin-Ciocalteu reagent by the method of Taga, Miller, and Pratt (1984) and calculated using gallic acid as a standard. Methanolic extracts of ODHF (100 μl, 250 μg/ml) were added to 2 ml of 2% Na_2CO_3. After 2 min, 50% Folin-Ciocalteau reagent (100 μl) was added to the mixture which was then left to stand for 30 min. Absorbance was measured at 750 nm on a spectrophotometer and compared to gallic acid calibration curves. The content of total phenolics was expressed as gallic acid equivalents (GAE). All analyses were run in three replicates and mean values recorded.

2.7. Determination of total flavonoids

The spectrophotometic assay for the quantitative determination of flavonoid content was carried out as described by Zhishen, Mengcheng, and Jianming (1999). Briefly, the extract of ODHF (0.5 ml, 250 μg/ml) was diluted with 2.85 ml distilled water. At zero time, 0.15 ml 5% $NaNO_2$ was added to the mixture. After 5 min, 1.5 ml 10% $AlCl_3$ was added. After another 6 min, 1.5 ml of the mixture was removed and added to an equal volume of 1.5 ml 1 N NaOH. Immediately, the absorbance of the mixture, pink in colour, was determined at 510 nm versus the prepared water blank. Total flavonoids of fruits were expressed on a fresh weight basis as mg/100 g quercetin equivalents (QE).

2.8. Quantification of phenolic acids and flavonoids

The procedure was based on a previous report by Zhishen et al. (1999) with a slight modification. The contents of phenolic acids and flavonoids in the ODHF extracts were determined by HPLC, performed with a Hitachi liquid chromatograph (Hitachi Ltd., Tokyo, Japan), consisting of a model L-6200 intelligent pump, a Rheodyne model 7125 syringe-loading sample injector, a model D-2500 integrator and a model L-3000 photo-diode array detector set at 280 nm. Extracts were filtered through a 0.45 μm filter before use. The mobile phase utilized 2% (v/v) acetic acid in water as solvent A and 0.5% acetic acid in water and acetonitrile (50:50, v/v) as solvent B. The elution gradient program was as follows: 10% B–55% B (50 min), 55% B–100% B (10 min), 100% B–10% B (5 min) at a flow rate of 1 ml/min. Phenolic acids and flavonoids were identified by comparison of their retention times (Rt) and UV spectra with known standards and determined by peak areas from the chromatograms.

2.9. Quantification of betanin, isobetanin and ascorbic acid

Betanin and isobetanin in ODHF extracts were quantified by HPLC using a LiChrospher 100 RP-18 reversed-phase column (5 μm, 25 cm × 5 mm i.d., Merck, Darmstadt, Germany). The separation was performed isocratically using a mixture of 90% solvent A (0.5% aqueous TFA) with 10% solvent B (acetonitrile) for 35 min at a flow rate of 0.5 ml/min and an injection volume of 10 μl. The HPLC apparatus was a Hitachi liquid chromatograph (Hitachi Ltd., Tokyo, Japan), consisting of a model L-6000 intelligent pump, a Rheodyne model 7125 syringe-loading sample injector, a model D-2500 integrator and a model L-4200 UV–Vis detector set at 538 nm.

Ascorbic acid was analyzed by HPLC. The ODHF extracts were filtered (0.45 μm) and injected into a Hitachi HPLC apparatus with a UV detector. The column used was LiChrocharts 250-4 LiChrosphar RP-18, 5 μm (Merck, Darmstadt, Germany). A buffer of 0.2% $NH_4H_2PO_4$ (pH 2.8) with flow rate of 1.0 ml/min, was used as the mobile phase. Quantification was carried out by an external standard method.

2.10. Statistical analysis

All analyses were run in triplicate and mean values recorded. Statistical analyses were performed according to the SAS Institute User's Guide. Analyses of variance were performed using the ANOVA procedure. Significant differences ($P < 0.05$) between the means were determined using Duncan's multiple range test.

3. Results

3.1. Antioxidant activity of extracts from Opuntia dillenii Haw fruit (ODHF)

The scavenging abilities of extracts from ODHF on $ABTS^{\cdot+}$ radicals are shown in Table 1. It was found that the scavenging effect (%) of ODHF increased with concentration (data not shown). As shown in Table 1, the Trolox equivalent antioxidant capacity (TEAC) of methanolic extracts from ODHF, compared with betanin and catechin, were in the order of catechin > seed > betanin > peel and pulp ($P < 0.05$). The scavenging activity of seed extracts was observed to be higher than that of betanin, peel and pulp extracts.

The abilities of ODHF extracts to scavenge peroxyl radical were measured by monitoring the loss of fluorescein induced by AAPH. The rapid decomposition of fluorescein was induced by addition to the solution, and 2.8% fluorescence remained after incubation for 40 min, when compared with the control (data not shown). As shown in Table 1, the ORAC value for the positive control (catechin) was higher than the others; it was 0.226 mM. Among three parts of the extracts, seeds exhibited the strongest efficiency and showed 0.175 mM at a concentration of 1000 μg/ml. Therefore, the seeds had the highest peroxyl radical capacity, followed by the peel and pulp. This trend is similar to the results obtained by the TEAC assay.

3.2. Effect of ODHF on inhibition of LDL peroxidation induced by Cu^{2+}

The formation of LDL conjugated dienes (CD) under the induction of Cu^{+2} was inhibited by ODHF (Table 2).

Table 1
Antioxidant activity of betanin, catechin, and the methanolic extracts from different parts of ODHF

Sample	TEAC (mM)[A]	ORAC (mM)[A]
Peel	1.24 ± 0.15^{d}	0.14 ± 0.01^{d}
Pulp	1.01 ± 0.13^{de}	0.10 ± 0.00^{e}
Seed	2.15 ± 0.04^{b}	0.18 ± 0.01^{b}
Betanin	0.87 ± 0.09^{e}	0.15 ± 0.00^{c}
(+)-Catechin	2.44 ± 0.22^{a}	0.23 ± 0.01^{a}

Each value represents mean ± standard deviation ($n = 3$). Data in the same column with the different superscripts are significantly different at $p < 0.05$.

[A] The concentration of methanolic extracts of ODHF used in antioxidant activity assays was 1 mg/ml.

Table 2
Effect of betanin, catechin, and the methanolic extracts from different parts of ODHF on the inhibition of Cu^{2+}-induced LDL oxidation

Sample	Lag time (min)[a]	TBARS level (% of control)[b]
Native LDL	–	3.3 ± 0.3
Control[c]	154.1	100 ± 1.5
Peel	190.2	23.6 ± 3.2
Pulp	163.9	26.8 ± 1.7
Seed	514.8	7.7 ± 0.9
Betanin	236.1	19.8 ± 0.6
Catechin	590.2	3.6 ± 0.2

[a] LDL (100 μg protein/ml) was incubated with 15 μM $CuSO_4$ at 37 °C in the absence or presence of 10 μg/ml methanolic extracts of ODHF, betalin, catechin, and epicatechin.

[b] LDL (100 μg protein/ml) was incubated with 15 μM $CuSO_4$ at 37 °C in the absence or presence of 10 μg/ml methanolic extracts of ODHF, betalin and catechin for 2 h.

[c] LDL was incubated with 15 μM $CuSO_4$ without samples.

Compared to the lag time (154.1 min) as revealed by the LDL control (LDL with Cu^{2+}), the lag time of LDL with different degrees of peroxidation was significantly suppressed by the addition of ODHF extracts. Seed extracts had the best inhibitory effect on CD formation compared to the other two extracts. Seed extracts extended the lag time to 514.8 min (the lag time increased 3.34-fold) in the presence of the inducer Cu^{2+}. The order of efficiency of the ODHF extracts was seed (514.8 min) > peel (190.2 min) > pulp (163.9 min).

As shown in Table 2, seed extracts possessed a remarkable inhibitory effect on the formation of TBARS from LDL peroxidation induced by Cu^{2+}. It showed 92.3% inhibition at a concentration of 10 μg/ml when compared to the positive control. Peel and pulp extracts of ODHF also showed significant inhibitory effects. This inhibitory trend of TBARS formation was similar to the results of CD formation in LDL peroxidation induced by Cu^{+2}.

Fig. 1 shows the effect of ODHF on the relative electrophoretic mobility (REM) of LDL peroxidation induced by Cu^{2+}. Taking the REM of native LDL to be 1, the REM increased to 5.2 with the addition of Cu^{2+}. The data showed that LDL peroxidation can be suppressed by the addition of seed extracts of ODHF, and the value of REM was reduced to 2.1 at a concentration of 100 μg/ml.

3.3. Total phenolics and flavonoids contents

The amounts of total phenolics and flavonoids in the ODHF are summarized in Table 3. The contents of total phenolic compounds and flavonoids in different parts of ODHF were decreased in the order of seeds > peel > pulp. A high content of total phenolics was observed in seed in comparison with other parts, followed by the peel and pulp. The content of flavonoids in seeds was about 5-fold that of pulp and peel.

To isolate the phenolics and flavonoids, the ODHF was separated by HPLC. As shown in Table 4, seeds contained

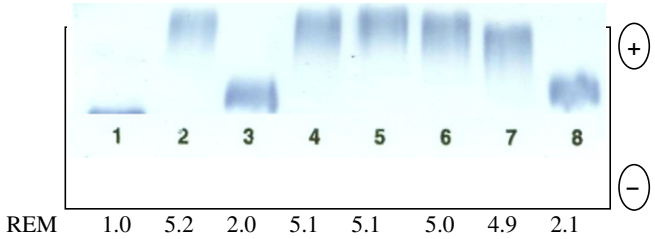

Fig. 1. The relative electrophoretic mobility (REM) of human LDL incubated with Cu^{2+} with or without seed extracts of ODHF. LDL (100 μg protein/ml) was oxidized with 15 μM $CuSO_4$ at 37 °C in the presence of seed extracts for 24 h. Lane 1: native LDL; Lane 2: LDL and Cu^{2+}; Lane 3: LDL and Cu^{2+} and 100 μg/ml catechin; Lanes 4–8: LDL and Cu^{2+} and 2.5, 5, 10, 50, 100 μg/ml of seed extracts, respectively.

Table 3
Antioxidant compounds in different parts of ODHF

Sample	Contents (mg/100 g fresh sample)	
	Total phenolics[A]	Flavonoids
Peel	133.4 ± 1.6^{b}	32.5 ± 1.6^{b}
Pulp	91.5 ± 1.5^{c}	29.2 ± 1.5^{c}
Seed	212.8 ± 10.4^{a}	144.1 ± 10.3^{a}

Data in the same column with the different superscripts are significantly different at $p < 0.05$.
[A] Each value is the mean ± standard deviation ($n = 3$).

higher amounts of phenolics and flavonoids than that of the peel and pulp. Catechin, epicatechin, ferulic acid, sinapinic acid and quercetin were the major compounds in seeds and were present in higher amounts than in the peel and pulp. These results are consistent with the data shown in Table 1 that the contents of total phenolic compounds and total flavonoids were both in the order of seed > peel > pulp.

Table 4
Flavonoids and phenolic compound contents in different parts of ODHF

Compound	Retention time (min)	*Opuntia dillenii* Haw (mg/100 g fresh sample)[a]		
		Peel	Pulp	Seed
Gallic acid	4.67	2.7 ± 0.03	4.0 ± 0.6	2.6 ± 0.1
Protocatechuic acid	9.33	ND	ND	ND
Gentisic acid	16.03	ND	ND	ND
Catechin	19.21	18.0 ± 0.2	22.7 ± 0.7	35.6 ± 3.7
Syringic acid	25.07	ND	ND	ND
Epicatechin	27.39	17.1 ± 0.1	10.9 ± 0.2	31.8 ± 1.1
p-Coumaric acid	31.33	0.6 ± 0.0	ND	2.2 ± 0.1
Ferulic acid	35.57	4.0 ± 0.1	ND	10.2 ± 1.2
Sinapinic acid	37.49	ND	ND	26.8 ± 1.4
Rutin	38.99	ND	ND	0.3 ± 0.0
Quercetin	56.56	4.6 ± 0.1	ND	33.5 ± 1.6

ND – not detectable (below detection limit).
[a] Each value is the mean ± standard deviation ($n = 3$).

Table 5
The contents of betanin, isobetanin and ascorbic acid in different parts of ODHF

Sample	Contents (mg/100 g fresh sample)		
	Betanin	Isobetanin	Ascorbic acid
Peel	15.7 ± 1.8	19.2 ± 0.1	1.2 ± 0.1
Pulp	18.2 ± 2.5	19.1 ± 1.0	15.1 ± 0.6
Seed	ND	ND	ND

ND – not detectable (below detection limit).

3.4. Quantitation of betanin, isobetanin and ascorbic acid

The HPLC chromatogram of ODHF showed the presence of betanin and isobetanin with retention times of 8.25 and 10.12 min, respectively (data not shown). The content of betanin and isobetanin in seeds was not detectable (Table 5). The peel contained most of the betanin and isobetanin with 15.7 and 19.2 mg/100 g fresh sample, respectively. The contents of betanin and isobetanin in pulp were 18.2 and 19.7 mg/100 g fresh sample, respectively. The content of ascorbic acid in seed was not detectable (Table 5). However, the peel and pulp contained 1.2 mg and 15.1 mg/100 g fresh sample of ascorbic acid, respectively.

4. Discussion

The Trolox equivalent antioxidant capacity (TEAC) assay, using free blue-green $ABTS^{\cdot+}$ as the radical, is shown to be a very useful tool in expeditiously measuring the antioxidant activity of individual chemical compounds or complex extracts (Kim, Lee, Lee, & Lee, 2002). The oxygen radical absorbance capacity (ORAC) test is an *in vitro* assay to measure the total antioxidant power of substances and it measures both lipophilic and hydrophillic extracts (Prior et al., 2003). The ORAC assay measures antioxidant inhibition of peroxyl radical-induced oxidation and therefore reflects the classical radical chain-breaking antioxidant activity by hydrogen atom transfer (Huang, Ou, & Prior, 2005). TEAC values ranging from 0.13 to 0.65 mmol/kg have been reported for four types of Chinese bayberries, and 2.2 to 3.6 mmol/kg pulp has been reported for differently coloured cactus pears (Bao, Cai, Sun, Wang, & Corke, 2005). In the present study, the TEAC values for three extracts of ODHF were close to those of cactus pears. The trend of TEAC for the three extracts of ODHF was similar to the result of the ORAC. Seed extracts exhibited the best effects in both test methods.

LDL is the major cholesterol carrier in the blood, and it is well established that elevated plasma levels of LDL correlate with an increased risk of atherosclerosis. Oxidative modification of LDL is recognized to be one of the main causes of coronary heart disease (Steinberg, 1997). Consequently, dietary antioxidants that protect LDL from oxidation may help to reduce atherogenesis and prevent coronary heart disease. Numerous in vitro studies have

shown that polyphenols from red and white wine (Vinson & Hontz, 1995), rapeseed and pine bark phenols and raspberry (Vuorela et al., 2005), coffee, cocoa, and tea beverages (Richelle, Tavazzi, & Offord, 2001) are recognized as bioactive components with antioxidant properties. In this study, the ability of ODHF to scavenge free radicals was further confirmed by the inhibition of LDL cholesterol peroxidation. These results revealed that ODHF extracts could react with free radicals, converting them to more stable products and terminating the radical chain reaction and supplying antioxidant action.

Phenolic compounds, such as quercetin, rutin, narigin, catechins, caffeic acid, gallic acid and chlorogenic acid, are very important plant constituents because of their antioxidant activities (Paganga, Miller, & Rice-Evans, 1999). (+)-Catechin and (−)-epicatechin are found in many foods and may have important effects on human health. Yilmaz and Toledo (2004) indicated that peroxyl radical scavenging activities of phenols present in grape seeds or skins in decreasing order were resveratrol > catechin > epicatechin = gallocatechin > gallic acid = ellagic acid. It has been revealed that various phenolic antioxidants, such as flavonoids, tannins, coumarins and more recently procyanidins scavenge radicals dose-dependently. Thus, they are viewed as promising therapeutic drugs for free radical pathologies (VanderJagt, Ghattas, VanderJagt, Crossey, & Glew, 2002). In our results, seeds possessed the highest amount of polyphenols, followed by the peel and pulp, which showed the marked antioxidant activities in the model systems. Phenolic acids and flavonoids might account for the bulk of the antioxidant capacity in seed extracts of ODHF. The antioxidant action of seed extracts may be attributed to their free radical-scavenging ability. Our results provide evidence that negligible amounts of polyphenols are present in the peel and pulp.

Betalains have recently been shown to be antioxidants in a number of lipid oxidation systems. Betalains occur in a number of natural sources such as prickly pears and beets (Butera et al., 2002). In the present study, we found values of 15.7 and 19.2 mg/100 g fresh sample (peel) for betanin and isobetanin, respectively, and the values of 18.2 and 19.7 mg/100 g fresh sample (pulp) for betanin and isobetanin, respectively. These contents were lower than the 28–30 mg/100 g from the edible portion of prickly pear (*Opuntia ficus-indica*) fruit (Butera et al., 2002), but compared well with amounts found in peaches and pears (Carbonaro, Mattera, Nicoli, Bergamo, & Cappelloni, 2002). Therefore, ascorbic acid, betanin and isobetanin may account for most of the antioxidant capacity of the peel and pulp extracts of ODHF, which has effective components different from the seed extracts. Leonard et al. (2002) indicated that ascorbic acid is only one facet of the protective effects of fruit and vegetable juices against lipid peroxidation induced in cell membranes. It appears that consumption of whole fruits and vegetables would be superior to an ascorbic acid supplement for antioxidant effectiveness.

In conclusion, the methanolic extracts of seeds from ODHF had a marked activity in ORAC, TEAC and anti-LDL cholesterol peroxidation systems in vitro. Our findings suggest that the phenolic acids and flavonoids, but not ascorbic acid, betanin and isobetanin, play an important role in antioxidant activity and anti-LDL peroxidation. Although the antioxidant contribution of other compounds in the extracts cannot be ruled out in this study, our findings suggest that ascorbic acid and betalain pigments may be important antioxidant components in the pulp and peel. On the basis of the results obtained, ODHF byproducts may play potential role as a source of health-promoting phenolics associated with antioxidant activity. Therefore, there is great promise for the utilization of ODHF seeds and peels for creating new beneficial health products for nutraceutical markets in the future.

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