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ARTICLE

Extraction, Quantification, and Antioxidant Activities of Phenolics from Pericarp and Seeds of Bitter Melons (*Momordica charantia*) Harvested at Three Maturity Stages (Immature, Mature, and Ripe)

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Bitter melon (*Momordica charantia*) is an exotic vegetable used for consumption and medicinal purposes mainly throughout Asia. Phenolics were extracted from pericarp (fleshy portion) and seeds of bitter melons harvested at three maturation stages (immature, mature, and ripe) using ethanol and water solvent systems. Total phenolic assessment demonstrated 80% of ethanol to be the optimal solvent level to extract phenolics either from pericarp or seed. Main phenolic constituents in the extracts were catechin, gallic acid, gentisic acid, chlorogenic acid, and epicatechin. Free radical scavenging assay using 2,2-diphenyl-1-picrylhydrazyl (DPPH) demonstrated the bitter melon extracts as slow rate free radical scavenging agents. There were low correlations between the total phenolic contents and antiradical power values of the extracts, suggesting a possible interaction among the phenolic constituents occurred. Bitter melon phenolic extracts contain natural antioxidant substances, and could be used as antioxidant agents in suitable food products.

KEYWORDS: Bitter melon; phenolics; antioxidant; DPPH; maturity

INTRODUCTION

Bitter melon (*Momordica charantia* Linn.) or bitter gourd, a member of the cucurbitaceae, is best known for its medicinal properties and has been used in traditional medicines in Asia, Africa, and West Indies (*1*). Bitter melon though bitter is consumed as a vegetable when it is unripe, and the seeds of ripe bitter melons are used as condiments (*2*).

Natural products from plants containing compounds such as polyphenols have potential as antioxidant agents. Some studies have shown that phenolic extracts from various plants have antioxidant activities (*3*−*9*). Bitter melon contains higher amounts of phenolics, and has been demonstrated to have a strong antioxidant activity (*7*). Some solvents that are commonly used to extract phenolics are methanol, ethanol, acetone, water, ethyl acetate, propanol, dimethylformamide, and their combination (*10*). Methanol has been used to extract free and simple phenolics in fruits, vegetables, and legume seeds including bitter melon for quantification and identification (*5*, *7*−*9*, *11*). Pericarp and seed of bitter melon are rich in phenolic compounds, including catechin, epicatechin, and gallic acid, and the methanolic extracts of these tissues from selected bitter melon varieties were demonstrated to have good antioxidant activities in a model system (*7*). However, the use of methanol for extraction is not acceptable for food uses due to the toxicity of methanol. Extraction of phenolics from bitter melon using "green solvents" including water and ethanol needs to be evaluated for application as an antioxidant in food products.

The amount and types of phenolics may change during the growth and maturity of bitter melon. These changes could affect the antioxidant activity of the extracted phenolics. Antioxidant activity of the phenolic extract that gives the maximum antioxidant activity is desirable for food applications. Studies on the total phenolics and antioxidant activity of the phenolics in bitter melons at different maturity stages have not been reported. The objectives of this study were to extract phenolics from pericarp and seed of the immature, mature, and ripe bitter melons using water and ethanol at varying levels as the extracting solvents, determine total phenolics and phenolic constituents of the extracts, and investigate antioxidant activities of the extracts.

MATERIALS AND METHODS

Materials. Bitter melons from var. Sri Lanka (Thinneyville White) planted at the Arkansas Agricultural Experiment Station (Fayetteville, AR) were harvested from 3 years of crops (2004, 2005, and 2006) at three maturity stages: immature when pericarp (fleshy portion) was green and seeds were not fully developed (∼2 weeks post flowering), mature when pericarp was green and seeds was completely developed (∼3−4 weeks post flowering), and ripe when pericarp turned yellow and aril/seed coat or inner tissue turned red (∼4−5 weeks post flowering).

Fourteen standard phenolics (gallic acid, protocatechuic acid, gentisic acid, (+)-catechin, vanillic acid, chlorogenic acid, syringic acid, (−)-epicatechin, *p*-coumaric acid, benzoic acid, sinapinic acid,

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o-coumaric acid, *t*-cinnamic acid, and *t*-ferulic acid) purchased from Sigma-Aldrich, Inc. (St. Louis, MO) were used as standards for HPLC determination of individual phenolics. Trifluoroacetic acid, acetonitrile, methanol, and water were HPLC grade. All other chemicals for total phenolic content determination and free radical scavenging assay were purchased from VWR International, Inc. (Suwanee, GA) and Sigma-Aldrich, Inc. (St. Louis, MO).

Bitter Melon Seed and Pericarp Preparation for Phenolic Extraction. Pericarp from immature, mature, and ripe bitter melons was collected and used as raw material, while seeds were collected from mature and ripe bitter melons only, because seeds from immature bitter melon were not completely developed, and could not be separated from aril. Pericarp was separated manually from the other portions of bitter melon, and sliced vertically into pieces (1−2 mm thickness) using a food processor (model FP1200, The Black & Decker Corp., Towson, MD), while seeds were manually separated from aril. The cut pericarp and seeds were oven-dried on a stainless steel tray at 40 °C in a dehydrator (Harvest Saver model R-4, Commercial Dehydrator Systems, Inc., Eugene, OR) for 24 h, ground using an sample grinder (IKA WERKE model M20, Ika Works, Inc., Wilmington, NC), and passed through a 60-mesh sieve (W. S. Tyler Inc., Mentor, OH) to obtain a uniform particle size. These fine dried bitter melon samples, designated as bitter melon products, were stored at 4 °C until analysis.

Water−Ethanol Extraction of Total Phenolics. "Green solvents", namely, water and ethanol, were used to extract phenolics from the bitter melon pericarp and seed products. Ten grams of the dried products were placed in an Erlenmeyer flask connected to a condenser with water coolant. Two hundred milliliters of ethanol at the concentrations of 0/20/40/60/80/95% were added into the products. The dispersions were stirred and heated for 2 h in a water bath at 80 °C, and then vacuum-filtered to separate the extracted phenolics from residue. The residues were re-extracted twice with 150 mL of the same solvents. Ethanol was then evaporated from the extracts using a vacuum distillation unit (Büchi Rotavapor model 011, Brinkman, Wesbury, NY). The extracts were freeze-dried to remove the remaining water, and the dried extracts were stored at 4 °C under nitrogen gas until further analysis.

Determination of Total Phenolics by Folin−Ciocalteu Reagent. Total phenolic contents of the extracts were determined using a Folin−Ciocalteau method (*12*). Fifty milligrams of each extract was weighed into a test tube and vortexed with 5 mL of methanol to make 1% of extract solution. Two hundred milliliters of the solution was added with 1.0 mL of 0.2 N Folin−Ciocalteau reagent in a test tube. The solution was vortexed and incubated for 5 min, followed by addition of 1 mL of sodium carbonate (0.5 N). Absorbances of the solutions were taken after 2 h incubation at room temperature using a spectrophotometer (Shimadzu model UV-1601, Kyoto, Japan) at 765 nm. Total phenolic content was expressed as gentisic acid equivalents (GAE) in mg/g extract in dry basis, and calculated as follows: total phenolic content (GAE in mg/g extract) = $(A \times 0.1477 - 0.0146)/0.05$, where A is the absorbance at 765 nm wavelength.

Phenolic Constituent Determination by HPLC. Phenolic constituents of the extracts were determined using a HPLC method of Cai and others (*11*) with some modification. Twenty milligrams of the extracts were dissolved in 0.2 mL of methanol, and the solution was filtered through a 0.2 μm PVDF target syringe filter (Natl. Scientific, Duluth, GA). The phenolic compounds were quantified using Hewlett-Packard liquid chromatograph model 1090 equipped with a UV detector (Agilent Technologies, Inc., Palo Alto, CA), and absorbance was monitored at 254 nm. TSK-GEL Super ODS (Supelco, Bellefonte, PA) column at a maintained temperature (37 °C) was used. Gradient solvents of 0.1% trifluoroacetic acid in acetonitrile (solvent A) and 0.1% trifluoroacetic acid (solvent B) in HPLC grade water were used to elute the sample at a flow rate of 1.0 mL/min, and methanol was used to flush the column between runnings. Total run time was 35 min, and the gradient was set to vary from solvent A to solvent B from 0 to 100%.

Antioxidant Activity by Free Radical Scavenging Assay. Antioxidant activities of the extracts were evaluated using a free radical scavenging assay described by Brand-Williams and others (*13*) with some slight modification. 2,2-Diphenyl-1-picrylhydrazyl (DPPH) was used as a free radical. Varying concentrations of each extract solution in methanol were prepared, and 0.1 mL of the extract solution was added into 3.9 mL of a 6×10^{-5} mol/L of DPPH solution in methanol. The decrease in absorbance was read at 515 nm using a spectrophotometer (Shimadzu model UV-1601, Kyoto, Japan) after 180 min of reaction. The exact initial and remaining DPPH concentrations (C_{DPPH}) in the solution were calculated from a calibration curve using the following equation: $Abs_{515nm} = 12{,}509 \times C_{DPPH} - 0.00258$, as determined by a linear regression. For each concentration, the percent of remaining DPPH after 180 min reaction was considered as the free radical scavenging activities. These percents of the remaining DPPH concentrations were then plotted against the extract levels to compute EC_{50} (efficient concentration in mg extract/mg DPPH, defined as the amount of extracts necessary to decrease the initial DPPH concentration by 50%) of the extracts. Antioxidant activities were also expressed as antiradical powers, calculated as $1/EC_{50}$ (in mg DPPH/mg extract).

Statistical Analysis. All values are reported as means of three determinations from 3 years of crops (2004, 2005, and 2006). Analysis of variance (ANOVA) was conducted by JMP 7 software package (SAS Institute Inc., Cary, NC) for total phenolic contents and antiradical power values of extracts using a Tukey honestly significantly different (HSD) procedure for the significance of differences among the extracts, obtained from different maturity stages within tissues and between tissue portions, using varying ethanol levels at a 5% significance level. Least significant difference (LSD) values were provided for mean comparisons; if the actual difference of the means was greater than the LSD, then the difference would be significant.

RESULTS AND DISCUSSION

Total Phenolic Content. Total phenolic contents of the extracts from pericarp and seeds of bitter melons harvested at different maturation stages are shown in **Table 1**. In the extracts from the bitter melon pericarp obtained using varying levels of ethanol as the extraction solvent, the total phenolics ranged from 6.9 to 15.7, 6.4 to 14.8, and 4.3 to 14.9 mg as GAE/g extract for the extracts from immature, mature, and ripe bitter melons, respectively. In general, there were significant effects of the ethanol levels on the total phenolics of the extracts from bitter melon pericarp of any maturity stages (LSD values were 0.78, 0.49, and 0.63 for immature, mature, and ripe pericarps, respectively, P values < 0.0001). The data from the extracts of the pericarp from each maturity stage and the average total phenolics of the pericarp extracts from multiple maturity stages also showed that 80% ethanol extracted a higher amount of phenolics. There was an increase in the total phenolic contents of the extracts when the ethanol levels increased from 0% to 80% and a decrease when the ethanol level was 95% (the calculated total phenolics were 5.9, 6.7, 8.1, 13.0, 15.1, and 13.6 mg GAE/g extract for the pericarp extracts obtained using 0%, 20%, 40%, 60%, 80%, and 95% of ethanol, respectively) (LSD value was 1.23, P value < 0.0001). The variability in the total phenolic contents caused by the ethanol concentration levels could be the result of the varying solubility of the phenolic compounds; this variation in solubility may be driven by the solvent polarity (*14*). Some studies showed that methanol and ethanol that are polar solvents were better extraction solvents for phenolics from plant materials than less polar solvents including acetone and hexane (*15*, *16*); however, according to another study conducted by Liu and others (*17*), a less polar solvent such as acetone could extract more phenolic compounds from a plant material/lychee flowers than more polar solvents, including methanol and water. These differences may be due to the types of phenolic compounds in the plant materials. In general, a good balance in polarity is needed in extracting phenolics from plant sources. An 80% concentration of ethanol may have the right degree of polarity for the extraction of most phenolic compounds from the pericarp of the bitter melon.

Total phenolic contents of seed extracts from mature and ripe bitter melons ranged from 6.4 to 18.0 and 6.1 to 20.9 mg GAE/g of extract, respectively (**Table 1**). Similar to the extracts from pericarp, the ethanol levels significantly affected the amount of the total phenolics extracted from the bitter melon seed

Table 1. Total Phenolic Contents (in mg GAE/g of Extract), EC_{50} (Efficient Concentration in mg extract/mg DPPH), and Antiradical Power (mg DPPH/mg extract $\times$ 10^{-2}) of Extracts from Pericarp and Seeds of Bitter Melons Harvested at Immature (~2 Weeks Postflowering), Mature (~3−4 Weeks Postflowering), and Ripe (~4−5 Weeks Postflowering) Stages, Obtained Using Varying Levels of Ethanol[a]

maturity	% ethanol	total phenolics	EC_{50}	antiradical power
		Pericarp		
immature	0	6.9 ± 0.1	13.8 ± 0.5	7.3 ± 0.3
	20	7.3 ± 0.2	14.0 ± 1.0	7.2 ± 0.5
	40	8.9 ± 0.1	15.0 ± 0.5	6.7 ± 0.2
	60	14.2 ± 0.6	15.6 ± 0.8	6.4 ± 0.3
	80	15.7 ± 0.1	14.5 ± 0.5	6.9 ± 0.3
	95	13.0 ± 0.2	16.0 ± 1.2	6.3 ± 0.5
mature	0	6.4 ± 0.1	16.0 ± 0.7	6.2 ± 0.3
	20	7.4 ± 0.1	14.1 ± 0.5	7.1 ± 0.2
	40	7.8 ± 0.1	15.8 ± 0.6	6.3 ± 0.2
	60	13.2 ± 0.2	15.2 ± 0.6	6.6 ± 0.2
	80	14.8 ± 0.3	12.6 ± 0.5	8.0 ± 0.3
	95	13.8 ± 0.2	14.5 ± 0.4	6.9 ± 0.2
ripe	0	4.3 ± 0.2	23.3 ± 1.1	4.3 ± 0.2
	20	5.2 ± 0.2	22.6 ± 1.0	4.4 ± 0.2
	40	7.6 ± 0.2	25.3 ± 1.8	4.0 ± 0.3
	60	11.8 ± 0.1	17.2 ± 1.2	5.8 ± 0.4
	80	14.9 ± 0.2	15.8 ± 0.8	6.3 ± 0.3
	95	14.1 ± 0.0	12.4 ± 1.2	8.1 ± 0.7
		Seed		
mature	0	14.8 ± 0.5	35.6 ± 1.5	2.8 ± 0.1
	20	15.9 ± 0.1	22.5 ± 0.6	4.4 ± 0.1
	40	17.7 ± 0.1	16.9 ± 0.4	5.9 ± 0.1
	60	17.9 ± 0.0	15.8 ± 0.3	6.3 ± 0.1
	80	18.0 ± 0.1	17.5 ± 0.2	5.7 ± 0.1
	95	6.4 ± 0.1	27.3 ± 0.5	3.7 ± 0.1
ripe	0	14.4 ± 0.1	40.4 ± 0.2	2.5 ± 0.0
	20	19.1 ± 0.1	31.6 ± 0.8	3.2 ± 0.1
	40	20.1 ± 0.1	20.7 ± 0.3	4.8 ± 0.1
	60	20.7 ± 0.1	19.6 ± 0.5	5.1 ± 0.1
	80	20.9 ± 0.1	18.2 ± 0.4	5.5 ± 0.1
	95	6.1 ± 0.1	51.8 ± 0.9	1.9 ± 0.0

[a] Values are means ± SD of three determinations from 3 years of crops (2004, 2005, and 2006). For total phenolics, LSD values across % ethanol (0 to 95%) in the same portion and maturity were 0.7752, 0.4946, 0.628, 0.466, and 0.25, respectively; For antiradical power, LSD values across % ethanol (0 to 95%) in the same portion and maturity were 0.954, 0.703, 1.086, 0.304, and 0.243, respectively.

(LSD values were 0.47 and 0.25 for mature and ripe seeds, respectively, P value < 0.0001). However, despite that there was a similar trend in the total phenolic contents in the pericarp extracts using various levels of ethanol, there were no significant differences among the average total phenolics of the seed extracts, calculated from multiple maturity stages obtained using 20, 40, 60, and 80% ethanol (P values were > 0.05) which were 17.5, 18.9, 19.3, and 19.5 mg GAE/g extract, respectively (LSD value was 2.24). These total phenolics were significantly higher than those of the extracts obtained using 0 or 95% of ethanol, which had the total phenolic contents of 14.6 and 6.3 mg GAE/g extract, respectively. When the total phenolic contents of these seed extracts were pooled and averaged from multiple maturation stages and all the ethanol levels, the phenolic content of the seed extracts was significantly higher than that of the pericarp extracts (16.0 vs 10.4 mg GAE/g extract with the P value < 0.0001). This difference may be due to the differences in the composition of the phenolic compounds in the bitter melon seed and pericarp.

Based on the total phenolic contents of the extract, 80% ethanol was the best extraction solvent to extract phenolics from either bitter melon seed or pericarp. **Table 2** shows the least-squares means (LSM) contrast for the total phenolic contents in the extracts from pericarp and seeds of bitter melons at different maturity stages, obtained using 80% ethanol. The LSM contrast analysis revealed that the mature and ripe seed extracts were significantly higher than either the extracts from pericarp (from immature, mature, and ripe) or the pericarp (from mature and ripe only) on their total phenolic contents (contained an average of 4.3 (±0.08) mg GAE/g extract (P value < 0.0001) and 4.6 (±0.09) mg GAE/g extract (P value < 0.0001), respectively) (**Table 2**). The estimate of average total phenolic contents for the mature vs ripe was 1.5 (±0.09) mg GAE/g extract (P value < 0.0001) (**Table 2**) indicates that there was a significant difference in the total phenolic contents of the extracts from mature and ripe bitter melons, when extracted with the 80% ethanol solvent.

Table 2. Least Squares Means Contrast for Total Phenolic Contents (in mg GAE/g extract) of Extracts from Pericarp and Seeds of Bitter Melons Harvested at Immature (~2 Weeks Postflowering), Mature (~3−4 Weeks Postflowering), and Ripe (~4−5 Weeks Postflowering) Stages, Obtained Using 80% Ethanol

test detail			
immature pericarp	0.3333	0	0
mature pericarp	0.3333	0.5	0.5
ripe pericarp	0.3333	0.5	−0.5
mature seed	−0.5	−0.5	0.5
ripe seed	−0.5	−0.5	−0.5
estimate	−4.329	−4.616	−1.525
std error	0.0781	0.0855	0.0855
t ratio	−55.46	−53.99	−17.83
Prob > |t|	9×10^{-14}	1×10^{-13}	6.6×10^{-9}
SS	67.456	63.935	6.9731

Phenolic Constituents. Individual phenolic compounds of the extracts were identified using HPLC based on retention times of the peak profile of the extracts in comparison to standard phenolic compounds, and these were confirmed by spiking the samples with the known standard phenolics. **Tables 3** and include the known phenolic constituents present in the pericarp and seed extracts quantified using calibration curves of the corresponding standards. The results showed that the most abundant phenolic in the extracts from immature, mature, and ripe bitter melon pericarp was catechin, which ranged from 116.3 to 145.8, 102.5 to 154.5, and 90.9 to 147.9 mg/100 g extract, respectively (**Table 3**). All values are expressed on a dry basis in mg/100 g. The catechin contents were higher in the extracts obtained from 80 and 95% of ethanol than in the extracts from the other ethanol levels. The other main phenolics in the pericarp extracts were gallic, gentisic, and chlorogenic acids and epicatechin. Gallic acid contents ranged from 30.1 to 49.9, 27.8 to 51.8, and 29.0 to 43.1 mg/100 g extract, gentisic acid contents ranged from 65.8 to 72.8, 61.4 to 71.9, and 50.4 to 69.5 mg/100 g extract, chlorogenic acid contents ranged from 32.8 to 62.0, 30.8 to 66.4, and 26.9 to 65.4 mg/100 g extract, and epicatechin contents varied from 30.9 to 44.6, 27.6 to 36.9, and 20.9 to 40.0 mg/100 g extract in the immature, mature, and ripe bitter melons, respectively. In addition to those phenolics, the pericarp extracts also contained other phenolics in relatively small quantities, including protocatechuic acid (12.9−21.1, 9.2−17.8, and 8.2−14.3 mg/100 g extract), vanillic acid (6.6−8.5, 4.7−10.2, and 4.2−6.3 mg/100 g extract), *p*-coumaric acid (0−16.2, 0−24.7, and 0−29.0 mg/100 g extract), and *o*-coumaric acid (11.4−18.6, 14.4−31.5, and 13.8−25.3 mg/100 g extract) in the immature, mature, and ripe bitter melons, respectively, and in very small amount as *t*-cinnamic acid (0−4.9, 0−8.5, and 0−4.3 mg/100 g extract from the immature, mature, and ripe bitter melons, respectively). In general, catechin,

Table 3. Phenolic Constituents (in mg/100 g extract)[a] of Extracts from Pericarp of Bitter Melons Harvested at Immature (~2 Weeks Postflowering), Mature (~3−4 Weeks Postflowering), and Ripe (~4−5 Weeks Postflowering) Stages, Obtained Using Varying Levels of Ethanol

solvent (% ethanol)	gallic acid	protocatechuic acid	gentisic acid	catechin	vanillic acid	chlorogenic acid	epicatechin	*p*-coumaric acid	*o*-coumaric acid	*t*-cinnamic acid
					Immature					
0	30.1 ± 2.5	21.1 ± 1.8	70.7 ± 5.9	121.0 ± 6.6	7.1 ± 0.1	32.8 ± 2.3	44.6 ± 1.8	7.5 ± 1.2	11.4 ± 0.2	nd
20	30.8 ± 0.4	18.3 ± 1.6	68.2 ± 0.6	123.0 ± 12.6	8.5 ± 0.6	33.1 ± 0.4	41 ± 1.5	7.2 ± 1.4	16.0 ± 1.1	nd
40	43.4 ± 1.0	12.9 ± 0.6	65.9 ± 1.6	126.3 ± 4.6	8.0 ± 0.4	32.9 ± 1.8	36.9 ± 1.7	14.9 ± 2.4	15.3 ± 0.4	3.9 ± 0.2
60	46.7 ± 2.6	14.4 ± 2.1	68.3 ± 2.7	116.3 ± 3.0	6.7 ± 1.1	40.7 ± 1.8	34.9 ± 1.2	16.2 ± 3.4	15.6 ± 1.0	3.9 ± 0.5
80	48.1 ± 3.7	15.0 ± 0.8	72.8 ± 1.5	145.8 ± 1.4	7.9 ± 0.7	62.0 ± 9.5	30.9 ± 0.7	nd	17.3 ± 1.7	4.9 ± 0.7
95	49.9 ± 3.4	14.3 ± 1.4	65.8 ± 1.5	139.4 ± 13.8	6.6 ± 1.2	48.5 ± 9.7	33.3 ± 0.8	nd	18.6 ± 2.9	4.9 ± 1.0
					Mature					
0	27.8 ± 2.7	17.8 ± 0.6	62.6 ± 6.3	112.8 ± 13.1	4.7 ± 0.2	30.8 ± 0.7	36.4 ± 0.7	12.3 ± 0.7	14.4 ± 0.7	nd
20	30.1 ± 2.9	17.3 ± 1.1	67.3 ± 4.4	137.7 ± 8.4	7.2 ± 2.5	34.6 ± 1.2	35.2 ± 2.4	10.4 ± 1.6	19.9 ± 0.9	nd
40	44.0 ± 3.1	15.8 ± 0.5	62.6 ± 4.0	102.5 ± 7.4	8.1 ± 1.9	31.1 ± 2.2	36.9 ± 1	15.2 ± 3.2	19.8 ± 2.3	3.3 ± 0.9
60	44.0 ± 2.6	15.5 ± 1.8	67.8 ± 1.7	122.2 ± 7.5	10.2 ± 1.8	47.3 ± 3.6	33.3 ± 1.3	24.7 ± 3.4	22.5 ± 1.8	5.2 ± 1.4
80	48.8 ± 1.4	11.8 ± 2.8	71.9 ± 3.7	154.5 ± 7.2	9.3 ± 2.1	66.4 ± 5.4	28.8 ± 0.5	nd	26.8 ± 5.9	8.5 ± 1.7
95	51.8 ± 2.9	9.2 ± 2.3	61.4 ± 3.5	140.1 ± 5.9	4.9 ± 0.6	50.4 ± 1.8	27.6 ± 1.4	nd	31.5 ± 5.8	6.4 ± 3.2
					Ripe					
0	29.0 ± 7.0	14.2 ± 0.8	50.4 ± 8.2	119.6 ± 1.3	4.2 ± 0.1	26.9 ± 1.0	36.4 ± 0.3	7.3 ± 2.2	13.8 ± 0.4	nd
20	29.6 ± 3.5	10.8 ± 1.5	61.5 ± 1.1	117.6 ± 17.9	4.7 ± 0.4	30.5 ± 2.1	34.3 ± 1.4	7.4 ± 1.9	17.7 ± 1.7	nd
40	40.6 ± 2.2	8.2 ± 1.2	55.1 ± 3.2	90.9 ± 9.5	6.3 ± 1.3	30.1 ± 2.7	40 ± 1.3	11.2 ± 3.1	16.4 ± 1.1	nd
60	43.1 ± 1.6	14.8 ± 0.4	63.0 ± 4.8	117.9 ± 9.4	5.9 ± 0.3	40.2 ± 1.5	24.8 ± 1.5	29.0 ± 3.3	17.5 ± 2.5	nd
80	41.8 ± 1.4	10.8 ± 1.9	69.5 ± 1.4	147.9 ± 4.1	4.5 ± 0.4	65.4 ± 3.5	20.9 ± 1.1	nd	24.3 ± 3.9	4.1 ± 0.3
95	38.3 ± 4.8	14.3 ± 0.7	65.7 ± 6.2	147.9 ± 10.4	4.9 ± 0.2	52.2 ± 2.2	26 ± 1.4	nd	25.3 ± 1.3	4.3 ± 0.2

[a] Values are means ± SD of three determinations from 3 years of crops (2004, 2005, and 2006); nd, not detectable.

Table 4. Phenolic Constituents (in mg/100 g extract)[a] of Extracts from Seeds of Bitter Melons Harvested at Mature (~3−4 Weeks Postflowering), and Ripe (~4−5 Weeks Postflowering) Stages, Obtained Using Varying Levels of Ethanol

solvent (% ethanol)	gallic acid	protocatechuic acid	gentisic acid	catechin	vanillic acid	chlorogenic acid	syringic acid	epicate-chin	*p*-coumaric acid	*o*-coumaric acid	*t*-cinnamic acid
					Mature						
0	15.9 ± 0.8	4.8 ± 1.3	76.1 ± 5.4	171.5 ± 5.7	2.9 ± 0.2	19.4 ± 1.6	8.0 ± 0.7	46.4 ± 1.2	24.2 ± 1.5	12.7 ± 1	4.5 ± 0.8
20	19.7 ± 5.4	5.2 ± 0.6	112.1 ± 6.6	229.4 ± 9.4	8.6 ± 0.2	19.6 ± 0.2	8.7 ± 0.9	57.5 ± 1.1	22.2 ± 0.6	10.9 ± 1.4	4.0 ± 0.1
40	21.2 ± 0.9	10.7 ± 0.3	107.4 ± 3.0	296.5 ± 14.5	5.4 ± 0.1	25.8 ± 0.5	10.2 ± 2.7	82.2 ± 1.7	20.4 ± 0.3	6.6 ± 0.1	8.3 ± 0.2
60	21.9 ± 2.0	6.2 ± 0.3	126.8 ± 13.5	294.2 ± 17.6	5.2 ± 0.1	21.0 ± 3.0	8.4 ± 2.4	73.9 ± 3.0	17.5 ± 1.3	5.9 ± 0.8	12.8 ± 0.2
80	10.2 ± 0.1	5.6 ± 0.0	100.5 ± 8.6	272.5 ± 14.5	3.4 ± 0.0	10.3 ± 0.2	5.4 ± 0.1	119.7 ± 0.3	7.4 ± 0.1	3.4 ± 0.0	17.1 ± 0.6
95	8.7 ± 0.3	2.3 ± 0.5	13.6 ± 0.7	142.8 ± 11.4	2.5 ± 1.0	6.8 ± 1.7	4.2 ± 1.4	49.0 ± 4.8	31.9 ± 1.5	11.8 ± 1.9	6.8 ± 0.9
					Ripe						
0	26.2 ± 0.8	12.3 ± 0.1	64.5 ± 1.6	177.6 ± 2.4	5.6 ± 0.4	29.8 ± 0.7	nd	121.2 ± 1.9	17.5 ± 1.9	9.4 ± 0.1	4.3 ± 0.1
20	30.1 ± 3.7	20.6 ± 1.5	133.5 ± 1.0	191.4 ± 4.9	7.2 ± 0.6	30.7 ± 0.1	9.8 ± 0.2	122.1 ± 3.9	21.8 ± 1.3	9.7 ± 0.5	9.3 ± 0.6
40	40.0 ± 0.8	22.1 ± 0.2	178.3 ± 1.2	255.9 ± 4.2	8.5 ± 0.3	22.8 ± 1.8	13.3 ± 1.3	152.4 ± 3.7	19.8 ± 0.2	18.0 ± 0.2	16.5 ± 0.3
60	40.6 ± 2.9	19.9 ± 0.3	214.6 ± 2.2	240.9 ± 24.8	8.3 ± 0.4	21.9 ± 0.5	15.1 ± 0.3	158.9 ± 1.8	19.3 ± 0.4	21.4 ± 0.2	17.2 ± 0.9
80	27.9 ± 3.7	19.5 ± 0.7	186.9 ± 1.4	243.8 ± 4.3	7.1 ± 0.1	19.2 ± 1.9	12.2 ± 0.1	146.0 ± 2.6	16.4 ± 0.2	20.1 ± 0.2	16.4 ± 0.5
95	10.0 ± 0.2	2.9 ± 0.1	80.0 ± 1.9	112.7 ± 9	6.9 ± 0.3	13.7 ± 0.2	nd	76.5 ± 6.9	19.9 ± 0.8	9.8 ± 0.2	6.8 ± 1.3

[a] Values are means ± SD of three determinations from 3 years of crops (2004, 2005, and 2006); nd, not detectable.

gallic acid, and chlorogenic acid contributed mostly to the phenolic contents of the pericarp extract. From 80% ethanol extracts, an increase in these three phenolics was observed as bitter melon reached the mature stage, but as the maturity progressed further these phenolic contents decreased. Other phenolics did not show the same trend indicating that probably there was a conversion from other phenolics to these three phenolics as the maturity developed.

The most abundant phenolic in the extracts from mature and ripe seeds was catechin, which was similar to the extracts from bitter melon pericarp. The catechin contents in the seed extracts were 142.8−296.5 and 112.7−255.9 mg/100 g extract from the mature and ripe bitter melons, respectively (**Table 4**). These values were much higher than those from pericarp extracts. Unlike pericarp extracts, seed extracts also contained a large amount of gentisic acid and epicatechin. The gentisic acid contents of the mature and ripe seed extracts were 13.6−126.8 and 64.5−214.6 mg/100 g extract, respectively; while the epicatechin contents ranged from 46.4 to 119.7 and 76.5 to 158.9 mg/100 g extract, respectively. The other major phenolics in the seed extracts were gallic acid (8.7−21.9 and 10.0−40.6 mg/100 g extract), chlorogenic acid (6.8−25.8 and 13.7−30.7 mg/100 g extract), and *p*-coumaric acid (7.4−31.9, and 16.4−21.8 mg/100 g extract) from the mature and ripe bitter melons, respectively. Minor phenolics in the seed extracts were protocatechuic acid (2.3−10.7 and 2.9−22.1 mg/100 g extract), *o*-coumaric acid

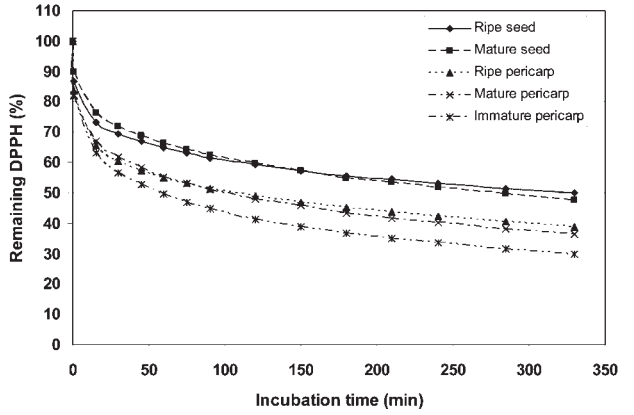

Figure 1. Effects of incubation time on remaining DPPHs in solutions containing bitter melon seed and pericarp extracts, obtained using 80% ethanol.

(3.4−11.8 and 9.4−21.4 mg/100 g extract), *t*-cinnamic acid (4.0−17.1 and 4.3−17.2 mg/100 g extract), and syringic acid (4.2−10.2 and 0−15.1 mg/100 g extract) from the mature and ripe bitter melons, respectively. Vanilic acid was present in a small amount that ranged from 2.5 to 8.6 and 5.6 to 8.5 mg/100 g extract from the mature and ripe bitter melons, respectively. In general, these results were correlated well to the phenolic constituents from bitter melons obtained from different varieties as reported previously (*7*). Overall, catechin, the most abundant phenolic compound in the seed extract, and epicatechin decreased as the maturity progressed to ripe stage in all ethanol solvents with the exception of 0% ethanol. Gentisic acid as the second abundant phenolic in the seed, gallic acid, protocatechuic acid, chlorogenic acid, and epicatechin contents showed an increase when bitter melon reached the ripe stage. These composition changes that occurred as bitter melon developed may be the result of a conversion of catechin into other phenolics as the maturity progressed.

Free Radical Scavenging Activity. Figure 1 associated with the free radical scavenging method demonstrates the % of remaining DPPH decreased over time due to the presence of phenolic compounds from bitter melon pericarp and seed extracts in the solution. It should be noted that an extended length of time was needed to decrease DPPH levels to reach a steady state. Twenty compounds were evaluated for antiradical activity using DPPH by Brand-Williams and others (*13*), and they found that there were only three compounds (ascorbic acid, isoascorbic acid, and isoeugenol) that could reach a steady state in less than 1 min; while rosmarinic acid and δ-tocopherol reached a steady state within 5 and 30 min, respectively, and were considered as having an intermediate kinetic behavior; the remaining compounds showed a slow kinetic behavior, taking 1 to 6 h to get to a plateau. Some of the antioxidants among this slow rate group were gallic, gentisic, protocatechuic, vanillic, coumaric, and ferulic acids (*13*). These results correlate well with those obtained using bitter melon extracts. Not only were the extremely slow rates observed, but the same phenolics that contributed to slower rates were found to exist in the bitter melon extracts. The steady state was not reached even after 6 h of reaction for all the extracts because of their slow kinetic behavior reactions as seen in **Figure 1**. However, only an insignificant decrease was observed after 3 h of incubation. For evaluation of the free radical scavenging assay of the extracts, 3 h of reaction was taken as a fixed reaction time for the absorbance measurement to determine the remaining DPPH in the solution, containing varying levels of the extracts. This incubation time would give enough time for the phenolic extract to quench a maximum amount of the free radical. A modified DPPH assay method with a fixed reaction time ranging from 0.5 to 3 h for the absorbance reading has been used to evaluate the radical scavenging capacity of many extracts recently (*15*, *17*−*21*).

Table 5. Bivariate Fit of Antiradical Powers (mg DPPH/mg extract $\times 10^{-2}$) by Total Phenolic Contents (mg GAE/g extract) of Extracts from Pericarp and Seeds of Bitter Melons Harvested at Immature (∼2 Weeks Postflowering), Mature (∼3−4 Weeks Postflowering), and Ripe (∼4−5 Weeks Postflowering) Stages, Obtained Using Varying Levels of Ethanol

		parameter estimate		
maturity	R^2	total phenolics	intercept	*P* value
		Pericarp		
combined	0.2607	0.157	4.743	<0.0001
immature	0.2357	−0.066	7.508	0.0411
mature	0.3258	0.103	5.766	0.0134
ripe	0.7005	0.297	2.623	<0.0001
		Seed		
combined	0.4075	0.188	1.310	<0.0001
mature	0.4126	0.202	1.752	0.0040
ripe	0.7264	0.221	0.098	<0.0001
		Pericarp and Seed		
combined	0.0013	0.012	5.410	0.7365

Table 1 includes EC_{50} and antiradical power values of the extracts from pericarp and seed of bitter melons using varying levels of ethanol. EC_{50} parameter was commonly presented together with other parameters derived from EC_{50} values, including antiradical power, to express the antioxidant power, capacity, activity, or properties (*13*, *20*, *22*). While EC_{50} value would give a reverse correlation to antioxidant activity (higher EC_{50} value means lower antioxidant activity), antiradical power value would provide a positive correlation to the antioxidant activity. For a reason of straightforwardness, the antiradical power is a better expression in relation to the antioxidant activity of the extracts than EC_{50} due to their positive correlation; larger antiradical power values reflect higher antioxidant activities (*13*).

As shown in **Table 1**, all the examined extracts had antioxidant activities. However, variability was observed among the extracts from different portions of the bitter melons, from different maturity stages, and within varying levels of ethanol as the extraction solvent. The antiradical powers of the extracts from immature, mature, and ripe pericarp obtained using varying levels of ethanol ranged from 6.3 to 7.3, 6.3 to 8.0, 7, and 4.0 to 8.1 $\times 10^{-2}$ mg DPPH/mg extract, respectively, while the extracts from mature and ripe seeds ranged from 2.8 to 6.3 and 1.9 to 5.5 $\times 10^{-2}$ mg DPPH/mg extract, respectively. Similar to the total phenolic contents, there were significant effects of the ethanol levels as the extraction solvents on the antiradical power values of the extracts. LSD values for the extracts from immature, mature, and ripe pericarp, and mature and ripe seeds were 0.954 (*P* value = 0.0223), 0.703 (*P* value < 0.0001), 1.086 (*P* value < 0.0001), 0.304 (*P* value < 0.0001), and 0.243 (*P* value < 0.0001), respectively. However, unlike their total phenolic contents, despite that 80% ethanol was the most effective extraction solvent for phenolics, only extracts from mature pericarp and ripe seed using 80% ethanol showed the most effective antiradical power. An increase in the total phenolic contents of the extracts did not reflect an increase in their antiradical capacities. A linear relationship of the total phenolic contents and the antiradical power values of the extracts were statistically illustrated using a bivariate fit as in **Table 5**. Only the extracts from ripe pericarp and seed demonstrated a significant positive linear correlation between their total phenolic contents and antiradical powers with the R^2 values of 0.7005 (*P* value < 0.0001) and 0.7264 (*P* value < 0.0001), respectively,

while the total phenolic contents of the extracts from immature pericarp had a negative correlation with their antiradical power values. Despite some studies showing a high correlation between the antiradical activities and the total phenolic contents of plant extracts (*4*, *17*, *20*, *23*, *24*), some studies also reported a low correlation (*22*, *25*−*27*), and even no correlation or insignificant relationship between the total phenolic contents and antiradical activities of the plant extracts (*28*, *29*). Variability in their correlation could be the result of the possible interaction of the phenolic compounds in the extracts that reduces their free radical scavenging capacities (*27*), or even causes no synergistic or antagonistic effects (*30*, *31*). Our study has identified phenolic extracts as antioxidants that can find several applications, particularly in lipid based foods.

Phenolics extracted from pericarp and seed of bitter melons at three maturity stages using varying levels of ethanol (0/20/40/60/80/95%) resulted in variability in their total phenolic contents and phenolic constituents. The results demonstrated that the maximum total phenolics that could be extracted from either bitter melon pericarp or seeds, when 80% ethanol was used as the extraction solvent. The results also revealed main phenolic constituents of the bitter melon extracts to be catechin, gallic acid, gentisic acid, chlorogenic acid, and epicatechin. Kinetic behavior showed that all the examined extracts to have a slow free radical scavenging property. Even though the extracts obtained using 80% ethanol had the highest total phenolic contents, these extracts did not always give the highest antioxidant activity. In general, there were significantly low correlations between the total phenolic contents and antiradical power values of the extracts. These extracts could find application as natural antioxidants as an alternative to synthetic antioxidants to prevent or minimize oxidation in food systems for extending the food shelf life.

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