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Antioxidant Activity of Hazelnut Skin Phenolics

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Phenolic compounds were extracted from hazelnut skin using 80% (v/v) aqueous acetone or methanol. The crude extracts were applied onto a Sephadex LH-20 column for two fractionations (Fr. I and Fr. II). Fr. I consisting of low-molecular-weight phenolics was eluted by ethanol, whereas Fr. II consisting of tannins was obtained using acetone/water (1:1, v/v) as the mobile phase. UV spectra of phenolic compounds present in the crude extracts and their fractions exhibited a maximum absorbance at 282 nm. The crude extracts and their fractions were examined for phenolic and condensed tannin contents as well as total antioxidant activity (TAA), antiradical activity against the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, and reducing power. Results of these assays showed higher values when Fr. II containing tannins was tested, followed by crude extract, and Fr. I. Both 80% acetone and methanol were capable of extracting phenolics, but 80% acetone was a more effective solvent for the extraction of condensed tannins ($p < 0.05$). These results suggest that hazelnut skin can be considered as a value-added byproduct for use as dietary antioxidants.

KEYWORDS: Hazelnut skin crude extract; fractionations; UV spectra; total phenolics; condensed tannins; antioxidant activity; DPPH radical; reducing power

INTRODUCTION

Hazelnut (*Corylus avellana* L.) belongs to the *Betulaceae* family and is a popular tree nut worldwide. Turkey is the world's largest producer of hazelnuts, contributing to approximately three-quarters of the global production. Hazelnut is therefore of great importance to Turkey's economy (*1*).

The hazelnut hard shell, containing a kernel, is the nut of commerce. After cracking the hazelnut, the hazelnut kernel may be consumed raw (with brown skin) or preferably roasted (without skin). Hazelnut skin, which is a byproduct of roasting, represents about 2.5% (skin absorbs oil during roasting) of the total hazelnut kernel weight and is discarded upon roasting. Therefore, the use of hazelnut skin as a potential source of natural antioxidant and functional food ingredient is of great interest to the hazelnut industry.

Recent recognition of nuts as heart-healthy foods by the U.S. Food and Drug Administration (FDA) has provided a major boost to the image of nuts, including hazelnuts (*2*). Among tree nuts, hazelnuts have many beneficial health attributes (*3*) and are among the three most popular and commonly consumed tree nuts in Europe (*4*) and other Western countries (*5*). The health effects of hazelnuts have been well documented (*3*, *6*).

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Over the past few years, much attention has been paid to the skins of tree nut kernels. Studies have acknowledged that tree nut skins are rich sources of phenolic compounds and possess stronger antioxidant activities than those of their kernel and other tree nut byproducts (*7*−*13*). These phenolic compounds provide protection against harmful effects of free radicals and are known to reduce the risk of several diseases including certain types of cancer, coronary heart disease (CHD), type-2 diabetes, and inflammation, among others (*14*−*18*).

Although some papers have been published regarding the antioxidant activity and phenolic constituents of hazelnut skin (*10*, *12*), little is known about the low-molecular-weight phenolic and tannin fractions. Tannins have been reported to possess strong antioxidant and antiradical activities (*19*). Extracts of natural antioxidants from hazelnut skin could potentially be used as nutraceuticals and dietary supplements. Therefore, the objectives of this study were to investigate the antioxidant activity of the crude extract of hazelnut skin and its low-molecular-weight phenolic and tannin fractions using two different extraction solvents.

MATERIALS AND METHODS

Samples. The premium class natural Turkish Tombul hazelnuts (*Corylus avellana* L) were harvested from the Giresun Province of Turkey at the beginning of the harvest season of 2007. The sun dried (for 3 days at ~20−25 °C) unshelled hazelnuts were cracked and then roasted at 140 °C for 30 min with an air velocity of 1 m/s (model CSO2-KF Hazelnut Roasting Oven, Ceselsan Machinary Ltd., Giresun, Turkey) to obtain the skin.

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Chemicals. All chemicals were obtained from Sigma-Aldrich Co. Ltd. (Poznań, Poland), unless otherwise specified.

Extraction of Phenolic Compounds. Hazelnut skin was ground in a coffee grinder (200−400 mesh) (model CBG5 series, Black and Decker Canada Inc., Brockville, ON) for 3 min and then transferred to dark-colored flasks, extracted using two different solvent systems [80:20 (v/v) acetone/water and 80:20 (v/v) methanol/water] at a solid to solvent ratio of 1:10 (w/v), and subsequently placed in a shaking constant-temperature water bath (model Elpan 357, Wroclaw, Poland) at 50 °C for 30 min (*20*). The resulting slurries were centrifuged at 4000*g* for 15 min, and the supernatant was collected. The residue was re-extracted twice under the same conditions, and supernatants were combined. The solvent was then evaporated from the combined supernatants under vacuum at 40 °C (model Büchi Rotavapor R-114; Büchi Labortechnik AG, Flawil, Switzerland), and the remaining water in the concentrated extract was removed by lyophilization for 72 h at −48 °C and 0.046 mbar (Freezone 6, Model 77530; Labconco Co., Kansas City, MO). Finally, the prepared crude extracts were stored at −20 °C in vacuum-sealed pouches (in the dark) until they were used for further analysis.

Column Chromatography. Separation of crude extracts into low-molecular-weight phenolic and tannin fractions was carried out according to the method described by Strumeyer and Malin (*21*). A 2 g portion of the crude extract was suspended in 20 mL of 95% (v/v) ethanol and applied onto a chromatographic column (5 × 40 cm) packed with Sephadex LH-20 and equilibrated with 95% (v/v) ethanol. Low-molecular-weight phenolic compounds (Fr. I) were eluted from the column using 1 L of 95% (v/v) ethanol. To obtain tannins (Fr. II), the column was washed with 500 mL of 50% (v/v) acetone. Organic solvents were evaporated, and water from the tannin fraction was removed by lyophilization.

UV Spectra. Ultraviolet (UV) spectra of crude extracts and their individual fractions were recorded using a Beckman DU 7500 diode array spectrophotometer (Beckman Instruments Inc., Fullerton, CA), as reported by Amarowicz et al. (*22*).

Determination of Total Phenolics. The content of total phenolic compounds in the crude extracts and each fraction was estimated using the Folin−Ciocalteu's phenol reagent as described by Amarowicz et al. (*22*). The results were expressed as milligrams of catechin equivalents (CE) per gram of crude extract or its fractions.

Determination of Condensed Tannins. The content of condensed tannins in the crude extract and its fractions was determined according to a modified vanillin/HCI method (*23*). For this method, the crude extract and its fractions were dissolved in methanol (0.5 mg/mL). To 1 mL of the solution so prepared, 5 mL of vanillin/HCI reagent [0.5 g of vanillin in 4% HCI in methanol (v/v)] was added. Samples and controls (without vanillin) were allowed to stand for 20 min in darkness, and then, the absorbance at 500 nm was then recorded. Results were expressed as milligrams of CE per milligram of crude extract or its fractions.

Determination of Total Antioxidant Activity (TAA). The TAA in crude extract and its fractions was determined according to the Trolox equivalent antioxidant capacity (TEAC) assay as described by Re et al. (*24*). The TAA was expressed as millimoles of Trolox equivalents (TE) per gram of crude extract or its fractions.

Determination of DPPH Radical Scavenging Activity. The method described by Amarowicz et al. (*22*) was used to assess DPPH (2,2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extract and its fractions. Briefly, an aliquot (0.1 mL) of methanolic solution containing 0.004 to 0.020 mg of extract, Fr. I or Fr. II was mixed with 2 mL of methanol, and then a methanolic solution of DPPH radical (1 mM, 0.25 mL) was added. The mixture was vortexed for 1 min and then left to stand at room temperature for 20 min. Finally, the absorbance of the resulting solution was read spectrophotometrically (Beckman Instruments Inc.) at 517 nm. Results were expressed as the content of crude extract (milligram per assay) or its fractions versus absorbance at 517 nm.

From the graph of DPPH radical scavenging activity, EC_{50} (efficient concentration) was read as micromoles of CE of crude extract or its fractions required to scavenge the initial DPPH radical by 50%.

Determination of Reducing Power. The reducing power of the extract and its fractions was determined as described by Oyaizu (*25*). Briefly, the suspension of each extract and its fractions (Fr. I and Fr. II) in 1 mL of distilled water was mixed with 2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of 1% (w/v) solution of potassium ferricyanide [$K_3Fe(CN)_6$]. After incubation in a water bath at 50 °C for 20 min, 2.5 mL of 10% (w/v) trichloroacetic acid solution was added, and the mixture was then centrifuged at 1750*g* for 10 min. Following this, 2.5 mL of the supernatant was combined with 2.5 mL of distilled water and 0.5 mL of 0.1% (w/v) solution of ferric chloride ($FeCl_3$). Finally, absorbance of the reaction mixture was recorded spectrophotometrically (Beckman Instruments Inc.) at 700 nm. The increased absorbance of the reaction mixture indicates a greater reducing power. Results were expressed as the content of crude extract or its fractions per assay versus absorbance at 700 nm.

Statistical Analysis. Results were expressed as the mean ± standard deviation (SD) ($n = 3$) for each extract and its fractions. The statistical significance (*t*-test: two-sample equal variance, using two-tailed distribution) was determined using Microsoft Excel statistical software (Microsoft Office Excel 2003, Microsoft Corp., Redmond, WA). Differences at $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Ultraviolet Spectra. Figure 1 depicts the UV spectra of phenolic compounds extracted from hazelnut skin crude extract and its fractions. The UV spectra of crude extracts and their fractions depended upon the extraction solvents employed. For both extraction solvents used, the hazelnut skin crude extract exhibited maximum absorbance at 282 nm, followed by Fr. I, and Fr. II. However, Fr. I separated from methanol crude extract exhibited UV spectra characterized by a maximum at 278 nm. This could be due to better extractability of gallic acid and its derivatives in methanol than acetone. A similar trend was also found in almond seed extract and its fractions (*22*). In a previous study (*26*), we compared 80% (v/v) ethanol and acetone extracts for processing phenolic compounds from hazelnut kernel and hazelnut green leafy cover and found that acetone was a more effective solvent than ethanol.

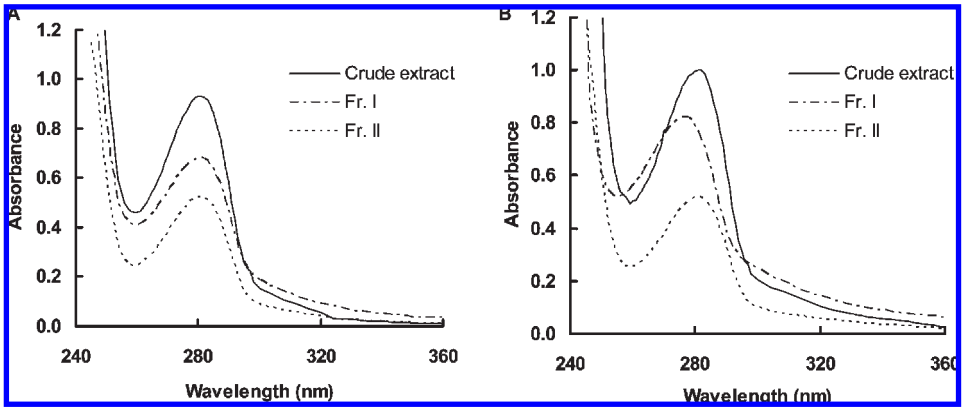

Figure 1. UV spectra of hazelnut skin crude extract and its fractions (**A**, acetone extraction and **B**, methanol extraction).

Table 1. Total Phenolics and Condensed Tannins in Hazelnut Skin Crude Extract and its Fractions[a]

		acetone extraction	methanol extraction
total phenolics (mg CE/g)	crude extract	686 ± 7 a	701 ± 13 a
	Fr. I	441 ± 12 a	442 ± 12 a
	Fr. II	697 ± 11 a	746 ± 3 b
condensed tannins (mg CE/mg)	crude extract	2.83 ± 0.04 a	2.73 ± 0.01 b
	Fr. I	1.57 ± 0.02 a	1.38 ± 0.01 b
	Fr. II	2.94 ± 0.01 a	2.89 ± 0.01 b

[a] Data are expressed as the means ± the standard deviation ($n = 3$) of a crude extract or its fractions. Means ± standard deviations followed by the same letter, within a row, are not significantly different ($p > 0.05$). Total phenolics are expressed as milligrams of catechin equivalents (CE) per gram of crude extract or its fractions. Condensed tannins are expressed as milligrams of catechin equivalents (CE) per milligram of crude extract or its fractions.

Total Phenolics. Total phenolic content was measured in acetone and methanol extracts. Low-molecular-weight phenolic fraction (Fr. I) and tannin fraction (Fr. II) were separated from each extract using Sephadex LH-20 column chromatography. The total phenolic contents in the crude acetone extract and its Fr. I and Fr. II were 686, 441, and 697 mg of CE/g, respectively. Methanol crude extract and its Fr. I and Fr. II were characterized by 701, 442, and 746 mg of CE/g, respectively (**Table 1**). No significant differences ($p > 0.05$) existed between acetone and methanolic crude extracts and their Fr. I, whereas differences ($p < 0.05$) existed for Fr. II. In both solvents used, the content of total phenolics in Fr. II was higher than those in the crude extract and its Fr. I.

Contini et al. (*12*) measured total phenolic contents of hazelnut shell waste, skin waste from whole roasted hazelnuts, and skin waste from chopped hazelnuts using three different extraction solvents [80% (v/v) of aqueous acetone, ethanol, and methanol] and found that the skin waste from whole roasted hazelnuts was approximately 7.4- and 2.9-fold higher than those of hazelnut shell waste and skin waste from chopped hazelnuts, respectively. With regard to the solvents used, the highest concentration of total phenolic content in skin waste from roasted hazelnuts using 80% (v/v) ethanol extract (588 mg of CE/g extract) was obtained, followed by 80% (v/v) acetone (547 mg of CE/g extract), and 80% (v/v) methanol (500 mg of CE/g extract). The values obtained in the present study for crude extracts (acetone and methanol) were higher than those reported by Contini et al. (*12*). The existing differences could be due to the extraction procedure used or varietal differences.

In a previous study (*26*), we measured total phenolic content of hazelnut kernel and hazelnut green leafy cover using two extraction solvents [80% (v/v) of aqueous acetone and ethanol]. Total phenolic content of hazelnut kernel and hazelnut green leafy cover extracts ranged from 23.2 to 103 and 156 to 201 mg of CE/g extract, respectively. Extracts obtained from 80% (v/v) acetone were characterized as having a higher content of total phenolics compared to those of extracts obtained from 80% (v/v) ethanol. Recently, Shahidi et al. (*10*) measured total phenolic contents of hazelnut kernel with skin (natural), hazelnut skin, hazelnut hard shell, hazelnut green leafy cover, and hazelnut tree leaf using 80% (v/v) ethanol extract. Hazelnut skin extract contained the highest total phenol content (578 mg of CE/g of extract), which was 42-fold higher than that of the hazelnut kernel extract.

Total phenolic content of different tree nut skins from almond, chestnut, and cashew have been examined using different extraction solvents. The total phenolic content in the extracts of almond skin (extracted with 80%, v/v, ethanol), chestnut skin (extracted with 50%, v/v, water), and walnut skin (extracted with 95%, v/v, ethanol) was 87.8 mg of CE/g of extract (*27*), 510 mg of gallic acid equivalents (GAE)/g of extract (*12*), and 623 mg of GAE/g of extract (*13*), respectively. Compared to the cited studies, the content of total phenolics in hazelnut skin crude extract, much higher than those of almond, chestnut, and walnut skin crude extracts, was detected regardless of the solvents used.

Condensed Tannins (Proanthocyanidins). The content of condensed tannins in the crude extract and its fractions was characterized by color reaction with vanillin/HCI reagent (**Table 1**). Fr. II (tannin fraction) contained a higher amount of condensed tannins than extracts obtained from crude and Fr. I. In both assays used, acetone was a more effective solvent ($p < 0.05$) for the extraction of condensed tannins than methanol.

The content of condensed tannins using the vanillin/HCI method was measured among almond, hazelnut, and walnut crude extracts [80% (v/v) acetone] (*28*). Hazelnut was found to possess the highest content of condensed tannins. Hazelnut skin extract and its fractions contained a higher content of condensed tannins (**Table 1**) than that of natural hazelnut itself (unpublished data).

Although a range of solvents (acetone, ethanol, methanol, and water) have been used for the extraction of various groups of phenolic compounds from plant materials (*29*), 80% (v/v) acetone is the most effective solvent for extraction of condensed tannins from hazelnut kernel and hazelnut green leafy cover (*26*), a majority of leguminous seeds (*30*), pulses, and tree nuts (*28*), as well as other plant materials (*31*). The reason why acetone is more effective than other solvent extracts is that condensed tannins are relatively high-molecular-weight compounds and hence better extracted into acetone with an appropriate polarity.

The health aspect of tannins has been thoroughly reviewed by Chung et al. (*32*) and Amarowicz (*19*). Hagerman et al. (*33*) reported that tannins were 15−30 times more effective for quenching peroxyl radicals than simple phenolics and Trolox. Therefore, tannins should be considered as potentially important biological antioxidants.

TAA. Total phenolic content and TAA value of hazelnut skin crude extract and its fractions followed a similar trend (**Tables 1** and **2**). At a given extract concentration, Fr. II exhibited the highest TAA in relation to that of other extracts. No significant differences ($p > 0.05$) existed between acetone and methanol extracts, except for Fr. I. In the present study, hazelnut skin crude extracts and their fractions showed much higher TAA than hazelnut kernel and hazelnut green leafy cover extracts (*26*). In a cited study, the extracts obtained from 80% (v/v) ethanol were characterized as having significantly lower ($p < 0.05$) TAA compared to those of extracts obtained from 80% (v/v) acetone.

Contini et al. (*12*) observed that the extracts [80% (v/v) of aqueous acetone, ethanol, and methanol] from the skin of whole roasted hazelnuts exhibited a stronger antioxidant activity, similar or superior to that of butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), Torolox, and α-tocopherol,

Table 2. Antioxidant and Antiradical Activities in Hazelnut Skin Crude Extract and its Fractions[a]

		acetone extraction	methanol extraction
total antioxidant activity (mmol TE/g)	crude extract	6.33 ± 0.10 a	6.36 ± 0.12 a
	Fr. I	4.77 ± 0.09 a	4.02 ± 0.11 b
	Fr. II	6.77 ± 0.11 a	6.47 ± 0.30 a
EC_{50}	crude extract	0.026	0.027
	Fr. I	0.027	0.029
	Fr. II	0.024	0.026

[a] Data are expressed as the means ± the standard deviation ($n = 3$) of a crude extract or its fractions. Means ± standard deviations followed by the same letter, within a row, are not significantly different ($p > 0.05$). Total antioxidant activity is expressed as millimoles of trolox equivalents (TE) per gram of crude extract or its fractions. EC_{50} was read as micromoles of catechin equivalents (CE) of crude extract or its fractions required to scavenge the initial DPPH radical by 50%.

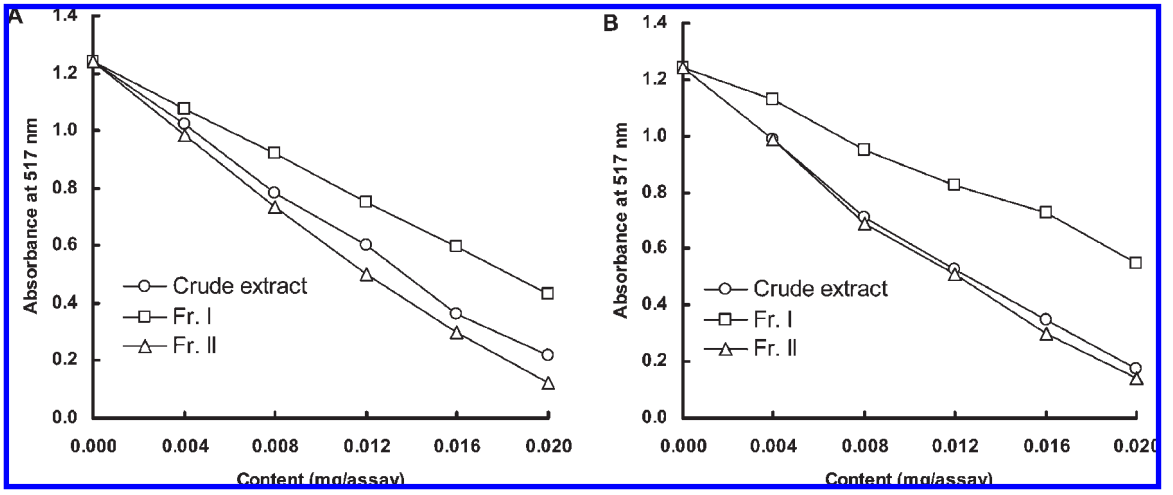

Figure 2. Scavenging activity of hazelnut skin crude extract and its fractions on the DPPH radical (**A**, acetone extraction and **B**, methanol extraction).

at equivalent concentrations. Siriwardhana and Shahidi (*27*), who evaluated the TAA of almond and its byproduct extracts, found that the 80% (v/v) ethanol extracts at the same concentration were in the order of brown skin > green shell cover > whole seed. Chestnut skin extracts [50% (v/v) water] exhibited higher antioxidant properties than the extracts of chestnut flower, leaf, and fruit (*11*). Similar results were also found for walnut skin extract [95% (v/v) ethanol] compared to whole nut and kernel extracts (*13*). These results show a clear indication that skin extracts from tree nuts possess stronger antioxidant activity than their byproduct extracts, regardless of the solvents used.

Scavenging of DPPH Radicals. The DPPH radical scavenging assay is commonly employed in evaluating the ability of antioxidants to scavenge free radicals. The change in absorbance at 517 nm is used as a measure of the scavenging effect of a particular extract for DPPH radicals (*34*). The absorbance at 517 nm decreases as the reaction between antioxidant molecules and DPPH radical progresses. Hence, the more rapidly the absorbance decreases, the more potent the antioxidant activity of the extract in terms of its hydrogen atom-donating capacity.

As shown in **Figure 2**, Fr. II and the crude extract exhibited a greater scavenging activity than Fr. I in both extraction solvents used. The low-molecular-weight phenolic fraction (Fr. I) was a weak scavenger of the DPPH radical. No significant differences ($p > 0.05$) were exhibited between Fr. II and the crude extracts at all concentrations tested. Similar trends were also observed for EC_{50} values (**Table 2**), which are micromoles of CE of crude extract or its fractions required for 50% scavenging of DPPH. The lower the EC_{50}, the higher the antiradical activity. For both extraction solvents used, Fr. II revealed the lowest EC_{50}, followed by crude extract and Fr. I. Barreira et al. (*11*) measured antioxidant activity with EC_{50} values of chestnut flowers, leaves, outer and inner skins, and fruits by different biochemical assays. Chestnut skins exhibited the best antioxidant properties and thus had much lower EC_{50} values. Cashew nut skin extract has been reported as having promising antioxidant activity with EC_{50} (*8*). Hazelnut skin can be used as an economical source of natural antioxidants.

Amarowicz et al. (*22*) studied the antioxidant activity of almond seed extract and its fractions (Fr. I and Fr. II separated using Sephadex LH-20 column chromatography) and found that acetone extract (80%, v/v) of crude almond seeds exhibited a stronger DPPH radical scavenging activity than that of its Fr.1, but rendered a weaker activity than that of its Fr. II. Similar trends were also found for adzuki bean acetone extract (80%, v/v) and its fractions (*35*). In addition, Siriwardhana and Shahidi (*27*) reported that a 100% scavenging activity of the DPPH radical was observed for brown skin ethanol extract (80%, v/v) at 100 and 200 ppm concentrations; and whole seed extracts scavenged 21% of the DPPH radical at 100 ppm and 73% at 200 ppm. Recently, Alasalvar et al. (*26*) showed that the hazelnut green shell cover extracts [both acetone (80, v/v) and ethanol (80, v/v)] exhibited a greater scavenging activity than hazelnut kernel extracts, acetone being more effective than ethanol. These results clearly show that skin extracts scavenged organic free radicals more effectively than seed/kernel and green shell/leafy cover extracts.

Reducing Power. Figure 3 depicts the reducing power of the crude extracts and their fractions of hazelnut skin examined as a function of their concentrations. The coefficient of determination (r^2) obtained for all acetone and methanol extracts were higher than 0.997. In this assay, the yellow color of the test solution changes to various shades of green and blue depending upon the reducing power of each extract and its fractions. At the same dose,

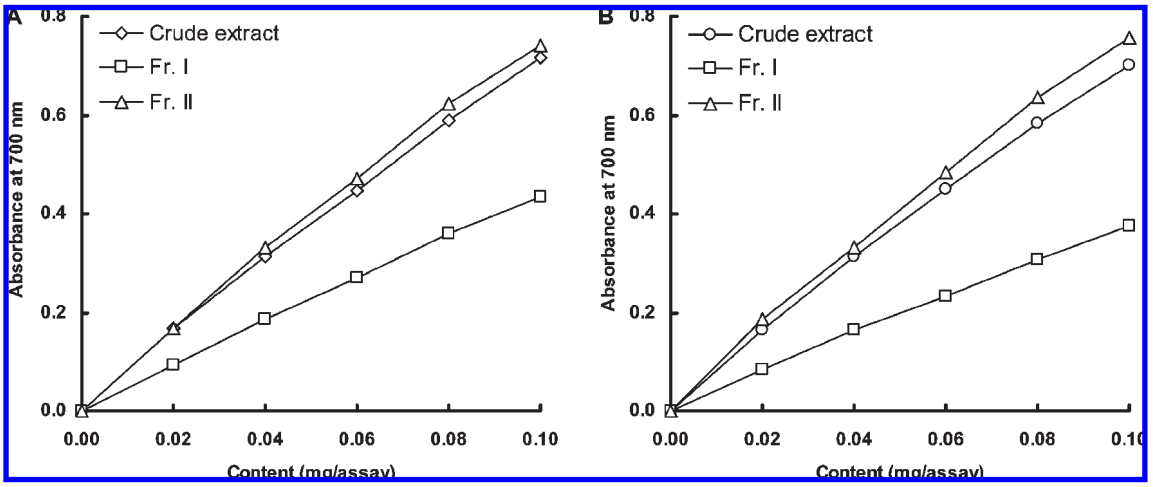

Figure 3. Reducing power of hazelnut skin crude extract and its fractions (**A**, acetone extraction and **B**, methanol extraction).

though the reducing power of Fr. II was superior to that of any other extracts or Fr. I that were investigated, no significant differences ($p > 0.05$) existed between Fr. II and the crude extract for both extracting solvents. Both acetone and methanol were effective at extracting antioxidants from hazelnut skin and its fractions. Phenolics present in the crude extract and Fr. II (**Table 1**) displayed considerable reducing power, primarily due to their effect as electron donor and thereby suppressing radical chain reactions by converting free radicals to more stable products. Thus, reducing activity leads to the termination of the radical chain reactions that may otherwise be very damaging (*36*).

The reducing power of the crude extract and its fractions determined in this study were found to be higher than that of 80% (v/v) acetone extract of almond seed and its fractions (*22*) and that of 80% (v/v) acetone and ethanol extracts of hazelnut kernel and green leafy cover (*26*). Chestnut flower and skin extracts [50% (v/v) water] exhibited higher reducing power than that of its leaf and fruit (*11*). Therefore, in general, the skin extract is a good source of natural antioxidants with respect to its reducing power.

In summary, different assays used for examining antioxidant efficacies of hazelnut skin crude extract and its fractions revealed that Fr. II (tannin fraction) exhibited the highest antioxidant activities, followed by the crude extract and Fr. I (low-molecular-weight phenolic compounds). Therefore, hazelnut skin could potentailly be considered as inexpensive source of natural antioxidants. All hazelnut skin crude extracts and their fractions performed differently. UV spectra of phenolic compounds present in the crude extracts and their fractions exhibited a maximum absorbance at 282 nm. These results also indicate that both 80% (v/v) acetone and methanol extracts are effective for extraction of phenolic compounds from hazelnut skin and its fractions. However, solvents with different polarity had some effects on total phenolics, extracted compounds, and antioxidant activities. Further research is required for qualitative and quantitative determination of free, esterified, glycoside, and ester-bound phenolic acids as well as flavonoinds present in hazelnut skin.

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