Turkish Journal of Biology

Volume 36 | Number 2 Article 8

1-1-2012

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Turk J Biol
36 (2012) 201-209

doi:10.3906/biy-1101-174

Properties of biological activity of ten wild almond (*Prunus amygdalus* L.) species

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Received: 10.01.2011

Abstract: To compare the antioxidant and antiradical activity of phenolic extracts of different species of wild almond kernels (including the brown skin), 10 wild almond species were selected from Shahindezh and Qasemloo Valley in West Azarbaijan Province, Iran. The fruits of these almonds were collected, their kernels were separated from their hulls and shells and then ground, and methanolic extracts were prepared from the kernels. The total phenolic and flavonoid contents were determined using the Folin-Ciocalteu method and a colorimetric method, respectively. The extracts were also evaluated on their reducing power and their capacity to scavenge for DPPH radicals. Significant differences were found in the phenolic and flavonoid contents of kernels from various species. The radical-scavenging capacity percentage also varied among 6 species at 50 ppm, although 100% radical scavenging was recorded for all species at 100 ppm. The results obtained were quite heterogeneous, revealing significant differences among the species assayed. *A. pabotti* Browicz and *A. orientalis* Duhamel revealed better antioxidant properties and the highest antioxidant contents. Therefore, these wild almonds can be used in breeding cultivated almonds and for rootstock improvement.

Key words: Biological activity, species, wild almonds

Introduction

There has been strong evidence indicating that free radicals cause oxidative damage to lipids, proteins, and nucleic acids (1). Though antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase play an important role in scavenging oxidants and preventing cell injury, these defense mechanisms are not adequate. Consequently, cellular macromolecules are easily subject to oxidative damage (2). Several epidemiological studies suggest that a high intake of foods rich in natural antioxidants increases the antioxidant capacity of the plasma and reduces the risk of some, though not all, cancers, heart diseases, and stroke (3). In addition to α-tocopherol, ascorbic acid, and carotenoids, plant tissues synthesize a wide variety of phenolic compounds. Several studies have revealed that a major part of the antioxidant activity may be from compounds such as flavonoids, isoflavones, flavones, anthocyanin, catechin, and other phenolics (4) with mechanisms involving both free radical scavenging and metal chelation (5). A great number of aromatic, spicy, medicinal, and other plants, such as nuts and cereals, contain chemical compounds exhibiting antioxidant properties and demonstrating such protective effects (3). Nuts are foods traditionally associated with the Mediterranean-type diet. Their regular consumption, in moderate doses, is related to a lower risk of cardiovascular diseases. The anticancer activity of nuts has also been demonstrated in experimental animals. These beneficial effects are mainly attributed to their lipid profile, arginine, fiber,

and vitamin E contents as well as to other compounds with antioxidant properties, such as polyphenols (6).

Almonds, *Prunus dulcis* (Mill.) D.A.Webb syn. *P. amygdalus* (L.) Batsch, are among the most popular tree nuts on a worldwide basis and rank first in tree nut production. They belong to the family Rosaceae, which also includes apples, pears, prunes, and raspberries (7-9). The United States is the largest almond producer in the world, with most US almonds being grown in California (10). Although the exact origin of almonds has been difficult to determine, it has been suggested that almonds are native to the temperate, desert areas of western Asia, from which they gradually spread to other regions of the world. Domesticated almonds have been documented from Bronze Age sites in Greece and Cyprus and were common in Palestine by 1700 BC. In addition to the cultivated almond, *Prunus dulcis*, more than 30 wild or minor cultivated almond species are known to exist (11). Wild populations of almond species, representing a wide range of morphological and geographical forms, have evolved throughout southwest and central Asia from Turkey and Syria into the Caucasus Mountains, through Iran, and into the deserts of the Tian-Shan and Hindu Kush Mountains of Tajikistan, Uzbekistan, and Afghanistan. Over 30 species have been described by botanists and may represent subspecies or ecotypes within a broad collection of genotypes that are adapted to a range of ecological niches in the deserts, steppes, and mountains of central Asia (12). Wild almond species commonly grow in areas between 28°N and 38°N and between 41°E and 54°E, and at altitudes from 1100 m to 2700 m (13). Iran is optimally situated for growing almonds. Nearly 20 of the wild species have been reported from Iran, indicating that the country is within the center of origin for almonds (14).

Almond fruit consists of 3 or, more accurately, 4 portions: the kernel or meat, the middle shell, the outer green shell cover or almond hull, and a thin leathery layer known as the brown skin of the meat or the seed coat (15). The nutritional importance of the almond fruit is related to its kernel. The hull splits open when the fruit reaches maturity and is then separated from the shelled almond (the whole natural almond). During some industrial almond processing, the skin, or seed coat, is removed from the kernel by blanching and then discarded. For roasted almonds and other appetizers, these skins are not removed. The skin, which has very low economic value, represents 4% of the total almond weight but contains 70%-100% of the total phenols present in the nut. Almonds are typically used as a snack and as ingredients in a variety of processed foods, especially in bakery and confectionery products (8,9). When incorporated in the diet, these nuts have been reported to reduce colon cancer risk in rats (16) and to increase high-density lipoprotein cholesterol and reduce low-density lipoprotein cholesterol levels in humans (17). Extracts of the whole almond seed, including the brown skin, shell, and green shell cover (hull), possess potent free radical-scavenging capacities (8,9,18-22). These activities may be related to the presence of flavonoids and other phenolic compounds in nuts. There is no study in the literature demonstrating the variations of antioxidant and antiradical characteristics of wild almond species. Therefore, the objective of this work was to provide a more thorough assessment of the diversity of antioxidant content (phenols and flavonoids) in kernels of wild almonds (including the brown skin) growing in West Azarbaijan Province, Iran, and to evaluate their potential use in the breeding of cultivated almonds.

Materials and methods

Samples and sample preparation

Almond fruits were collected in August-September 2007 from Shahindezh and Qasemloo Valley, located in West Azarbaijan Province in northwestern Iran. The botanical names, synonym names, local names, kernel tastes, and global distributions of these wild almonds are shown in Table 1 (13,14,23). The selected trees had not been irrigated and no phytosanitary treatments were applied. The fruits of these almonds were collected, dried at room temperature, and exposed to the sun, as is common practice in the region. For extraction of the antioxidant compounds, a fine dried powder of the sample (3 g) was extracted using 50 mL of methanol at 25 °C for 60 min. The extracts were filtered through Whatman No. 4 paper and evaporated at 40 °C to dryness. All of the samples were redissolved in water and analyzed for their contents in terms of phenols, flavonoids, DPPH radical-scavenging activity, and reducing power.

Table 1. The botanical names, synonym names, local names, kernel tastes, and global distributions of the wild almonds collected.

No.	Botanical name	Synonym name	Local name	Kernel taste	Global distribution
1	*Amygdalus lycioides* Spach	*Prunus lycioides* (Spach) C K.Schneid.	Badame Khardar, Tangras	Bitter	Native to Iran
2	*Amygdalus eburnea* Spach	*Amygdalus scorpius* Spach *Prunus eburnea* (Spach) C.K.Schneid. *Amygdalus spathulata* Boiss.	Arzhan, Badame Kohi	Bitter	Native to Iran
3	*Amygdalus carduchorum* Bornm.	*Prunus carduchorum* (Bornm.) Meikle	–	Bitter	Iran, Iraq
4	*Amygdalus fenzliana* (Fritsch) Lipsky	*Prunus fenzliana* Fritsch	–	Bitter	Northwest Iran, northeast Anatolia, Georgia
5	*Amygdalus orientalis* Duhamel	*Amygdalus argenta* Lam. *Amygdalus variabilis* Bornm. ex C.K.Schneid. *Prunus orientalis* Koehne	Badame Shargi, Bekhourak	Bitter	Iran, Iraq
6	*Amygdalus kotschyi* Boiss. & Hohen.	*Prunus Kotschyi* (Boiss. & Hohen.) Nab. *Amygdalus elaeagnifolia* Spach Var. *Kotschyi* (Boiss. & Hohen.) Boiss.	Badame kordestani	Bitter	Northeast Iraq, southeast Anatolia, western Iran
7	*Amygdalus trichamygdalus* (Hand.-Mazz.) Woronow	*Prunus trichamygdalus* Hand.-Mazz.	Badame Makhmali	Bitter	Western Iran, southeast Anatolia
8	*Amygdalus pabotti* Browicz	–	–	Bitter	Native to Iran
9	*Amygdalus urumiensis* (Bornm.) Browicz.	*Amygdalus spinosissima* Bunge var. *urumiensis* Bornm.	Badame Oroomie	Bitter	Native to Iran
10	*Amygdalus korschinskii* (Hand.-Mazz.) Bornm.	*Prunus Korschinskii* Hand.-Mazz. *Amygdalus communis* L.var. *microphylla* Post.		Bitter	Anatolia, Syria, Palestine, Mediterranean regions

Determination of phenol content

The phenolic concentration of the extracts was estimated by a colorimetric assay based on the procedures described by Singleton and Rossi (24) with some modifications. Basically, 1 mL of sample was mixed with 1 mL of Folin-Ciocalteu phenol reagent. After 3 min, 1 mL of saturated sodium carbonate solution was added to the mixture, and the mixture was then adjusted to 10 mL with distilled water. The reaction was kept in the dark for 90 min, after which the absorbance was read at 725 nm (S2100 Diode Array Biowave Spectrophotometer, Biochrom WPA, Cambridge, UK). Gallic acid was used for constructing the standard curve (0.01-0.4 mM; $y = 2.94848x - 0.09211$; $R^2 = 0.99914$) and the results were expressed as milligrams of gallic acid equivalents per gram of extract (mg GAEs/g extract).

Determination of flavonoid content

The flavonoid contents of the extracts were determined by the colorimetric method described by Jia et al. (25), with some modifications. The almond extract (250 µL) was mixed with 1.25 mL of distilled water and 75 µL of a 5% $NaNO_2$ solution. After 5 min, 150 µL of a 10% $AlCl_3$ H_2O solution was added. After 6 min, 500 µL of 1 M NaOH and 275 µL of distilled water were added to the mixture. The solution was mixed well and the intensity of the pink color was measured at 510 nm. Catechin was used to calculate the standard curve (0.250-2.500 mM; $y = 0.2903x$; $R^2 = 1.0000$) and the results were expressed as milligrams of catechin equivalents per gram of extract (mg CEs/g extract).

DPPH radical-scavenging activity

Various concentrations of almond extracts (0.3 mL) were mixed with 2.7 mL of a methanolic solution containing DPPH radicals (6×10^{-5} mol/L). The mixture was shaken vigorously and incubated, allowed to stand in the dark for 60 min until stable absorption values were obtained. The reduction of DPPH radicals was determined by measuring the absorption at 517 nm. The radical-scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation $RSA\% = [(A_{DPPH} - A_S)/A_{DPPH}] \times 100$, where A_S is the absorbance of the solution when the sample extract has been added at a particular level and A_{DPPH} is the absorbance of the DPPH solution (26). The extract concentration providing 50% inhibition was calculated from the graph of the scavenging effect percentage against the corresponding extract concentration. BHA was used as the reference compound.

Reducing power

Various concentrations of almond constituent extracts (2.5 mL and 50 ppm) were mixed with 2.5 mL of 200 mmol/L sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. After 2.5 mL of 10% trichloroacetic acid (w/v) was added, the mixture was centrifuged at 1000 rpm for 8 min (BHG 1100 centrifuge, Rotina 35R, Hettich, Germany). The upper layer (5 mL) was mixed with 5 mL of deionized water and 1 mL of 0.1% of ferric chloride, and the absorbance was measured spectrophotometrically at 700 nm (27).

Statistical analysis

For all of the experiments, 3 samples of each almond species were analyzed and all of the assays were carried out in triplicate. The results are expressed as mean values and standard error of the mean or standard deviation of the mean. The differences between the almond species were analyzed using one-way analysis of variance.

Results and discussion

Phenol content

Phenolic compounds are commonly found in both the edible and inedible parts of plants and are known to possess antioxidant activity. Phenolics are also responsible for inhibiting or delaying the oxidation of different biomolecules important for life. Natural antioxidants present in foods of plant origin that scavenge reactive oxygen species may be of great value in preventing the onset and/or occurrence of oxidative stress and its related diseases. Nutritional epidemiology has shown that nut consumption protects against cardiovascular disease, stroke, and other chronic ailments (28,29).

Using the high-performance liquid chromatography method, phenolic compounds such as vanillic, caffeic, *p*-coumaric, ferulic acids (after basic hydrolysis), quercetin, kaempferol, isorhamnetin (after acidic hydrolysis), delphinidin, and cyanidin (after *n*-butanol/HCl hydrolysis) were identified in

the almond extracts, as were procyanidins B_2 and B_3. The dominant compounds were procyanidins B_2 and B_3 and, on the whole, the phenolic constituents present in almond extract are not well known (20). Four flavonol glycosides were identified in the almond seed coats and quantitatively determined through a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry methodology (30). Nine phenolic compounds were isolated from almond skin and identified by nuclear magnetic resonance and mass spectrometry data, which demonstrated that they were glycosides of quercetin, kaempferol, naringenin, catechin, protocatechuic acid, vanillic acid, and *p*-hydroxybenzoic acid (7).

Table 2 shows the total phenolic content of the 10 wild almond kernel extracts. The maximum total phenolic content was 482.3 ± 1.22 mg GAEs/g extract for *A. pabotti* and the minimum total phenolic content was 184.1 ± 2.32 mg/g for *A. urumiensis*. The total phenolic content in almond kernel extract without the brown skin was reported by Amarowicz et al. (20), Siriwardhana and Shahidi (18), Wijeratne et al. (8), and Siriwardhana et al. (21) to be 16.1 ± 0.4 mg CEs/g in an 80% aqueous acetone extract, 8.1 ± 1.75 mg CEs/g in an ethanolic extract, 8 ± 1 mg quercetin equivalents/g in an ethanolic extract, and 8 ± 1 mg quercetin equivalents/g in an ethanolic extract, respectively. Barreira et al. (31) evaluated 10 almond cultivars (both commercial and regional) and showed that the phenolic content of extracts from almond kernels retaining the brown skin can range from 9.22 ± 1.04 to 163.71 ± 3.04 mg/g. American and Spanish varieties were studied by Monagas et al., who reported 242-413 μg/g of total phenolic content in almond skins (6). The total phenolic content in the different wild almond species investigated here was higher than those of cultivated almonds and the results mentioned above.

Flavonoid content

Table 2 shows the flavonoid values obtained from the 10 almond species. The highest total flavonoid content in the almond kernel extracts was 35.6 ± 1.11 mg CEs/g for *A. pabotti* and the lowest flavonoid content, 10.3 ± 1.16 mg/g, was obtained for *A. korschinskii*. The total flavonoid content for the almond kernel extract reported by Barreira et al. (31) was from 6.24 ± 1.36 to 25.02 ± 8.43 mg CEs/g extract.

Reducing power

The Fe^{3+} to Fe^{2+} transformation in the presence of methanolic extract was investigated using the method of Oyaizu (27). The antioxidant properties of phenolic compounds are associated with their reducing power (32), which is associated with the presence of reductones (33). The reducing power of almond species increased significantly according to their phenol content. In addition, the phenolic content and reducing power of wild almonds varied among the species studied (Table 2). The maximum reducing power was 0.883 for *A. pabotti* and the minimum reducing power was 0.389 for *A. urumiensis*.

Radical-scavenging activity

This study showed that most of the different wild almond species are capable of scavenging DPPH radicals at 50 ppm. Complete radical-scavenging activity was achieved for all wild almonds species at 100 ppm. In the DPPH assay, the concentrations of almond kernel extracts required to scavenge 50% of radicals (SC50) varied between 0.26 and 0.81 mg/mL (Table 2). Samples with higher total phenols showed the strongest free radical-scavenging effect (lower SC50 values). The obtained results showed strong antioxidant potential when compared to the values obtained for the standard (BHA: 97% at 3.8 mg/mL). Siriwardhana and Shahidi (18) reported that whole almond seed extract scavenged 21% of the DPPH radicals at a concentration of 100 ppm and 73% at 200 ppm. A 100% scavenging activity of DPPH radicals was observed for brown almond skin and green shell extracts at 100 and 200 ppm, respectively, by the same authors. The DPPH radical-scavenging activity of almond seed extract (20) and hull phenolics was also reported by Pinelo et al. (19).

The antioxidant activity of phenolics may be related to their redox properties, which allow them to act as reducing agents or hydrogen-atom donors, or their ability to chelate metals, inhibit lipoxygenase, and scavenge free radicals (34). Thus, natural antioxidants function as free radical scavengers and chain breakers, complexers of prooxidant metal ions, and quenchers of singlet-oxygen formation. In food systems, flavonoids can act as free radical scavengers and terminate the radical chain reactions that occur during the oxidation of triglycerides (35), and they also appear to possess a variety of biological activities,

Table 2. Comparison of phenol content (mg GAEs/g extract), flavonoid content (mg CEs/g extract), reducing power, and DPPH radical scavenging (percentage and SC50) for different species of wild almond kernels.

No.	Almond name	Phenol content[a] (mg GAEs/g extract)	Flavonoid content[a] (mg CEs/g extract)	Reducing power[b] (700 nm)	DPPH		
					Radical scavenging (%)		SC50 (mg/mL)
					50 ppm	100 ppm	
1	*Amygdalus lycioides* Spach	233.3 ± 1.24	13.4 ± 1.33	0.639	65.4 ± 2.23	100.0 ± 0.00	0.58
2	*Amygdalus eburnea* Spach	336.9 ± 2.35	20.2 ± 2.16	0.684	100.0 ± 0.00	100.0 ± 0.00	0.37
3	*Amygdalus carduchorum* Bornm.	365.5 ± 3.21	28.3 ± 0.55	0.743	100.0 ± 0.00	100.0 ± 0.00	0.38
4	*Amygdalus fenzliana* (Fritsch) Lipsky	195.8 ± 2.33	11.3 ± 2.73	0.444	59.6 ± 1.22	100.0 ± 0.00	0.73
5	*Amygdalus orientalis* Duhamel	458.3 ± 0.32	29.4 ± 0.65	0.832	100.0 ± 0.00	100.0 ± 0.00	0.33
6	*Amygdalus kotschyi* Boiss. & Hohen.	311.9 ± 1.11	21.3 ± 2.33	0.705	92.3 ± 1.15	100.0 ± 0.00	0.42
7	*Amygdalus trichamygdalus* (Hand.-Mazz.) Woronow	203.1 ± 3.24	13.5 ± 0.44	0.475	69.3 ± 1.14	100.0 ± 0.00	0.60
8	*Amygdalus pabotti* Browicz	482.3 ± 1.22	35.6 ± 1.11	0.883	100.0 ± 0.00	100.0 ± 0.00	0.26
9	*Amygdalus urumiensis* (Bornm.) Browicz.	184.1 ± 2.32	12.9 ± 0.39	0.389	58.3 ± 1.34	100.0 ± 0.00	0.81
10	*Amygdalus korschinskii* (Hand.-Mazz.) Bornm.	296.5 ± 0.29	11.3 ± 1.16	0.639	85.1 ± 3.32	100.0 ± 0.00	0.53

[a] Each value is expressed as mean ± standard error, n = 3, $P < 0.05$.

[b] Each value is expressed as mean ± standard deviation, n = 3, $P < 0.05$.

Table 3. Correlations among the total phenol content, flavonoid content, reducing power, and DPPH radical scavenging of 10 wild almond kernel extracts.

	Phenol content (mg GAEs/g extract)	Flavonoid content (mg CEs/g extract)	Reducing power (700 nm)	DPPH radical scavenging (50 ppm)
Phenol content	1	0.946	0.961	0.908
Flavonoid content		1	0.893	0.833
Reducing power			1	0.913
DPPH radical scavenging				1

Correlation is significant at the 0.05 level.

including antioxidant, antiinflammatory, and vasodilatory actions (36). These natural constituents of plant foods have been carefully studied in fruits and vegetables, but less attention has been paid to their presence in whole grains and tree nuts.

Correlations between bioactivity results

The results of correlation analyses among the total phenolic content, the flavonoid content, and the reducing power and antiradical activities are depicted in Table 3. Statistically significant ($P < 0.05$) correlation was found among the total phenolics, flavonoid, reducing power, and antiradical activity. Some authors have reported a direct correlation between antioxidant activity and total phenolic content (37). Velioglu et al. (38) examined 28 plant products and found a significant relationship between the total antioxidant activity and the total phenolics in flaxseed and cereal products. In the case of leguminous seeds extracts, a statistically significant ($P \leq 0.01$) correlation was determined for total phenolics versus total antioxidant activity (39). A strong correlation between the content of total phenolics and reducing power was found in the extracts of selected plant species from the Canadian prairies and in the phenolic extracts of hulls and shells of 4 wild almonds (*P. amygdalus* L.), as reported by Amarowicz et al. (40) and Jahanban Isfahlan et al. (41), respectively.

Lastly, this study revealed that some of the investigated wild almond species can be used as rootstock almonds after testing their effects on scion productivity, nut quality, and tolerance to soil-borne diseases. In these practices, it should be considered that the concentration and composition of phenolic compounds in plants is influenced by a large number of factors, such as climate and agricultural conditions. The use of pesticides, for instance, reduces the amounts of phenols, so the application of biological agriculture conditions is advised (42).

In this study, results were obtained for the first time regarding the total phenolic content, total flavonoid content, reducing power, and DPPH radical-scavenging effects of 10 extracts of wild almond (*Prunus amygdalus* L.) kernels growing in Iran. A high correlation was found between the total phenolic and total flavonoid content, as well as between the polyphenolic contents and the antioxidant activities. Due to the elevated values of their antioxidant activity, the species *A. pabotti*, *A. orientalis*, *A. carduchorum*, and *A. eburnea* can be considered interesting sources of antioxidants. Because of the adaptability of wild almond species to severe environmental conditions and their resistance to drought, salinity, and some pests and diseases, these almonds with high antioxidants content can be used as rootstock for almond cultivars and in breeding programs for rootstock improvement.

Acknowledgment

We would like to thank Hasan Jahanban Esfahlan for proofreading the manuscript.

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