Antioxidant Capacities of Phenolic Compounds and Tocopherols from Tunisian Pomegranate (*Punica granatum*) Fruits

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Abstract: This article aims to determine the phenolic, tocopherol contents, and antioxidant capacities from fruits (juices, peels, and seed oils) of 6 Tunisian pomegranate ecotypes. Total anthocyanins were determined by a differential pH method. Hydrolyzable tannins were determined with potassium iodate. The tocopherol (α-tocopherol, γ-tocopherol, and δ-tocopherol) contents were, respectively, 165.77, 107.38, and 27.29 mg/100 g from dry seed. Four phenolic compounds were identified and quantified in pomegranate peel and pulp using the high-performance liquid chromatography/ultraviolet method: 2 hydroxybenzoic acids (gallic and ellagic acids) and 2 hydroxycinnamic acids (caffeic and *p*-coumaric acids). Juice, peel, and seed oil antioxidants were confirmed by ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC) methods. The highest values were recorded in peels with 25.63 mmol trolox equivalent/100 g and 22.08 mmol TE/100 g for FRAP and ORAC assay, respectively. Results showed that the antioxidant potency of pomegranate extracts was correlated with their phenolic compound content. In particular, the highest correlation was reported in peels. High correlations were also found between peel hydroxybenzoic acids and FRAP ORAC antioxidant capacities. Identified tocopherols seem to contribute in major part to the antioxidant activity of seed oil. The results implied that bioactive compounds from the peel might be potential resources for the development of antioxidant function dietary food.

Keywords: antioxidant capacities, hydrolyzable tannins, *Punica granatum* L., tocopherols, total phenolics

Introduction

Considerable interest has been focused on the influence of antioxidants on cardiovascular diseases. Antioxidants are often referred as nonnutritive compounds thought to be produced by plants as means of protection mainly against pathogens and ultraviolet radiation (Wink 2010). A whole range of plant-derived dietary supplements, antioxidants, and provitamins that assist in maintaining good health and combating diseases are now being described as functional foods, nutriceuticals, and nutraceuticals (Ivanova and others 2005). Therefore, the chemistry and biology assays of antioxidants are of importance for evaluation of their potential health benefits to humans.

The pomegranate (*Punica granatum*) belongs to the Punicaceae family and is a nutrient dense food source rich in phytochemical compounds. Pomegranates are popularly consumed as fresh fruit, beverages, food products (jams and jellies), and extracts wherein they are used as botanical ingredients in herbal medicines and dietary supplements. Several studies reported that phytochemicals have been identified from various parts of the pomegranate tree and from pomegranate fruit: peel, juice, and seeds (Artik and others 1998; Seeram and others 2006).

Pomegranate trees have been cultivated and naturalized over the whole Mediterranean region. In Tunisia, they have been cultivated traditionally since ancient times under diverse agroclimatic conditions. Pomegranate is well known typically in the coastal regions and in many regions inside the country. Pomegranate may play a considerable role in preventing cancer and heart disease (Aviram and others 2002). Since ancient times, pomegranate juice has been used as a natural astringent for treating diarrhea and harmful internal parasites (Das and others 1999).

Pomegranate polyphenols include flavonoids (flavonols, flavanols, anthocyanins. . .), condensed tannins (proanthocyanidins), and hydrolyzable tannins (ellagitannins and gallotannins). Other phytochemicals identified from the pomegranate are organic and phenolic acids, sterols and triterpenoids, fatty acids, triglycerides, and alkaloids (Seeram and others 2006). Other major components of pomegranate juice are ellagic, caffeic, and punicic acids. These phenolic compounds belong to different representative chemical classes with known bioactivities.

Anthocyanins possess known pharmacological properties and are used by humans for therapeutic purposes (Porter and others 2001). Anthocyanins are the water-soluble pigments responsible for the bright red color of pomegranate juice. Several anthocyanin compounds were identified in pomegranate juice, include pelargonidin-3-glucoside, cyanidin-3-glucoside, delphinidin-3-glucoside, pelargonidin 3,5-diglucoside, cyanidin 3,5-diglucoside,

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and delphinidin 3,5-diglucoside (Miguel and others 2004; Hasnaoui and others 2010). Anthocyanins can be insect attractants in flowers but also they can be insecticidal and antimicrobial at the same time.

Tannins can be divided into 2 groups: hydrolyzable and nonhydrolyzable (or condensed) tannins. Hydrolyzable tannins have been shown to have high antioxidant and free radical-scavenging activities (Larrosa and others 2010). Tannins as well as anthocyanins have significant antiproliferative and proapoptotic effects in several different types of cancer cells *in vitro*, including colon cancer, prostate cancer, and head and neck cancer (Seeram and others 2006).

Traditional medicine practitioners consider pomegranate juice as a provider of natural antiviral, antifungal, and antibacterial benefits (Seeram and others 2006). Recently, there has been an increase in the use of pomegranate fruit extracts as botanical ingredients in herbal medicines and dietary supplements. The main objective of this research was to compare 6 Tunisian pomegranate ecotypes in terms of their tannins, anthocyanins, phenolic acids content in juices and peels. The antioxidant capacities have been compared on 6 pomegranate ecotypes with commonly used spectrophotometric methods. Tocopherols in pomegranate seed oils were evaluated. As well this study aims to assess the relationship between the oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) antioxidant activities and quantified phenolic classes.

Materials and Methods

Chemicals and reagents

All solvents were of reagent grade without any further purification. Cyanidin-3-glucoside, tannic, caffeic, gallic, ellagic, and *p*-coumaric acids were purchased from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). Trolox and rutin were obtained from Aldrich (Milwaukee, Wis., U.S.A.). α-, γ-, and δ-tocopherols were purchased from Sigma-Aldrich (St. Louis, Mo., U.S.A.). The analytical reagent grade acetonitrile and methanol were obtained from Lab-Scan (Labscan Ltd., Dublin, Ireland). The water used in high-performance liquid chromatography (HPLC) and sampling was prepared with a Millipore Simplicity system (Millipore S.A.S., Molsheim, France). All chemicals used in antioxidant activity assays were of chromatography grade and were purchased from Sigma Chemical Co. (Poole, Dorset). Spectrophotometric measurements were performed on Shimadzu UV-1600 spectrometer (Shimadzu, Kyoto, Japan).

Plant material

Six pomegranate ecotypes were used in this work: Chetoui (CH), Gabsi2 (GB2), Gabsi3 (GB3), Garsi (GR), Mezzi (MZ), and Zehri (ZH). All ecotypes were grown in the same collection at Gabès oasis (southeast of Tunisia). Pomegranates were randomly picked from each tree in October 2009. Samples from fully mature fruits were included in the present study. Fresh pomegranates were stored at room temperature (18 to 20 °C) for a few days until used.

Methanolic extracts preparation

Methanolic extracts were prepared from juice, peel, and pulp. Pomegranate juice extract and pomegranate peel extract were prepared following the method described previously by Elfalleh and others (2009). The pulps (30 g) were pitted, crushed, and cut to small pieces with a sharp knife and blended for 3 min and extracted as reported by Chaira and others (2009). All extracts were kept in dark glass at −4 °C until used for further analyses.

Determination of total anthocyanin content (TAC)

TAC of pomegranate juice and peel extract were determined by a pH differential method using 2 buffer systems: potassium chloride buffer (pH 1.0, 0.025 M) and sodium acetate buffer (pH 4.5, 0.4 M). Methanolic pomegranate juice and peel extract were mixed with 3.6 mL of corresponding buffers and read against water as a blank at 510 and 700 nm (Çam and others 2009).

Absorbance (A) was calculated using $A = [(A_{510} - A_{700})\ pH_{1.0} - (A_{510} - A_{700})\ pH_{4.5}]$ with a molar extinction coefficient of 29600. Results were expressed as mg of cyanidin-3-glucoside equivalents (CGE) per L of juice and per g of dry weight basis (dw) of peel.

Determination of hydrolyzable tannin content (HTC)

HTC were determined by modified method of Çam and Hişil (2010). One milliliter of 10-fold diluted extracts and 5 mL of 2.5% KIO_3 were added into a vial and vortexed for 10 s. In the reaction optimum, absorbance of the red-colored mixture was determined at 550 nm versus the prepared water blank. Optimum reaction, time to gain maximum absorbance value, was determined to be 2 min for pomegranate peel extracts and 4 min for standard solutions of tannic acid. Different concentrations of tannic acid solutions (100 to 1600 mg/L) were used for calibrations. The final results were expressed as mg tannic acid equivalent (TAE) per L of juice and per g of dw of peel.

Simple polyphenol compositions

The phenolic composition was determined by HPLC/ultraviolet analysis described by Chaira and others (2009). Separation was carried out at room temperature on a C18 column (Knauer, Berlin, Germany). Prior to use, solvents were filtered over a 0.45 μm membrane filter and degassed for 15 min in an ultrasonic bath Cleaner Model SM 25E-MT (Branson Ultrasonics Corp., Dambury, Conn., U.S.A.). The mobile phase consisted of methanol/acetonitrile 50/50 (A) and acetic acid in ultrapure water, pH 3.2 (B). Detection was monitored by an UV Detector (Knauer). The flow rate and the injection volume during the experiment were 1.0 mL/min and 20 μL, respectively. The pump gradient during analyses was: 5% to 30% (A), 0 to 25 min; 30% to 38% (A), 25 to 35 min; 38% to 45% (A), 35 to 45 min; and 45 to 52% (A), 45 to 50 min.

The integrator was calibrated with external standards consisting of caffeic, gallic, ellagic, and *p*-coumaric solutions. The concentration of each standard was 330 μg/mL in methanol. Simple polyphenols were identified by comparison of their retention times with standards. They were quantified according to their percentage area obtained by integration of the peaks. In pomegranate pulps, phenolic acid concentrations were reported to fresh weight basis (fw) in pulp and to dw in peel. All samples were analyzed in triplicate.

Oil extraction

Lipids were extracted by the method of Folch and others (1957). All solvents were of reagent grade purchased from Merck Chimie S.A.S. and were used without any further purification. A total of 30 mL of methanol was added to 10 g of ground seeds and mixed for at least 30 min at room temperature in Erlenmeyer flask. Then, 60 mL of chloroform were added and the solution was stirred for 30 min. Next, each sample was aliquoted into

2 Falcon 50-mL conical tubes. The total chloroform-methanol-NaCl (1%) homogenate was centrifuged at 1000 *g* for 15 min, and the lower chloroform phase containing the total lipids was saved. Solvents were removed using a rotary evaporator at 50 °C, and lipid fractions were stored in chloroform at –20 °C for tocopherol analysis.

Determination of tocopherol contents

Tocopherols were determined by HPLC with fluorescence detection according to Nasri and others (2009). The HPLC system consisted of a Lichrospher 100 RP-18 silica column (5 μm, 250 × 4 mm) equipped with a Rheodyne 7125 sample injector fitted with a LC-7A pump, and a Fluorescence Detector with the excitation and emission wavelength set at 290 and 330 nm, respectively, and a C-R6A Chromatopac integrator. The chromatograms were recorded automatically by a chromate-integrator. The isocratic mobile phase used was hexane/propane-2-ol (99.5:0.05, v/v) at a flow rate of 2 mL/min. Tocopherol peaks were identified and quantified with the help of an external standard solution of α-, γ-, δ-tocopherols purchased from Sigma-Aldrich.

Determination of FRAP antioxidant activity

Antioxidant activity in the samples was determined using FRAP assay of Benzie and Strain (1999) with modification. The FRAP reagent containing 2.5 mL of a 10 mM TPTZ [2,4,6-tris(2-pyridyl)-1,3,5-triazine] solution in 40 mM HCl, 2.5 mL of 20 mM ferric chloride, and 25 mL of 0.1 M acetate buffer (pH 3.6) was freshly prepared and warmed at 37 °C. Then, 0.3 mL of methanolic extracts of samples and 2 mL of FRAP reagent were transferred into a 10-mL volumetric flask and made up to volume with distilled water. The blue solutions obtained were kept at room temperature for 5 min and centrifuged at 10500 *g* for 10 min in Eppendorf 5804 centrifuge (Eppendorf, Hamburg, Germany) to remove solids. The absorbance of the reaction mixture was determined at 593 nm in a Shimadzu UV-1600 spectrometer (Shimadzu). The change in absorbance was calculated and related to the standard curve generated with Trolox. Results were expressed as Trolox equivalent antioxidant capacity (TEAC). All assays were run in triplicate, and averages were reported.

Determination of ORAC antioxidant activity

ORAC in the samples was determined using the method of Prior and others (2003). ORAC assays were performed using a multiwell plate reader (Fluoroskan Ascent FL fluoro-luminometer, Thermo Labsystems). The antioxidant capacity of the samples was measured by the inhibition of the decrease in the fluorescence. Briefly, fluorescein (FL) was used as a target of free radical attack, with AAPH, 2,2′-azobis(2-amidinopropane) dihydrochloride, as a peroxyl radical generator. In the absence of an inhibitor, these radicals will rapidly destroy the fluorescence of the FL dye.

By following the time course of the fluorescence decay, with and without added test substances, a measure of the radical-trapping ability of the test substance can be estimated. The net area under the curve (net AUC) was determined to be the difference between the area under the FL decay curve and the blank. Using Trolox standard, a calibration curve was generated using the net AUC ($AUC_{Trolox} - AUC_{blank}$). ORAC values were calculated using the regression equation between Trolox concentration and the net AUC. Results were expressed as TEAC. All assays were run in triplicate, and averages were reported.

Statistical and chemometric methods

All tests were carried out in triplicate and the results were presented as mean ± standard deviation (SD). Differences at $P < 0.05$ were considered statistically significant. Ecotype values for each compound were compared to the mean of all ecotypes by calculating a confidence interval. An analysis of variance was used to compare ecotypes.

Results and Discussion

Total anthocyanins and tannins from juice and peel

Mean values of total anthocyanin and tannin contents of studied pomegranates ecotypes are shown in Table 1. Total peel anthocyanins ranged from 63.76 ± 2.47 as mg CGE per g dw (CGE mg/g dw) in GB3 ecotype to 84.31 ± 1.91 mg CGE/g dw in GR ecotype.

Color is one of the most important parameters in sensorial evaluation of juice quality. The bright color of pomegranate fruit and juice is mainly due to anthocyanins, so their stability through juice processing is of major importance. Miguel and others (2004) reported a drastic decrease of cyanidin 3,5-duglucoside level in pomegranate juice during storage at 4 °C.

In juice, the highest value of anthocyanin was in CH ecotype (48.27 ± 5.01 CGE mg/L) and the lowest value was 28.15 ± 5.12 CGE mg/L of juice in GB3 ecotype. Quantitatively, our results are in agreement with a recent study on anthocyanin content of juice from Tunisian pomegranate accessions (Hasnaoui and others 2010). They reported that TAC ranged from 9 to 115 mg/L. The levels of anthocyanins obtained in the current study are lower than those reported by Gil and others (2000) (306.0 mg/L). They reported that cyanidin 3-glucoside is the major compound with 128.3 mg/L of juice. Hasnaoui and others (2010) reported 6 anthocyanin compounds found in Tunisian pomegranate juice with cyanidin-3,5-diglucoside as the major compound (3.1 to 74.4 mg/L). Miguel and others (2004) reported that delphinidin-3-glucoside is the major anthocyanin in juice (45 to 69 mg/L). The anthocyanin fingerprints among pomegranate ecotypes were quite different. Hasnaoui and others (2010) and Mousavinejad and others (2009) reported a high genetic heterogeneity in juice anthocyanin for Tunisian and Iranian pomegranate ecotypes. Furthermore, anthocyanin content was known to be affected by several parameters such as harvest maturity, storage temperature, and relative humidity (Shin and others 2008).

Table 1–Mean values of total anthocyanins and total tannins of juice and peel from 6 Tunisian pomegranate ecotypes.

			CH	GB2	GB3	GR	MZ	ZH	Mean ± SD ($n = 6$)
Total anthocyanins[1]	(mg/g)	Peel	80.90 ± 3.73^{A}	$71.67 \pm 8.03^{A,B}$	63.76 ± 2.47^{B}	84.31 ± 1.91^{A}	$72.58 \pm 8.27^{A,B}$	$70.17 \pm 5.35^{A,B}$	73.90 ± 8.24
	(mg/L)	Juice	48.27 ± 5.01^{A}	$38.06 \pm 6.72^{A,B}$	28.15 ± 5.12^{B}	43.29 ± 3.19^{A}	$41.62 \pm 6.53^{A,B}$	$35.79 \pm 4.65^{A,B}$	39.19 ± 7.69
Hydrolyzable tannins[2]	(TAE mg/g)	Peel	$132.47 \pm 7.52^{A,B}$	$121.05 \pm 4.92^{B,C}$	111.23 ± 10.22^{C}	140.55 ± 7.37^{A}	$121.41 \pm 9.84^{B,C}$	$117.75 \pm 0.59^{B,C}$	124.08 ± 11.53
	(TAE mg/mL)	Juice	3.38 ± 0.67^{A}	$2.60 \pm 0.09^{A,B}$	$2.40 \pm 0.21^{A,B}$	$2.97 \pm 0.16^{A,B}$	$2.82 \pm 0.79^{A,B}$	1.97 ± 0.48^{B}	2.69 ± 0.58

[1]Total anthocyanin content was expressed, respectively, in mg cyanidin-3-glucoside of g of peel dw and per L of juice.
[2]Hydrolyzable tannin content was expressed as mg tannic acid equivalent (TAE) per g of peel dw and per mL of juice.
Each value in the table is represented as mean ± standard error (SE) ($n = 3$).
Superscript letters with different letters in the same row of ecotype, respectively, indicate significant difference ($P < 0.05$) analyzed by Duncan's multiple range test.

The hydrolyzable tannins in peels as TAE varied from 111.23 ± 10.22 mg/g dw (GB3) to 140.55 ± 7.37 mg/g dw (GR), with an average of 124.08 ± 7.69 mg/g dw (ca. 12.4%). These results are slightly lower than those obtained by Çam and Hişil (2010) in pomegranate peel (260.2 ± 12.6 mg/g TAE in methanolic extract and 82.6 ± 5.6 mg/g TAE in aqueous extract). HTC depends on solvent and extraction method. Çam and Hişil (2010) reported that 262.7 ± 11.5 mg/g TAE (ca. 26%) can be recovered from dry pomegranate peel as hydrolyzable tannins using pressurized water extraction. In juice, hydrolyzable tannins ranged from 1.97 ± 0.48 mg TAE/mL to 3.38 ± 0.67 mg TAE/mL with an average of 2.69 ± 0.58 mg/mL (ca. 2.7%). Gil and others (2000) reported lower total hydrolyzable tannins in juice from fresh arils (0.539 mg/mL).

Both anthocyanins and tannin contents in pomegranate peel are higher than juice. In a previous study, we reported greater total polyphenols and flavonoids content in peel compared to juice (Elfalleh and others 2009). It implies that pomegranate peel can be subjected to phenolic compounds extraction for additional benefits. In particular, it makes commercialization of under-used cultivars after processing possible.

Individual phenolic compounds content from pulp and peel

Four phenolic compounds were identified and quantified in pomegranate peel and pulp using the HPLC/ultraviolet method (Figure 1). These compounds include 2 hydroxybenzoic acids (gallic and ellagic acids) and 2 hydroxycinnamic acids (caffeic and *p*-coumaric acids). Table 2 gives the concentrations of individual phenolic compounds (mean ± SD) identified in pomegranate peel and pulp of different ecotypes. Overall mean concentrations of phenolic compounds in dw pomegranate peel were: gallic acid 123.79 ± 9.56 mg/100 g, ellagic acid 35.89 ± 2.42 mg/100 g, caffeic acid 20.56 ± 1.54 mg/100 g, and *p*-coumaric acid 4.48 ± 0.39 mg/100 g. Phenolic compounds in pomegranate pulp (fw) were: gallic acid 17.50 ± 2.62 mg/100 g, ellagic acid 23.10 ± 0.44 mg/100 g, caffeic acid 13.52 ± 0.72 mg/100 g, and *p*-coumaric acid 11.45 ± 0.80 mg/100 g. Pulp gallic, ellagic, and caffeic acid contents explain most content differences between ecotypes. For

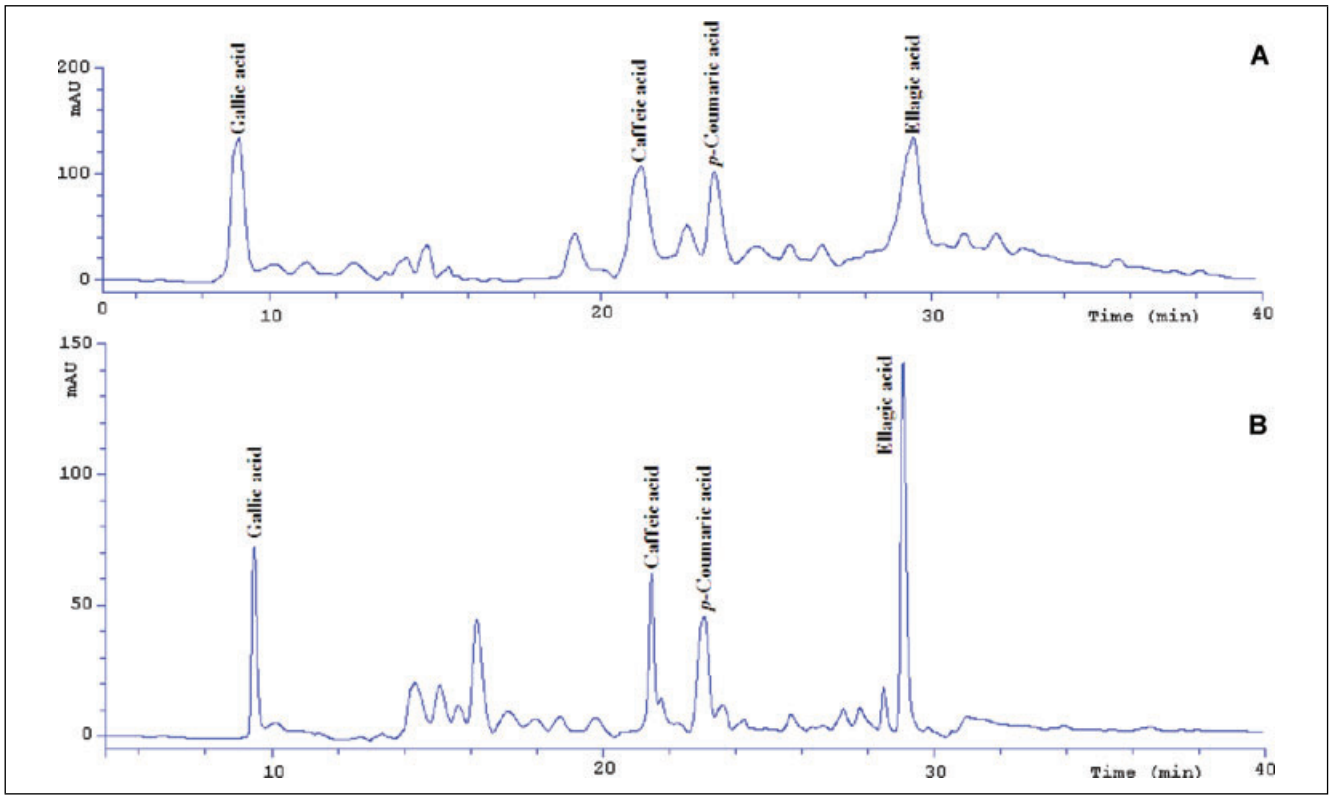

Figure 1–Chromatogram of individual phenolic acid from peel (A) and pulp (B) of Gabsi3 pomegranate cultivar.

Table 2–Individual phenolic compounds in peel and pulp (mg/100 g dw) from 6 Tunisian pomegranate ecotypes.

		CH	GB2	GB3	GR	MZ	ZH	Mean ± SD (n = 6)
Gallic acid	Peel	128.57 ± 3.13^{A}	127.65 ± 13.17^{A,B}	109.61 ± 10.34^{B}	131.63 ± 2.91^{A,B}	121.54 ± 2.43^{A,B}	120.01 ± 5.32^{A,B}	123.79 ± 9.56
	Pulp	20.55 ± 1.50^{A}	18.76 ± 1.29^{A}	13.71 ± 1.40^{C}	18.56 ± 0.69^{A,B}	15.61 ± 1.32^{B,C}	17.70 ± 0.70^{A,B}	17.50 ± 2.62
Ellagic acid	Peel	36.86 ± 2.20^{A}	36.53 ± 3.04^{A}	34.07 ± 4.00^{A}	36.63 ± 3.37^{A}	35.63 ± 2.51^{A}	34.19 ± 1.63^{A}	35.89 ± 2.42
	Pulp	23.43 ± 0.10^{A,B}	23.42 ± 0.46^{A,B}	22.69 ± 0.13^{B}	22.77 ± 0.15^{B}	22.85 ± 0.33^{B}	24.35 ± 0.82^{A}	23.10 ± 0.44
Caffeic acid	Peel	22.33 ± 0.62^{A}	20.62 ± 1.37^{A}	19.96 ± 2.64^{A}	20.58 ± 0.51^{A}	20.42 ± 1.57^{A}	18.85 ± 0.65^{A}	20.56 ± 1.54
	Pulp	13.63 ± 0.52^{A,B,C}	13.84 ± 0.15^{A,B,C}	12.97 ± 0.76^{B,C}	14.03 ± 0.49^{A,B}	12.68 ± 0.65^{C}	14.48 ± 0.04^{A}	13.52 ± 0.72
p-Coumaric acid	Peel	4.65 ± 0.74^{A}	4.20 ± 0.07^{A}	4.64 ± 0.81^{A}	4.43 ± 0.11^{A}	4.50 ± 0.09^{A}	4.90 ± 0.59^{A}	4.48 ± 0.39
	Pulp	12.58 ± 1.15^{A}	10.78 ± 0.16^{A}	11.20 ± 0.30^{A}	11.12 ± 0.84^{A}	11.41 ± 0.56^{A}	11.22 ± 0.87^{A}	11.45 ± 0.80

Each value in the table is represented as mean ± SE (n = 3).
Superscript letters with different letters in the same row of ecotype, respectively, indicate significant difference ($P < 0.05$) analyzed by Duncan's multiple range test.

peel, differences were not significant based on Duncan's test except for the gallic acid content. In previous studies, gallic acid was reported by Artik and others (1998) in pomegranate juice and by Ben Nasr and others (1996) in pomegranate peel.

Our results show that gallic and ellagic acids contents from Tunisian pomegranate peel and pulp were comparable with others studies. Mousavinejad and others (2009) have been reported that from Iranian pomegranate juice, the highest ellagic acid content was 16 mg/100 mL. Poyrazoğlu and others (2002) reported that gallic acid is the major phenolic compounds in pomegranate from Turkey (45.57 ± 85.5 mg/100 mL of juice). Present findings are greater than those reported by Poyrazoğlu and others (2002). They reported 0.67 ± 0.7 mg/100 mL of *p*-coumaric acid in pomegranate juice and 7.87 ± 7.9 mg/100 mL of caffeic acid.

Based on phenolic compounds from peel, our results are greater than those reported by Ben Nasr and others (1996) in Tunisian pomegranate fruit. They reported 11.7 ± 0.1 mg/100 dw ellagic acid and 3.0 ± 0.1 mg/100 dw gallic acid. Pomegranate peel can be subjected to phenolic compounds extraction and added to commercial pomegranate juice. Consequently, the increase in the soluble polyphenol content includes mainly ellagic tannins, gallic and ellagic acids, anthocyanins, and catechins. Instead, Shabtay and others (2008) reported that boosting antioxidant levels in the diet of cattle may help improve their health. Those findings seemed to make pomegranate peel, a waste product of the pomegranate industry, an attractive candidate as a nutritional supplement for cattle feed.

Tocopherol composition

Results in Table 3 show that seed oil of pomegranate contained high amounts of tocopherols ranging from ca. 277 mg/100 g dw of seed to ca. 318 mg/100 g dw with an average of ca. 300 mg/100 g dw. The predominant isomer was α-tocopherol at more than 53% (ca. 165 mg/100 g dw). γ-tocopherol ranged between ca. 95 mg/100 g dw (34.5%) and ca. 115 mg/100 g dw (37%) with an average of 107.38 mg/100 g dw, while δ-tocopherol ranged from 24.19 mg/100 g dw (8.7%) to 29.91 mg/100 g dw (10%), with an average of 27.29 mg/100 g dw.

Recently, tocopherol (as vitamin E) has attracted scientific attention for its antiaging, anticancer, and antiatherosclerosis effect. Because of its richness in tocopherols (as vitamin E), the seed oil of pomegranate would be a potential natural source for nutritional and medicinal uses. Tocopherol has been proposed to be a very important lipid-soluble radical-scavenging antioxidant in cellular and subcellular membranes and also in plasma lipoproteins (Burton and others 1983).

ORAC and FRAP values of pulp (juice), seed oil, and peel from pomegranate ecotypes

The free radical-scavenging capacities of pomegranate extracts were evaluated with the FRAP and ORAC tests (Table 4). They were expressed as TEAC. FRAP and ORAC radical-scavenging activity values are comparable in the 6 ecotypes.

From pomegranate juice, FRAP radical-scavenging activities ranged from 6.36 mmol/L in ZH ecotype to 8.65 mmol/L in GR ecotype, with an average of 7.24 ± 1.24 mmol/L. In seed oil, FRAP values ranged from 5.38 mmol/100 g in MZ ecotype to 10.04 mmol/100 g (seed oil) in GB3 ecotype. In peel, FRAP values ranged from 21.24 mmol /100 g in MZ to 29.8 mmol/100g in GR with an average of 25.63 ± 3.67 mmol/100 g (peel). From 133 Indian medicinal plants, Surveswaran and others (2007) reported pomegranate peel with the 3rd highest antioxidant capacity based on the FRAP assay with a TEAC of 9.07 mmol/100 g. However, they reported an inferior activity in seed, only 0.94 mmol/100 g. Our finding was similar to results reported in the FRAP assay by Ozgen and others (2008), in 6 pomegranate cultivars from southern Turkey with an average FRAP value of 7.35 ± 0.21 mmol TE/L.

In pomegranate juice, ORAC activities ranged from 5.94 mmol/L in GB3 to 8.18 mmol/L in GR ecotype, with an average of 7.24 ± 1.24 mmol/L. In seed oil, ORAC values ranged from 5.55 mmol/100 g in MZ ecotype to 7.65 mmol/100 g (seed oil) in GB3 ecotype. In peel, ORAC values ranged from 19.27 mmol/100 g in GB3 to 23.72 mmol/100 g (peel) in GR. Sepulveda and others (2010) reported that the antioxidant capacity of the pomegranate juices measured as ORAC value was between

Table 3–Tocopherol composition (mg/100 g) of dry pomegranate seeds.

Ecotypes	α-tocopherols	%	γ-tocopherols	%	δ-tocopherols	%	Σ tocopherols
CH	164.56 ± 13.06^{A}	53	115.45 ± 9.44^{A}	37	29.91 ± 3.41^{A}	10	309.91 ± 7.03
GB2	174.29 ± 6.55^{A}	55.5	$110.75 \pm 11.95^{A,B}$	35.3	28.67 ± 2.31^{A}	9.2	313.70 ± 7.71
GB3	178.53 ± 1.37^{A}	56	$109.83 \pm 2.09^{A,B}$	34.5	29.69 ± 3.62^{A}	9.5	318.05 ± 2.91
GR	161.20 ± 5.61^{A}	55	$107.17 \pm 2.75^{A,B}$	36.5	24.93 ± 2.11^{A}	8.5	293.29 ± 0.75
MZ	157.69 ± 6.55^{A}	56.8	95.71 ± 8.59^{B}	34.5	24.19 ± 1.58^{A}	8.7	277.58 ± 13.56
ZH	160.73 ± 13.81^{A}	55.8	$99.92 \pm 4.89^{A,B}$	34.7	27.22 ± 2.55^{A}	9.5	287.86 ± 11.48
Mean ± SD ($n = 6$)	165.77 ± 10.75	55.2	107.38 ± 8.78	35.7	27.29 ± 3.16	9.1	300.44 ± 17.40

Each value in the table is represented as mean ± SE ($n = 3$).
Superscript letters with different letters in the same column of ecotype, respectively, indicate significant difference ($P < 0.05$) analyzed by Duncan's multiple range test.

Table 4–ORAC and FRAP values of pulp, oil, and peel from pomegranate ecotypes.

		CH	GB2	GB3	GR	MZ	ZH	Mean ± SD ($n = 6$)
FRAP								
(TE mmol/L)	Juice	7.86 ± 0.95^{A}	7.31 ± 1.27^{A}	6.44 ± 0.01^{A}	8.65 ± 1.87^{A}	6.67 ± 0.83^{A}	6.36 ± 0.79^{A}	7.24 ± 1.24
(TE mmol/100 g)	Oil	$9.23 \pm 0.28^{A,B}$	$9.25 \pm 0.54^{A,B}$	10.04 ± 0.25^{A}	$7.12 \pm 1.93^{A,B,C}$	5.38 ± 1.53^{C}	$6.65 \pm 1.73^{B,C}$	8.17 ± 1.90
(TE mmol/100 g)	Peel	29.80 ± 0.09^{A}	$25.01 \pm 0.38^{B,C}$	$22.40 \pm 0.16^{C,D}$	29.26 ± 3.51^{A}	21.24 ± 0.25^{D}	$26.23 \pm 0.51^{A,B}$	25.63 ± 3.67
ORAC								
(TEAC mmol/L)	Juice	7.59 ± 2.26^{A}	6.50 ± 0.14^{A}	5.94 ± 1.29^{A}	8.18 ± 2.38^{A}	7.00 ± 2.05^{A}	6.62 ± 0.09^{A}	7.00 ± 1.52
(TEAC mmol/100 g)	Oil	7.02 ± 1.48^{A}	7.00 ± 0.62^{A}	7.65 ± 2.02^{A}	6.93 ± 3.21^{A}	5.55 ± 1.57^{A}	6.02 ± 4.99^{A}	6.43 ± 2.03
(TEAC mmol/100 g)	Peel	22.88 ± 2.20^{A}	22.53 ± 3.06^{A}	19.27 ± 1.17^{A}	23.72 ± 5.23^{A}	22.39 ± 2.83^{A}	21.58 ± 0.40^{A}	22.08 ± 2.73

Each value in the table is represented as mean ± SE ($n = 3$).
Superscript letters with different letters in the same row of ecotype, respectively, indicate significant difference ($P < 0.05$) analyzed by Duncan's multiple range test.

12.7 and 24.4 mmol/L. Similar results were obtained for pomegranate juice (25.0 ± 1.0 mmol/L) by Seeram and others (2008). In both FRAP and ORAC assays, the antioxidant capacity of CH and GR ecotypes was slightly higher than other ecotypes. However, no significant differences between ecotypes were reported with the Duncan test (Table 4).

These results bring attention to the richness of pomegranate peel, juice, and seed oil with natural antioxidant and can explain the interest of traditional medicine practitioners on pomegranate fruit and why they considered pomegranate as a medicinal plant. The antioxidant activity of phenolics is mainly due to their redox properties that make them act as reducing agents, hydrogen donors, singlet oxygen quenchers, and also may have a metallic chelating potential (Seeram and others 2006). In addition, synergism between the antioxidants in the mixture makes the antioxidant activity not only dependent on the concentration, but also on the structure and the interaction between the antioxidants (Seeram and others 2006).

Correlation coefficients of total anthocyanin, hydrolyzable tannins, tocopherols, and phenolic acid in peel and pulp (juice) to the FRAP and ORAC assays are shown in Table 5. Results show that the antioxidant potency of pomegranate extracts was correlated with their phenolic compound content. Particularly, the highest correlation was reported in peel. Peel anthocyanin and hydrolyzable tannins contribute to the antioxidant activities of pomegranate. Respectively, the correlation coefficients of TAC to FRAP and ORAC assay in peel were 0.800 and 0.898. The correlation of hydrolyzable tannins content to FRAP and ORAC capacities in peel was 0.788 and 0.870. The peel gallic and ellagic acids contribute to the antioxidant activities. ORAC assay show significant correlations at 1% of both acids (gallic acid $r = 0.971$; ellagic acid $r = 0.847$). Furthermore, with FRAP assay, only the gallic acid shows significant correlation at 5% ($r = 0.715$). It seems that hydroxybenzoic acids in peel contribute to the major antioxidant capacities of pomegranate. Both juice and peel extracts contain ellagic and gallic acids that may probably be produced from the hydrolysis of ellagitannins and gallotannins, respectively.

Table 5– Correlation coefficients of FRAP and ORAC assay to total anthocyanins, hydrolyzable tannins, individual phenolic compounds, and tocopherol composition of pomegranate peel, juice, and seed oil extracts.

	FRAP peel	ORAC peel
Total anthocyanin (peel)	**0.800***	**0.898****
Hydrolyzable tannins (peel)	**0.788***	**0.870****
Gallic acid (peel)	**0.715***	**0.971****
Ellagic acid (peel)	0.567	**0.847****
Caffeic acid (peel)	0.453	0.456
p-Coumaric acid (peel)	0.091	−0.388
	FRAP juice	**ORAC juice**
Total anthocyanin (juice)	0.693	**0.850****
Hydrolyzable tannins (juice)	−0.256	0.189
Gallic acid pulp (juice)	0.669	0.643
Ellagic acid pulp (juice)	−0.296	−0.150
Caffeic acid pulp (juice)	0.298	0.273
p-Coumaric acid pulp (juice)	0.213	0.377
	FRAP seed oil	**ORAC seed oil**
α-tocopherols	**0.884****	**0.813***
γ-tocopherols	**0.889****	**0.866****
δ-tocopherols	**0.908****	0.708

*Correlation is significant at the 0.05 level.
**Correlation is significant at the 0.01 level.

Seeram and others (2006) reported that hydrolyzable tannins were the predominant polyphenols found in pomegranate juice and account for 92% of its antioxidant activity. The predominant pomegranate hydrolyzable tannin as punicalagin was responsible for about half of the total antioxidant capacity of the juice. However, the peroxyl radicals used in the ORAC assay was effective in detecting the antioxidant activity of total anthocyanin ($r = 0.85$), other phenolic compounds are not significant contributor to the overall antioxidant capacity of our juices.

In a previous study, we reported the 2,2-diphenyl-1- picrylhydrazyl (DPPH) and 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) scavenging activity of the same ecotypes (Elfalleh and others 2009). Results clearly confirm that peel extract contains more antioxidants than the juice extract, which are confirmed in current study with the FRAP and ORAC assays. The range of tests used for antioxidant activity measurement is a testimony to the uncertainty surrounding the chemistry of antioxidant compounds. Besides, result showed that antioxidant activity does not necessarily correlate with high amounts of phenolics, and that is why both phenolic content and antioxidant activity information must be discussed when evaluating the antioxidant potential of extracts. The fact that there is a rather poor correlation between juice phenolic content and the FRAP and ORAC antioxidant activities may mean that other components, such as ascorbic acid, need to be investigated for their contribution to the antioxidant activity.

These results indicate that total anthocyanin, hydrolyzable tannins, and studied phenolic acid alone cannot explain the antioxidant activity of these natural food juices. Our observations indicate they are only one facet of the FRAP ORAC antioxidant capacities of pomegranate juices. Again, this showed that the constituents of a real matrix solution did not chemically interact with selected pure antioxidants, and that the antioxidant capacities were additive (Apak and others 2007).

Furthermore, identified tocopherols seem to contribute in major part to the antioxidant activity of seed oil. Table 5 shows the correlation of FRAP and ORAC assay of methanolic seed oil extract to tocopherol contents of pomegranate seeds. Concurrently, α-tocopherols, γ-tocopherols, and δ-tocopherols contributed to the antioxidant activity of seed oil and they expressed high correlation coefficients to FRAP and ORAC assays.

Conclusions

Anthocyanins and tannin contents in pomegranate peel are higher than juice. In previous study, we reported greater total polyphenols and flavonoids content in peel compared to juice (Elfalleh and others 2009). Four phenolic compounds were identified and quantified in pomegranate peel and pulp, including 2 hydroxybenzoic acids (gallic acid and ellagic acid) and 2 hydroxycinnamic acids (caffeic acid and *p*-coumaric acid). Both juice and peel extracts contain ellagic and gallic acids that may probably be produced from the hydrolysis of ellagitannins and gallotannins, respectively. It seems that hydroxybenzoic acids in peel contribute to the major antioxidant capacities of pomegranate.

Pomegranate peel can be subjected to phenolic compounds extraction and supplied to commercial pomegranate juice. Consequently, these can be an increase in the soluble polyphenol content that includes mainly ellagic tannins, gallic and ellagic acids, anthocyanins, and catechins. Furthermore, those findings seemed to make pomegranate peel, a waste product of the pomegranate industry, an attractive candidate as a nutritional supplement for cattle feed.

In both FRAP and ORAC assays, the antioxidant capacity of CH and GR ecotypes was slightly higher than other ecotypes. However, no significant differences between ecotypes were reported with the Duncan test.

Results show that seed oil of pomegranate contained high amounts of tocopherols. Identified tocopherols seem to contribute in a major part to the antioxidant activity of seed oil. Concurrently, α-tocopherols, γ-tocopherols, and δ-tocopherols contribute to the antioxidant activity of seed oil and they express high correlation coefficients to FRAP and ORAC assays. The richness in tocopherols (as vitamin E) makes seed oil of pomegranate a potential natural source for the production of these compounds for nutritional and medicinal uses.

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