

Full Length Research Paper

Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower

Walid Elfalleh^{1*}, Hédia Hannachi¹, Nizar Tlili², Yassine Yahia¹, Nizar Nasri² and Ali Ferchichi¹

¹Institut des Régions Arides de Médenine, Laboratoire d'Aridoculture et Cultures Oasiennes, 4119, Tunisia.

²Laboratoire de Biochimie, Département de Biologie, Faculté des Sciences de Tunis, Université de Tunis El Manar, 2092, Tunisia.

Accepted 3 January, 2012

Pomegranate (*Punica granatum* L.) is a nutrient dense food rich in beneficial phytochemicals. The aim of this study is to investigate and to determine antioxidant contents from local and highly consumed pomegranate variety called 'Gabsi'. Peels, seeds, leaves and flowers were used to quantify total polyphenols, flavonoids, anthocyanins and hydrolysable tannins. Antioxidants contents were as follows: peel > flower > leaf > seed. Total polyphenols contents from peels were 85.60 ± 4.87 mg gallic acid equivalents per g dry weight (mg GAE/g DW), flavonoids (51.52 ± 8.14 mg rutin equivalents per g DW (mg RE/g DW), anthocyanins (102.2 ± 16.4 mg cyanidin-3-glucoside equivalents per g DW (mg CGE/g DW) and hydrolysable tannins (139.63 ± 4.25 mg tannic acid equivalent per g of DW (mg TAE/g DW). High free radical scavenging activity is reported in peels and flowers. Effective concentration at 50% (EC₅₀) was 3.88 ± 0.33 µg/ml (peels) and 4.55 ± 0.97 µg/ml (flowers). Antioxidant capacity value was respectively 7.50 ± 0.83 Trolox equivalent antioxidant capacity (TEAC) mg/g DW (peels) and 6.39 ± 0.83 TEAC mg/g DW (flowers). Less important values were obtained from leaves (4.16 ± 1.35 TEAC mg/g DW) and seeds (1.10 ± 0.23 TEAC mg/g DW). Peels and flowers extract exhibited higher activities than seeds and leaves. All of these findings implied that bioactive compounds from pomegranate peels, flowers, leaves and seeds might be potential resources for the development of antioxidant function dietary foods. Extraction process of whole fruits may provide a commercial pomegranate juice with high antioxidants and consequently high usefulness antioxidant activities.

Key words: Pomegranate fruit and leaves extracts, polyphenols, flavonoids, anthocyanins, total tannins, antioxidant activity.

INTRODUCTION

Plants are always a rich source of compounds that do not appear essential for primary metabolism, including thousands of secondary metabolites and several macromolecules, such as peptides, proteins, enzymes, lignin and cellulose. Phytochemicals are often referred to non-nutritive compounds thought to be produced by plants as means of protection against such dangers as harmful ultraviolet radiation, pathogens and herbivorous predators.

The consumption of a plant-based or phytochemical-rich diet has been associated with a reduced risk of chronic human illnesses such as certain types of cancers, inflammation, cardiovascular and neurodegenerative diseases (Kong et al., 2003; Beretta et al., 2009). Therefore, the chemistry and biology of phytochemicals are of highest importance for evaluation of their potential health benefits to humans. Phenolic compounds, including flavonoids, anthocyanins and tannins, are the main group of antioxidant phytochemicals with interesting properties and have deeply value due to their biological and free radical scavenging activities (Elfalleh et al., 2011). Traditional medicine practitioners consider pomegranate as a provider of natural antiviral, antifungal,

*Corresponding author. E-mail: walid.elfalleh@fst.rnu.tn. Tel: +216 97 606 803.

and antibacterial benefits. Since ancient times, pomegranate juice has been used as a natural astringent for treating diarrhoea and harmful internal parasites (Das et al., 1999). The pomegranate (*Punica granatum* L.), which belongs to the Punicaceae family, is a nutrient dense food source rich in phytochemical compounds (Seeram et al., 2006; Miguel et al., 2010). Pomegranates are popularly consumed as fresh fruit, as beverages (for example, juices and wines), as food products (for example, jams and jellies), and as extracts wherein they are used as botanical ingredients in herbal medicines and dietary supplements. The major source of dietary pomegranate phytochemicals is the fruit (peel, seeds and juice). Pomegranate tree has been cultivated and naturalized over the whole Mediterranean region. In Tunisia, it has been cultivated traditionally since ancient time under diverse agroclimatic conditions. Pomegranate is well known typically in the coastal regions and in many regions inside the country (Mars and Marrakchi, 1999). Previous studies reported that phytochemicals have been identified from various parts of the pomegranate tree and from pomegranate fruit: peel, juice and seeds (Singh et al., 2002; Elfalleh et al., 2009).

The main objective of this research was to quantify total phenols, flavonoids, anthocyanin and hydrolysable tannins in pomegranate aqueous and methanolic extract from peel, flower, seed and leaf. We compared the antioxidant capacity of different extracts from the different part of the plant. Particularly, we focus in relationships between the content of phenolic compound and the antioxidant activities.

MATERIALS AND METHODS

Plant materials

Leaves, peels, seeds and flowers of pomegranate were harvested in October, 2010 from pomegranate trees in 'Gabès' province (Southern Tunisia: 33°40'N, 10°15'E). The sampling was done from different trees of commercial "Gabsi" variety. Samples were collected, sun-dried and powdered. Leave, peel, seed and flower powders (10 g) were extracted by maceration in 100 ml of methanolic extracts at 30°C for one night. Separately, the same amount (10 g) were extracted and stirred with 100 ml of water at 30°C for one night. In each case, the solution was covered with parafilm to prevent the solvent evaporation and taken in continuous agitation for one night. Phenolic compounds' extractions from different tissues were done under homogenous conditions. The extracts were filtered through Whatman No.1 filter paper for removal of particles. Then extracts were pooled and concentrated under vacuum.

Chemicals

All solvents were of reagent grade without any further purification. Gallic acid, cyanidin-3-glucoside, tannic acid and Folin-Ciocalteu's phenol reagent were purchased from Sigma Chemical Co. (St Louis, MO, USA). Trolox and rutin were purchased from (Aldrich, Milwaukee, WI). The analytical reagent grade methanol was obtained from Lab-Scan (Labscan Ltd, Dublin, Ireland). The water

used in sampling was prepared with a Millipore Simplicity (Millipore S.A.S., Molsheim, France). All chemicals used in antioxidant activities were of chromatography grade quality and were purchased from Sigma Chemical Co. (Poole, Dorset). Spectrophotometric measurements were performed on Shimadzu ultraviolet (UV)-1600 spectrometer (Shimadzu, Kyoto, Japan).

Qualitative phytochemical screening

Each extract was screened for the presence of key families of phytochemicals according to the method cited by Marzouk et al. (2009) and previously reported by Trease and Evans (1984) and Sakar and Tanker (1991) using the related reagents and chemicals. Alkaloids were analyzed using Dragendorff's reagent confirmed with Bouchardat's (I_2/MgI_2) and with Meyer's reagents ($KI/MgCl_2$). Based on Wilstater test, flavonoids were analyzed with metallic magnesium and hydrochloric acid (HCl). Saponins were tested for their ability to produce suds. Tannins tested with ferric chloride (confirmed with concentrated hydrochloric acid, Bath-Smith reaction).

Determination of total polyphenol content (TPP)

TPP were estimated by the Folin-Ciocalteu method reported in Elfalleh et al. (2009). From each sample, 0.5 ml of methanolic solution to 0.5 ml of Folin-Ciocalteu (Prolabo) reagent was added. We add 4 ml of a solution of sodium carbonate (1 M). The tubes were laid for 5 min in a water bath at 45°C and then put in a cold water bath. The reading of the absorbance was made at 765 nm using a Shimadzu 1600-UV spectrophotometer. TPP of each fraction were converted into mg gallic acid equivalents per g dry weight (mg GAE/g DW).

Determination of total flavonoids content (TF)

The amount of TF in the extracts was measured spectrophotometrically following the method of Djeridane et al. (2006). This method was based on the formation of a complex flavonoid-aluminium, having the maximum absorbance at 430 nm. Rutin was used to make a calibration curve. 1 ml of methanolic extract was mixed with 1 ml of 2% $AlCl_3$ methanolic solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm using a Shimadzu 1600-UV spectrophotometer. TF was expressed as mg rutin equivalents per g DW (mg RE/g DW).

Determination of total anthocyanin content (TA)

TA content was determined by pH differential method using two buffer systems: potassium chloride buffer (pH 1.0, 0.025 M) and sodium acetate buffer (pH 4.5, 0.4 M). Methanolic extract were mixed with 3.6 ml of corresponding buffers and read against water as a blank at 510 and 700 nm (Elfalleh et al., 2011; Çam et al., 2009). Absorbance (A) was calculated using this formula $A = [(A_{510} - A_{700})_{pH_{1.0}} - (A_{510} - A_{700})_{pH_{4.5}}]$ with a molar extinction coefficient of 29600. Results were expressed as mg of cyanidin-3-glucoside equivalents per g DW (mg CGE/g DW).

Determination of hydrolysable tannins content (HTs)

HTs were determined by the method of Çam and Hişil (2010). 1 ml 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

Table 1. Qualitative phytochemical screening in pomegranate seed, leaf, flower and peel.

Antioxidants content	Alkaloids		Flavonoids		Saponins		Tannin	
	Aq.	MeOH	Aq.	MeOH	Aq.	MeOH	Aq.	MeOH
Seed	+	+	+	+	++	++	+	+
Leave	+	+	+	+	+	+	++	++
Flower	++	++	++	++	++	++	++	++
Peel	++	++	++	++	++	++	++	++

Aq., Aqueous extract; MeOH, methanolic extract; (++) , highly presence; (+), presence; (-), absence.

the red colored mixture was determined at 550 nm versus the prepared water blank. Optimum reaction defined as the 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 per g of DW (mg TAE/g DW).

2,2-Diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging activity

The scavenging activity on DPPH radical of different extracts was determined following the method reported by Okonogi et al. (2007). A test solution of deferent concentrations was prepared from a stock solution of methanolic and aqueous extracts (1 mg of dry powder per ml). DPPH (100 µM) was dissolved in methanol and mixed with an aliquot of 100 µl of each dilution. The mixture was shaken vigorously and left to stand for 30 min in the dark at room temperature. After the reaction was allowed to take place in the dark for 30 min, the absorbance at 517 nm was recorded to determine the concentration of remaining DPPH. The radical-scavenging activity was calculated as % inhibition by the following formula:

$$\text{DPPH}_{\text{radical-scavenging}}(\%) = \left[1 - \frac{\text{Abs}_{\text{sample}}}{\text{Abs}_{\text{Control}}} \right] \times 100$$

Effective concentration at 50% (EC₅₀) values calculated denote the effective concentration of a sample required to decrease the absorbance at 517 nm by 50%. All measurements were performed in triplicate.

2, 2'-Azinobis (3-ethylbenzothiazoline)-6-sulfonic acid (ABTS⁺) radical scavenging activity

ABTS⁺ assay was based on the method of Re et al. (1999) modified. ABTS⁺ radical cation was produced by reacting 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature before use. The ABTS⁺ solution was diluted with ethanol to an absorbance of 0.70 ± 0.02 at 734 nm. After addition of 25 µl of sample or Trolox standard to 2 ml of diluted ABTS⁺ solution, absorbance at 734 nm was measured at 5 min. Results were expressed as Trolox equivalent antioxidant capacity (TEAC).

Reducing power assay

The reducing power of aqueous and methanolic extracts was

quantified according to the method cited by Ferreira et al. (2007) and Singh and Rajini (2004). 1 ml of reaction mixture, containing various concentrations of samples (0.05, 0.1, 0.2, 0.4, 0.8 and 1.2 mg/ml) in phosphate buffer (0.2 M, pH 6.6), was incubated with potassium ferricyanide (1% w/v) at 50°C for 20 min. The reaction was terminated by adding trichloroacetic acid (TCA) solution (10% w/v) and the mixture was centrifuged at 2000 g for 10 min. The supernatant was mixed with distilled water and ferric chloride (0.1% w/v) solution and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power. The reducing power was calculated and EC₅₀ values denote the effective concentration at which the absorbance is 0.5.

Statistical analyses

All samples were analyzed in triplicate. The analysis of variance (ANOVA) was conducted to compare the data between peel, seed, leave and flowers of the same pomegranate variety. Statistical analyses were performed using XLSTAT 2009 (www.xlstat.com). Data were expressed as mean ± SD using ANOVA. Differences at *p* < 0.05 were considered statistically significant by Duncan's new multiple range test.

RESULTS AND DISCUSSION

Qualitative phytochemical screening

Results (Table 1) show that there is no difference between extraction method (aqueous extracts and methanolic ones). Differences were noted between plant organs: alkaloids and flavonoids in peel and flower were high than seeds and leaves. Saponins were highly present in peel, seeds and flower than leaves, tannin were highly present in peel, leaves and flower than seeds. Indeed, alkaloids are commonly found to have antimicrobial properties (Omulokoli et al., 1997). Flavonoids have been found to possess antitumoral, anti-allergic, and anti-inflammatory activities (Ferrandiz and Alcaraz, 1991; Gil et al., 1994; Terao et al., 1994). Hydrolysable tannins, in particular, have anti-ischemic activity and an endothelium-dependent vasorelaxant effect (Beretta et al., 2009). For these reasons, activity cannot be imputed to one family of phytochemicals. The difference between parts can explain and support the uses by old people of specific part of the plant to treat specific illness and disease.

Table 2. Mean values of total polyphenols, flavonoids, anthocyanins and hydrolysable tannins of pomegranate seed, leaf, flower and peel.

Antioxidants content	Total polyphenol (TPP)			Total flavonoids (TF)			Total anthocyanins (TA)			Hydrolysable tannin (HT)		
	(GAE mg/g dry weight)			(RE mg/g dry weight)			(CGE mg/g dry weight)			(TAE mg/g dry weight)		
	Aq.	MeOH	p-value*	Aq.	MeOH	p-value*	Aq.	MeOH	p-value*	Aq.	MeOH	p-value*
Seed	7.94 ± 1.25 ^C	11.84 ± 1.92 ^C	0.042	3.30 ± 0.52 ^C	6.79 ± 0.57 ^D	0.001	19.62 ± 3.12 ^C	40.84 ± 7.77 ^C	0.011	32.86 ± 4.24 ^B	29.57 ± 4.54 ^C	NS
Leave	9.85 ± 0.82 ^C	14.78 ± 2.10 ^C	0.019	12.77 ± 0.23 ^B	26.08 ± 1.24 ^C	0.0001	40.91 ± 3.43 ^B	89.81 ± 7.50 ^B	0.0005	64.40 ± 4.85 ^A	128.02 ± 4.49 ^B	0.0001
Flower	42.70 ± 2.17 ^B	66.29 ± 3.06 ^B	0.0004	21.45 ± 0.58 ^A	72.52 ± 5.59 ^A	0.0001	80.20 ± 7.02 ^A	168.91 ± 3.13 ^A	0.0001	57.04 ± 3.41 ^A	148.24 ± 10.29 ^A	0.0001
Peel	53.65 ± 4.13 ^A	85.60 ± 4.87 ^A	0.001	21.03 ± 1.62 ^A	51.52 ± 8.14 ^B	0.003	51.02 ± 10.33 ^B	102.20 ± 16.42 ^B	0.01	62.71 ± 11.32 ^A	139.63 ± 4.25 ^{A^B}	0.0001

Each value in the table is represented as mean ± SE (n = 3). Superscript letters with different letters in the same column indicate significant difference (P < 0.05) analyzed by Duncan's multiple range test. *p-values were determined by Fisher's exact test, significantly different (P < 0.05); NS, not significant

Total phenols, flavonoids, anthocyanins and hydrolysables tannins contents

All studied phenol contents (Table 2) varied according to plant parts and also extraction solvent. Stintzing et al. (2005) suggested that a considerable diversity of opinion exists on the appropriate method to assess these antioxidants in plant tissues. Results show that with methanol we obtained the highest values of total polyphenols, flavonoids, anthocyanins and tannins except for the tannin in the seeds. This result is in agreement with Singh et al. (2002) who reported that the maximum antioxidant yield was obtained with methanol compared to acetone and water. Li et al. (2006) suggested that a combination of different solvents seems to be more efficient for extracting antioxidants. As shown in Table 2, the TPP expressed as mg GAE/g DW are highest in peel (85.60 ± 4.87), followed by flowers (66.29 ± 3.06), leaves (14.78 ± 2.10) and seeds (11.84 ± 1.92). Pande and Akoh (2009) reported that TPP in peel, seeds and leaves of Georgian pomegranate are 311, 89, 365 mg GAE/g FW, respectively. Li et al. (2006) reported that the content of TPP in the peel of Chinese pomegranate was 249.4 ± 17.2 mg/g

TAE/g FW. TF are 51.52, 6.79, 26.08 and 72.52 mg (mg RE/g DW) for peel, seeds, leaves and flowers, respectively. TA are 102.20 (± 16.42), 40.84 (± 7.77), 89.81 (± 7.50) and 168.91 (± 3.13) mg of mg CGE/g DW for peel, seeds, leaves and flowers, respectively. Indeed, anthocyanins also possess known pharmacological properties and are used by humans for therapeutic purposes (Kong et al., 2003). Anthocyanins are the water-soluble pigments responsible for the bright red colour of pomegranate juice. Noda et al. (2002) reported that three major anthocyanidins found in pomegranate juice were delphinidin, cyanidin and pelargonidin. Total hydrolysable tannins are expressed as mg TAE/g DW and varied according to the organ type: peel (139.63 ± 4.25), seeds (29.57 ± 4.54), leaves (128.02 ± 4.49) and flowers (148.24 ± 10.29). These results bring attention to the richness of the different part of pomegranate with natural antioxidant and can explain the interest of traditional medicine practitioners to pomegranate tree and why this plant is considered as medicinal plant. Furthermore, Gil et al. (2000) suggested that some pomegranate juices showed a high antioxidant activity (18 to 20 TEAC), nearly three times the antioxidants of green tea or red wine. Methanol is an effective

solvent for polyphenols, and then it is commonly used in the laboratory and in industrial extraction process, while, ordinary people when prepare pomegranate juice, use water. Indeed, water is not an effective solvent for the extraction of phenols compared to methanol. But, results obtained with water show also that different part of pomegranate contained a good quantities of natural antioxidants (Table 2). Moreover, these results encourage studying more, the possibility to improve the extraction of natural antioxidants using water and avoid organic solvent. Indeed, a suitable extracting procedure should be developed and improved to recover as many antioxidants as possible before an extract rich in natural antioxidants could be further explored for possible application in health-promoting supplements for the food industry (Li et al., 2006).

Antioxidant activities

DPPH is a free radical compound that has been widely used to determine the free radical-scavenging ability of various samples (Amarowicz et al., 2004). The free radical scavenging activity determined by DPPH was expressed as the

Table 3. DPPH/ABTS⁺ radical scavenging activities and reducing power of pomegranate seed, leaf, flower and peel.

Antioxidants content	DPPH (EC ₅₀) (µg/ml) ^a			ABTS ⁺ (TEAC mmol/100 g DW)			Reducing power (EC ₅₀) (µg/ml) ^b		
	Aq.	MeOH	p-Value*	Aq.	MeOH	p-value*	Aq.	MeOH	p-value*
Seed	45.05 ± 1.59 ^A	21.00 ± 1.07 ^A	0.0001	0.76 ± 0.06 ^C	1.10 ± 0.23 ^C	NS	321.15 ± 38.93 ^A	337.84 ± 38.93 ^A	NS
Leaf	26.65 ± 0.98 ^B	11.44 ± 1.04 ^B	0.0001	1.81 ± 0.17 ^B	4.16 ± 1.35 ^B	0.041	348.68 ± 24.69 ^A	293.63 ± 15.29 ^A	0.03
Flower	13.87 ± 3.48 ^C	4.55 ± 0.97 ^C	0.011	4.06 ± 0.85 ^A	6.39 ± 0.83 ^A	0.027	203.54 ± 30.58 ^B	180.18 ± 26.48 ^B	NS
Peel	11.48 ± 2.29 ^C	3.88 ± 0.33 ^C	0.005	3.80 ± 0.31 ^A	7.50 ± 0.83 ^A	0.002	163.50 ± 10.42 ^C	155.16 ± 13.24 ^C	NS

^aEC₅₀ (µg/ml), Effective concentration at which 50% of DPPH radicals are scavenged; ^bEC₅₀ (µg/ml), effective concentration at which the absorbance is 0.5. Each value in the table is represented as mean ± SE (n = 3). Superscript letters with different letters in the same column indicate significant difference (P < 0.05) analyzed by Duncan's multiple range test. *p-values were determined by Fisher's exact test, significantly different (P < 0.05); NS, not significant.

EC₅₀ value (the effective concentration of extract required to inhibit 50% of the initial DPPH free radical). Results are shown in Table 3. Results show that both aqueous and methanolic extract displayed good antioxidant activities for all parts of the plants. In both methanol and aqueous extract, the EC₅₀ value of seed and leaf were higher than flower and peel. The EC₅₀ value of seed and leaf aqueous extract were 45.05 ± 1.59 and 26.65 ± 0.98 µg/ml, respectively, which were higher than those of flower and peel (13.87 ± 3.48 and 11.48 ± 2.29 µg/ml, respectively). With methanol extract, the EC₅₀ value of seed and leaf (21.00 ± 1.07 and 11.44 ± 1.04 µg/ml, respectively) were also higher than those of flower and peel (3.88 ± 0.33 and 4.55 ± 0.97 µg/ml, respectively). In addition, relative activities of methanol extracts were significantly higher than those of water extracts. Indeed, due to different antioxidant potentials of different compounds, the antioxidant activity of extract strongly depends on the extraction solvent (Jang et al., 2007). Based on EC₅₀ value, the current study coordinates with previous study reporting higher DPPH radical scavenging activity of pomegranate peel compared to pulp in Tunisian pomegranate fruit (Elfalleh et al., 2009). The

ABTS free radical assay can be used to measure the antioxidant activity of a broad diversity of substances, for example, both aqueous phase radicals and lipid peroxyl radicals (Re et al., 1999; Rice-Evans et al., 1996). The methanol extracts of the different part showed stronger antioxidant activities than the water extracts (Table 3). With methanol extract, the ABTS activities of peel and flower (7.5 and 6.39 TEAC mmol/100 g DW, respectively) were higher than those of seed and leaf (1.1 and 4.16 TEAC mmol/100 g DW, respectively). Also, the ABTS activities with water extract of peel and flower are higher than seed and leaf. These differences are due to the content and quality of the phenols in the different part extract. The antioxidant activity of phenolics is mainly due to their redox properties which make them act as reducing agents, hydrogen donors, singlet oxygen quenchers and also may have a metallic chelating potential (Rice-Evans et al., 1996). In addition, synergism between the antioxidants in the mixture makes the antioxidant activity not only dependant on the concentration, but also on the structure and the interaction between the antioxidants (Djeridane et al., 2006). Figure 1 shows the reducing powers of

pomegranate seed, leaf, flower and peel in aqueous (Figure 1a) and methanolic extracts (Figure 1b). The reducing power of pomegranate extracts (as indicated by the absorbance at 700 nm) correlated well with increasing concentrations. Peel and flower extracts, at all concentrations, exhibited higher activities than seed and leaf. In Table 3, the reducing power was calculated and EC₅₀ values denote the effective concentration at which the absorbance is 0.5. Based on p-values, no difference were shown between aqueous and methanolic extract except for leaf (p-value = 0.03). The methanol extracts showed stronger reducing power than the aqueous extracts. EC₅₀ were respectively 337.84 µg/ml in seed, 293.63 µg/ml in leaf, 180.18 µg/ml in flower and 155.16 µg/ml in peel. In aqueous extracts, EC₅₀ were respectively 321.15 µg/ml in seed, 348.68 µg/ml in leaf, 203.54 µg/ml in flower and 163.50 µg/ml in peel. In literature, many authors reported correlation between antioxidant activity and reducing power of certain plant extracts (Pin-Der-Duh et al., 1999; Duh, 1998). The reducing properties are generally associated with the presence of reductones (Duh, 1998), which having antioxidant action by breaking the free

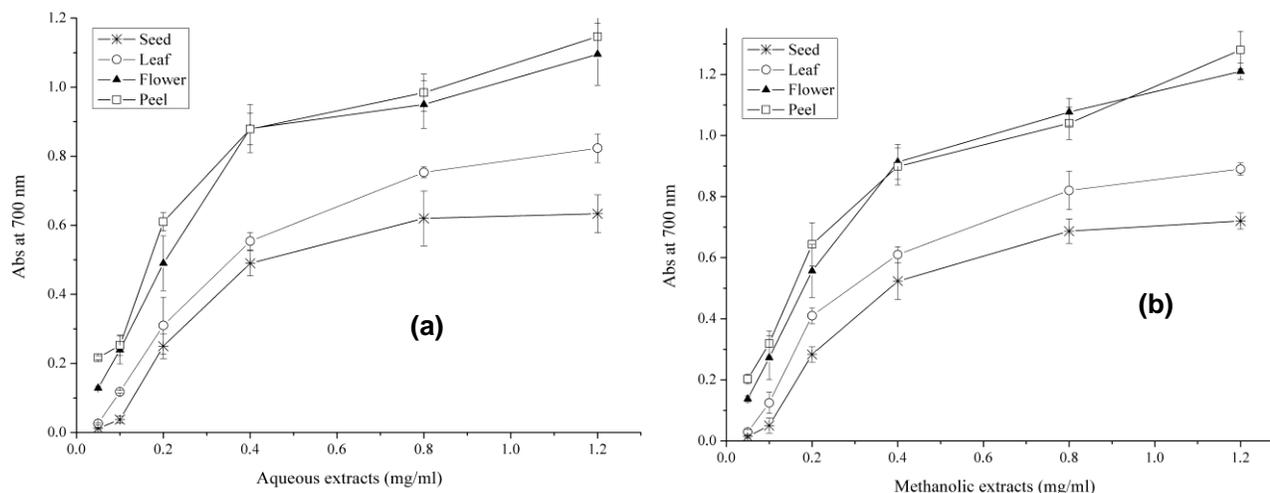


Figure 1. Reducing power of: (a), Aqueous extracts; (b), methanolic extracts from pomegranate seed, leaf, flower and peel. Each value is expressed as mean \pm standard deviation ($n = 3$).

radical chain by donating a hydrogen atom (Gordon et al., 1990). Fe^{2+} has been shown to produce oxyradicals and lipid peroxidation.

Consequently, reduction of Fe^{2+} concentrations in the Fenton reaction would protect against oxidative damage (Singh and Rajini, 2004). Reductones are also reported to react with certain precursors of peroxide, thus, preventing peroxide formation. Our data on the reducing power of pomegranate extracts suggest that it is likely to contribute significantly towards DPPH and ABTS radical scavenging activities.

ACKNOWLEDGMENTS

Authors wish to thank Dr. Khaled Jebahi for providing language help and L. Ben Yahia and N. Kbaier for advice and technical support.

REFERENCES

- Amarowicz R, Pegg RB, Rahimi-Moghaddam P, Barl B, Weil JA (2004). Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies. *Food Chem.* 84(4):551–562.
- Beretta G, Rossoni G, Alfredo Santagati N, Maffei Facino R (2009). Anti-ischemic activity and endothelium-dependent vasorelaxant effect of hydrolysable tannins from the leaves of *Rhus coriaria* (Sumac) in isolated rabbit heart and thoracic aorta. *Planta Med.* 75(14):1482–1488.
- Çam M, Hışıl Y (2010). Pressurised water extraction of polyphenols from pomegranate peels. *Food Chem.* 123(3):878–885.
- Çam M, Hışıl Y, Durmaz G (2009). Classification of eight pomegranate juices based on antioxidant capacity measured by four methods. *Food Chem.* 112(3):721–726.
- Das AK, Mandal SC, Banerjee SK, Sinha S, Das J, Saha BP, Pal M (1999). Studies on antidiarrhoeal activity of *Punica granatum* seed extract in rats. *J. Ethnopharmacol.* 68:205–208.
- Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N (2006). Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. *Food Chem.* 97(4):654–660.
- Duh PD (1998). Antioxidant activity of burdock (*Arctium lappa* Linne): its scavenging effect on free-radical and active oxygen. *J. Am. Oil Chem. Soc.* 75:455–461.
- Elfalleh W, Nasri N, Marzougui N, Thabti I, M'rabet A, Yahya Y, Lachiheb B, Guasmi F, Ferchichi A (2009). Physico-chemical properties and DPPH-ABTS scavenging activity of some local pomegranate (*Punica granatum*) ecotypes. *Int. J. Food Sci. Nutr.* 60(2):197–210.
- Elfalleh W, Tlili N, Nasri N, Yahia Y, Hannachi H, Chaira N, Ying M, Ferchichi A (2011). Antioxidant Capacities of Phenolic Compounds and Tocopherols from Tunisian Pomegranate (*Punica granatum*) Fruits. *J. Food Sci.* 76:707–713.
- Ferrandiz ML, Alcaraz MJ (1991). Anti-inflammatory activity and inhibition of arachidonic acid metabolism by flavonoids. *Agents Actions.* 32:283–288.
- Ferreira ICFR, Baptista P, Vilas-Boas M, Barros L (2007). Free-radical scavenging capacity and reducing power of wild edible mushrooms from northeast Portugal: Individual cap and stipe activity. *Food Chem.* 100(4):1511–1516.
- Gil B, Sanz MJ, Terencio MC, Ferrandiz ML, Bustos G, Paya M, Gunasegaran R, Alcaraz MJ (1994). Effects of flavonoids on *Naja naja* and human recombinant synovial phospholipases A2 and inflammatory responses in mice. *Life Sci.* 54(20):333–338.
- Gil MI, Tomas-Barberan FA, Hess-Pierce B, Holcroft DM, Kader AA (2000). Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. *J. Agri. Food Chem.* 48(10):4581–4589.
- Gordon MH (1990). The mechanism of antioxidant action in vitro. In BJJ Hudson (Ed.), *Food antioxidants* Elsevier. Appl. Sci, London pp. 1–18.
- Jang HD, Chang KS, Huang YS, Hsu CL, Lee SH, Su MS (2007). Principal phenolic phytochemicals and antioxidant activities of three Chinese medicinal plants. *Food Chem.* 103(3):749–756.
- Kong JM, Chia LS, Goh NK, Chia TF, Brouillard R (2003). Analysis and biological activities of anthocyanins. *Phytochemistry* 64(5):923–933.
- Li Y, Guo C, Yang J, Wei J, Xu J, Cheng S (2006). Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. *Food Chem.* 96(2):254–260.
- Mars M, Marrakchi M (1999). Diversity of pomegranate (*Punica granatum* L.) germplasm in Tunisia. *Genet. Resour. Crop. Evolution.* 46(5):461–467.
- Marzouk B, Marzouk Z, Decor R, Edziri H, Haloui E, Fenina N, Aouni M (2009). Antibacterial and anticandidal screening of Tunisian Citrullus

- colocynthis Schrad from Medenine. J. Ethnopharmacol. 125(2):344-349.
- Miguel GM, Neves AM, Antunes DM (2010). Pomegranate (*Punica granatum* L.): A medicinal plant with myriad biological properties – A short review. J. Med. Plants Res. 4(25):2836-2847.
- Noda Y, Kaneyuki T, Mori A, Packer L (2002). Antioxidant activities of pomegranate fruit extract and its anthocyanidins: delphinidin, cyanidin, and pelargonidin. J. Agric. Food Chem. 50(1):166–171.
- Okonogi S, Duangrat C, Anuchpreeda S, tachakittirungrod S, Chowwanapoonpohn S (2007). Comparison of antioxidant capacities and cytotoxicities of certain fruit peels. Food Chem. 103(3):839-846.
- Omulokoli E, Khan B, Chhabra S (1997). Antiplasmodial activity of four Kenyan medicinal plants. J. Ethnopharmacol. 56(2):133–137.
- Pande G, Akoh CC (2009). Antioxidant Capacity and Lipid Characterization of Six Georgia-Grown Pomegranate Cultivars. J. Agric. Food Chem. 57(20):9427–9436.
- Pin-Der-Duh X, Pin-Chan-Du X, Gow-Chin Yen X (1999). Action of methanolic extract of mung hulls as inhibitors of lipid peroxidation and non-lipid oxidative damage. Food Chem. Toxicol. 37(11):1055–1061.
- Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. 26(9-10):1231–1237.
- Rice-Evans C, Miller NJ, Paganga G (1996). Structure–antioxidant activity: activity relationships of flavonoids and phenolic acids. Free Radic. Biol. Med. 20(7):933–956.
- Sakar MK, Tanker M (1991). Fitokimya sal analizler. In: Ankara universitesi eczacilik fakultesi yayinlari no.67. Ankara, Turkey.
- Seeram NP, Henning SM, Zhang Y, Suchard M, Li Z, Heber D (2006). Pomegranate juice ellagitannin metabolites are present in human plasma and some persist in urine for up to 48 h. J. Nutr. 136:2481-2485.
- Singh N, Rajini PS (2004). Free radical scavenging activity of an aqueous extract of potato peel. Food Chem. 85(4):611–616.
- Singh RP, Murthy KNC, Jayaprakasha GK (2002). Studies on the antioxidant activity of pomegranate peel and seed extracts using in vitro models. J. Agric. Food Chem. 50(1):81–86.
- Stintzing FC, Herbach KM, Mosshammer MR, Carle R, Yi W, Sellappan S, Akoh CC, Bunch R, Felker P (2005). Color, betalain pattern, and antioxidant properties of cactus pear (*Opuntia* spp.) clones. J. Agric. Food Chem. 53(2):442-451.
- Terao J, Piskula M, Yao Q (1994). Protective effect of epicatechin gallate, and quercetin on lipid peroxidation in phospholipid bilayers. Arch. Biochem. Biophys. 308(1):278–284.
- Trease GE, Evans CW (1984). Pharmacognosy, 12th ed. Balliere Tindall, London, UK, London.