Full Length Research Paper

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

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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 as 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,
and antibacterial benefits. Since ancient times, pomegranate juice has been used as a natural astringent for treating diarrhea 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, 2010 from pomegranate trees in northern Tunisia: 33°40’N, 10°15’E. The sampling was done from different trees of different tissues were done under homogenous conditions. Agitation for one night.

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

<table>
<thead>
<tr>
<th>Antioxidants content</th>
<th>Alkaloids</th>
<th>Flavonoids</th>
<th>Saponins</th>
<th>Tannin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed</td>
<td>Aq. MeOH</td>
<td>Aq. MeOH</td>
<td>Aq. MeOH</td>
<td>Aq. MeOH</td>
</tr>
<tr>
<td>Leave</td>
<td>+ +</td>
<td>+ +</td>
<td>++ ++</td>
<td>+ +</td>
</tr>
<tr>
<td>Flower</td>
<td>++ ++</td>
<td>++ ++</td>
<td>++ ++</td>
<td>++ ++</td>
</tr>
<tr>
<td>Peel</td>
<td>++ ++</td>
<td>++ ++</td>
<td>++ ++</td>
<td>++ ++</td>
</tr>
</tbody>
</table>

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 different 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, leaf 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.

<table>
<thead>
<tr>
<th>Antioxidants content</th>
<th>Total polyphenol (TPP) (GAE mg/g dry weight)</th>
<th>Total flavonoids (TF) (RE mg/g dry weight)</th>
<th>Total anthocyanins (TA) (CGE mg/g dry weight)</th>
<th>Hydrolysable tannin (HT) (TAE mg/g dry weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aq.</td>
<td>MeOH</td>
<td>Aq.</td>
<td>MeOH</td>
</tr>
<tr>
<td>Seed</td>
<td>7.94 ± 1.25c</td>
<td>11.84 ± 1.92c</td>
<td>3.30 ± 0.52c</td>
<td>6.79 ± 0.57 D</td>
</tr>
<tr>
<td></td>
<td>0.042</td>
<td>0.01</td>
<td>0.001</td>
<td>0.001</td>
</tr>
<tr>
<td>Leave</td>
<td>9.85 ± 0.82c</td>
<td>14.78 ± 2.10c</td>
<td>12.77 ± 0.23c</td>
<td>26.08 ± 1.24c</td>
</tr>
<tr>
<td></td>
<td>0.019</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Flower</td>
<td>42.70 ± 2.17a</td>
<td>66.29 ± 3.06b</td>
<td>21.45 ± 0.58a</td>
<td>72.52 ± 5.59a</td>
</tr>
<tr>
<td></td>
<td>0.0004</td>
<td>0.0001</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Peel</td>
<td>53.65 ± 4.13a</td>
<td>85.60 ± 4.87a</td>
<td>21.03 ± 1.62a</td>
<td>51.52 ± 8.14a</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>0.003</td>
<td>0.003</td>
<td>0.003</td>
</tr>
</tbody>
</table>

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 hydrolysable 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 polyphenols 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.

<table>
<thead>
<tr>
<th>Antioxidants</th>
<th>DPPH (EC50) (µg/ml)a</th>
<th>ABTS* (TEAC mmol/100 g DW)</th>
<th>Reducing power (EC50) (µg/ml)b</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Aq.</td>
<td>MeOH</td>
<td>p-value*</td>
</tr>
<tr>
<td>Seed</td>
<td>45.05 ± 1.59B</td>
<td>21.00 ± 1.07B</td>
<td>0.0001</td>
</tr>
<tr>
<td>Leave</td>
<td>26.65 ± 0.98B</td>
<td>11.44 ± 1.04B</td>
<td>0.0001</td>
</tr>
<tr>
<td>Flower</td>
<td>13.87 ± 3.48C</td>
<td>4.55 ± 0.97C</td>
<td>0.011</td>
</tr>
<tr>
<td>Peel</td>
<td>11.48 ± 2.29C</td>
<td>3.88 ± 0.33C</td>
<td>0.005</td>
</tr>
</tbody>
</table>

*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.

The 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 dependent 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 (Elfailleh 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 theaqueous 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...
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

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REFERENCES


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