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Original Article

Comparative Antioxidant Activity and Total Flavonoid Content of Persian Pomegranate (Punica granatum L.) Cultivars

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Abstract

Pomegranate (Punica granatum L.), Lythraceae, is mainly grown in Mediterranean region. It is one of the major cultivated productions of Iran, which have been used in folk medicine for many centuries. It has been proved that pomegranate has a high antioxidant activity and is effective in the prevention of atherosclerosis. This study compares the antioxidant activity, total phenolic and flavonoid contents of nine different pomegranate cultivars grown in Iran. Aqueous solutions of known Fe+2 concentration, vitamin E, vitamin C, gallic acid and catechin were used for calibration. The results showed that Sour summer pulp cultivar had the most antioxidant effect with significant difference with the other cultivar (p < 0.05) which can be introduced as a potent source of natural antioxidants, and the peel of three cultivars (Sweet saveh malas, Sour summer and Black peel) as a suitable source for extraction and purification of phenolic and flavonoid compound. The antioxidant capacity of pomegranate peel extract is 10 times higher than the pulp extract.

Keywords: Antioxidant activity; Fruit extract; Punica granatum; Lythraceae.

Introduction

Epidemiological studies show that consumption of fruits and vegetables with high phenolic and flavonoid contents are correlated with reduced cardiovascular (1, 2), inflammation (3, 4), cancer mortality (5-8) and some other disease rates (9, 10).

Polyphenolic compounds consist of different phenolic rings, out of which one of the major subgroups of these secondary metabolites are flavonoids. They show some functionality in the plant related to interaction with environment such as plant protection against ultraviolet radiation (11-13) and antimicrobial properties to protect plants against micro organisms (14).

As human consumption aspects, flavonoids are one of the major groups of phytochemicals with high antioxidant activity; they have been interesting subjects for general studies in recent years. Pomegranate (Punica granatum L.), Lythraceae, is mainly grown in Mediterranean regions and is one of the major cultivated productions of Iran. It has been consumed for many centuries or perhaps millenniums as fruit, beverage and food-related product. Pomegranate has been used in Iranian traditional medicine for different therapies. For example,
The fruit was effective as diuretic and prokinetic agent and also as liver revival. Some other parts of pomegranate tree were also used in anti-parasite and anti-diarrhea formulations. It was also applied in cosmetic and toiletries because of its styptic properties. Today pomegranate is known as antimicrobial (15-17), antiviral (18, 19) and anticaner (5-8) substance which has led to being the center of attention in many studies. Both pomegranate pulp and peel contain different kinds of antioxidants (20), including those which have not possibly been well characterized so far. It has been acknowledged that phenolic compounds such as flavonoids and anthocyanins are the major class of effective antioxidants in many fruits and vegetables. In this paper the antioxidative activity, total phenolic and total flavonoid contents of 9 different Iranian pomegranate cultivars were studied.

Experimental

Chemicals

All reagents and solvents were purchased from Merck (Darmstadt, Germany) and Sigma (St. Louis, MO) unless otherwise mentioned. All chemicals used in the experiments were of analytical grade.

Sample preparation

Nine cultivars of pomegranate were donated from Saveh Agricultural Investigation Center during September 2007. A total of 27 pomegranate fruits (three numbers of each cultivar) were collected and washed three times with distilled water. To prepare pomegranate extract, fresh fruits were peeled and their edible portions (seed coats and juice) were separated. 30 g of pulps and peels were weighted and extracted separately for 4 h by Soxhlet apparatus with acetone, followed by ethyl acetate, methanol and water solvent respectively (four hours for each solvent) to extract different kinds of antioxidant components. The four different extracts of every cultivar were mixed and dried on a water bath. 1 g of pulp and peel extracts were dissolved and diluted with methanol 80% (v/v) to 25 mL. To assay total antioxidant and phenolic content, pulp extracts were diluted 1:10 (v/v) where it was 1:100 (v/v) for peel extracts by 80% methanol.

Total flavonoid assay

Total flavonoid content was measured by the aluminum chloride colorimetric assay (21). An aliquot (1 mL) of extracts or standard solution of catechin (50, 100, 150, 200, 250 and 300 mg/L) was added to 10 mL volumetric flask containing 4 mL of double distilled water. Then 0.3 mL 5% NaNO2 was added to the flask and after 5 min, 0.3 mL AlCl3 (10%) was also added. At 6th min, 2 mL NaOH (1 M) was added and the total volume was made up to 10 mL with double distilled water. The solution was mixed completely and the absorbance level was measured versus prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg catechin equivalents (CE) per one gram dry extract. The total flavonoid assay was measured three times for each pomegranate extract.

Total phenolic assay

Total phenolics were determined using Folin-Ciocalteu reagent as described by Velioğlu et al. (22) with slight modifications. The extract (200 µL) was mixed with 1.5 mL of Folin-Ciocalteu reagent (previously diluted 10 times with distilled water) and allowed to stand at room temperature for 5 min. 1.5 mL sodium bicarbonate solution (60 g/L) was added to the mixture and after incubation for 90 min at room temperature, the absorbance level was measured at 750 nm using a UV-Visible spectrophotometer (GBC, Cintra 40). Total phenolics were quantified by calibration curve obtained from measuring the absorbance of the known concentrations of gallic acid standard solutions (25-150 µg/mL in 80% methanol). The results were calculated as gallic acid equivalent (GAE) per one gram dry extract and reported as mean value ± SD.

Table 1. The total phenolic and flavonoid content of pulp extracts in nine different pomegranate cultivars.

| Cultivar           | Total phenolic mg GAE/g extract | Total flavonoids mg CE/g extract | Flavonoids/ Phenolics |
|--------------------|---------------------------------|---------------------------------|-----------------------|
| 1 Sweet white peel | 13.74 ± 1.21                    | 2.58 ± 0.21                     | 0.13                  |
| 2 Agha mohammad ali| 12.63 ± 0.63                    | 2.10 ± 0.14                     | 0.16                  |
| 3 North white peel | 14.94 ± 1.60                    | 2.34 ± 0.11                     | 0.14                  |
| 4 Sour white peel  | 16.92 ± 1.63                    | 2.08 ± 0.08                     | 0.11                  |
| 5 Sweet malas      | 17.65 ± 1.28                    | 1.10 ± 0.08                     | 0.06                  |
| 6 Sour Summer      | 21.03 ± 1.51                    | 1.46 ± 0.05                     | 0.09                  |
| 7 Sweet saveth malas| 19.22 ± 1.71                    | 1.05 ± 0.06                     | 0.06                  |
| 8 Sweet acum       | 19.93 ± 0.42                    | 1.34 ± 0.09                     | 0.07                  |
| 9 Black peel       | 19.06 ± 1.42                    | 1.09 ± 0.08                     | 0.07                  |

Table 2. The total phenolic and flavonoid content of peel extracts in nine different pomegranate cultivars.

| Cultivar           | Total phenolic mg GAE/g extract | Total flavonoids mg CE/g extract | Flavonoids/ Phenolics |
|--------------------|---------------------------------|---------------------------------|-----------------------|
| 1 Sweet white peel | 220.10 ± 11.23                  | 25.05 ± 0.56                    | 0.14                  |
| 2 Agha mohammad ali| 168.21 ± 6.39                   | 33.52 ± 0.41                    | 0.19                  |
| 3 North white peel | 192.72 ± 15.45                  | 26.94 ± 0.48                    | 0.14                  |
| 4 Sour white peel  | 198.24 ± 4.81                   | 28.30 ± 0.54                    | 0.28                  |
| 5 Sweet malas      | 212.11 ± 8.69                   | 18.61 ± 0.53                    | 0.15                  |
| 6 Sour Summer      | 226.56 ± 18.98                  | 35.92 ± 0.84                    | 0.15                  |
| 7 Sweet saveth malas| 216.74 ± 19.01                  | 34.71 ± 1.34                    | 0.13                  |
| 8 Sweet acum       | 184.10 ± 25.97                  | 30.36 ± 2.44                    | 0.16                  |
| 9 Black peel       | 250.13 ± 33.03                  | 36.40 ± 1.34                    | 0.14                  |
The flavonoid content in the pulp and peel extracts is expressed in terms of catechin equivalent (the standard curve equation: $y = 0.005x + 0.1478$, $r^2 = 0.9919$) ranged from 0.84 ± 0.08 to 2.14 ± 0.11 and 18.61 ± 0.53 to 36.40 ± 1.34 mg catechin equivalents per gram of extract respectively (Tables 1 and 2). Tables 1 and 2 also show the content of phenolic compounds that were measured in terms of gallic acid (the standard curve equation: $y = 0.005x - 0.0234$, $r^2 = 0.9975$). The total phenolic contents in the pulp and peel extracts varied from 11.62 ± 0.63 to 21.03 ± 1.51 and 98.24 ± 4.81 to 226.56 ± 18.98 mg gallic acid equivalents per gram of extract respectively.

Table 3. The total antioxidant activity of pomegranate pulp extract as FRAP value, in comparison with vitamins E and C.

| Cultivar          | micromol Fe/g extract | mg Vitamin E/g extract | mmol Vitamin C/g extract |
|-------------------|-----------------------|------------------------|-------------------------|
| 1 Sweet white peel| 325.697 ± 15.531      | 81.399 ± 4.796         | 0.160 ± 0.009           |
| 2 Agra mohammad ali| 276.333 ± 7.684       | 69.433 ± 2.172         | 0.136 ± 0.004           |
| 3 North white peel| 343.349 ± 18.311      | 85.953 ± 5.177         | 0.169 ± 0.010           |
| 4 Sour white peel | 347.255 ± 22.103      | 86.961 ± 5.704         | 0.171 ± 0.011           |
| 5 Sweet melas     | 316.938 ± 15.431      | 79.137 ± 3.246         | 0.155 ± 0.002           |
| 6 Sour Summer     | 467.817 ± 10.818      | 118.074 ± 3.058        | 0.234 ± 0.060           |
| 7 Sweet saveh melas| 347.104 ± 14.873     | 86.922 ± 4.205         | 0.171 ± 0.008           |
| 8 Sweet alac      | 410.349 ± 6.412       | 103.243 ± 1.813        | 0.204 ± 0.004           |
| 9 Black peel      | 312.052 ± 8.302       | 77.876 ± 2.347         | 0.153 ± 0.005           |

Table 4. The total antioxidant activity of pomegranate peel extract as FRAP value, vitamins E and C equivalent.

| Cultivar          | micromol Fe/g extract | mmol Vitamin E/g extract | mmol Vitamin C/g extract |
|-------------------|-----------------------|-------------------------|-------------------------|
| 1 Sweet white peel| 4560.331 ± 63.451     | 1150.363 ± 16.173       | 2.277 ± 0.033           |
| 2 Agra mohammad ali| 3401.354 ± 118.713   | 851.237 ± 30.635        | 1.675 ± 0.062           |
| 3 North white peel| 4685.185 ± 96.655     | 1162.715 ± 24.821       | 2.301 ± 0.050           |
| 4 Sour white peel | 4144.748 ± 73.967     | 1048.051 ± 18.712       | 2.071 ± 0.038           |
| 5 Sweet melas     | 3900.882 ± 433.021    | 979.798 ± 111.829       | 1.934 ± 0.225           |
| 6 Sour Summer     | 4788.401 ± 248.400    | 1209.126 ± 64.025       | 2.395 ± 0.129           |
| 7 Sweet saveh melas| 4313.445 ± 140.326   | 1086.586 ± 36.223       | 2.148 ± 0.073           |
| 8 Sweet alac      | 3953.205 ± 113.149    | 1003.871 ± 34.226       | 1.982 ± 0.069           |
| 9 Black peel      | 4607.206 ± 78.405     | 1162.325 ± 20.131       | 2.301 ± 0.040           |

Results and Discussion

The flavonoid content in the pulp and peel extracts is expressed in terms of catechin equivalent (the standard curve equation: $y = 0.005x + 0.1478$, $r^2 = 0.9919$) ranged from 0.84 ± 0.08 to 2.14 ± 0.11 and 18.61 ± 0.53 to 36.40 ± 1.34 mg catechin equivalents per gram of extract respectively (Tables 1 and 2). Tables 1 and 2 also show the content of phenolic compounds that were measured in terms of gallic acid (the standard curve equation: $y = 0.005x - 0.0234$, $r^2 = 0.9975$). The total phenolic contents in the pulp and peel extracts varied from 11.62 ± 0.63 to 21.03 ± 1.51 and 98.24 ± 4.81 to 226.56 ± 18.98 mg gallic acid equivalents per gram of extract respectively. As it can be seen in Tables 1, the range of phenolic and phenol content in pulp (R² = 0.94 and 0.84 ± 0.08 to 2.14 ± 0.11 mg catechin per gram of extract) and the flavonoid content was 249.4 ± 24.4 ± 2.7 and 17.2 ± 3.3 (mg tannic acid/g extract) and the flavonoid content was 249.4 ± 17.2 and 59.1 ± 4.8 (mg rutin/g extract). It is obvious that the flavonoid contents in the flour's reports were more than the present study. Stangeland et al. in 2009 (29) evaluated the total antioxidant activity in 35 Ugandan fruits and vegetables. Among the tested samples, antioxidant activity of pomegranate was 5.11 ± 0.61 mmol Fe2+ per 100 g fresh weight. In this report, antioxidant activity in Sor summer cultivar with FRAP value equivalent to 4.678 ± 1.818 mmol Fe2+ per 100 g fresh weight is comparable with the measure reported in Stangeland’s study. To sum up, Sour summer cultivar is a potent source of natural antioxidants, phenolic and flavonoid content for beverage industry and the peel of three cultivars (Sweet saveh melas, Sour Summer and Black peel) are suitable sources of phenolic and flavonoid compound. This study is the most comprehensive comparison among different pomegranate cultivars in the basis of antioxidant activity, phenolic and flavonoid contents. Further studies on the effective antioxidants contained in these fruit fractions and the mechanisms by which they protect against disease development are highly recommended.

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