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Antioxidant Properties of Peel and Pulp Hydro Extract in Ten Persian Pomegranate Cultivars

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Abstract: This study compares the antioxidant activity of ten different pomegranate cultivars grown in Iran using the ferric reducing power assay (FRAP assay), which is based on the reduction of a ferric-tripyridyl triazine complex to its ferrous, colored form in the presence of antioxidants. Aqueous solutions of known Fe^{+2} concentration, in the range of 100-1000 μmol L^{-1} were used for calibration. The results showed that among pulp and peel fractions the sour alac and sweet white peel cultivars had more FRAP value respectively. The pomegranate peel extract had markedly higher antioxidant capacity than the pulp extract. The peel extract of sweet white peel cultivar appeared to have more potential as a health supplement rich in natural antioxidants compared to the pulp and peel extracts of other pomegranate cultivars.

Key words: Antioxidant activity, fruit extract, *Punica granatum*

INTRODUCTION

Reactive Oxygen Species (ROS) and Reactive Nitrogen Species (RNS) are constantly generated *in vivo* for physiological purposes and often over-produced in pathological conditions, resulting in oxidative stress. To protect possible oxidative damage to biological molecules, lipids, proteins and especially deoxyribonucleic acids (DNA), all oxygen-consuming organisms are endowed with a well-integrated antioxidant system. This antioxidant system includes enzymatic and non-enzymatic components. The superoxide dismutase, glutathione peroxidase and catalase are the major antioxidant enzymes frequently mentioned in the literature (Fang *et al*., 2002; Jacob, 1995). The non-enzymatic components consist of macromolecules, such as albumin, ceruloplasmin and ferritin as well as an array of small molecules, like vitamin C, E, β-carotene and reduced glutathione (Fang *et al*., 2002; Jacob, 1995). Fruits can be rich sources of various vitamins, minerals and fibers required by human body for optimal health. In the recent years, more attention has been paid to the antioxidants contained in fruits. Epidemiological studies have shown that high fruit intake can be associated with reduced mortality and morbidity of cardiovascular disease and some types of cancer. One possible mechanism is attributed to the antioxidant activity (Lampe, 1999; Guo and Yang, 2001). The classic antioxidants are vitamin C, E and β-carotene. Other antioxidants include phenolic compounds which have been identified as important antioxidants found in fruits. Some of these compounds have been shown to have even more antioxidant activity than vitamin C and E *in vitro* and significant bioavailability has been demonstrated by animal and human studies (Bravo, 1998; Rice-Evans *et al*., 1996; Su *et al*., 2003; Ader *et al*., 2000; Cao *et al*., 1998; Mazza *et al*., 2002). Fruits are diverse in antioxidant composition and those with high antioxidant activity generally contain more antioxidants (Guo *et al*., 1997).

Pomegranate (*Punica granatum* L.) is native to the Mediterranean region and has been used extensively as a medicine in many countries. There are reports of the use of pomegranate as antiviral (Neurath *et al*., 2005) antimicrobial (Braga *et al*., 2005) and anticancer agent (Malik *et al*., 2005; Jeune *et al*., 2005). The pomegranate juice consumption has also shown to be effective for coronary heart disease (Fuhrman *et al*., 2005; Sumner *et al*., 2005) and chronic obstructive pulmonary disease (Cerda *et al*., 2006). It has been cultivated

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extensively in Mediterranean countries, Iran, India, Japan and Russia. The presence of antioxidants has been reported from pomegranate in juice, peel, pulp and seed fractions (Guo *et al*., 2003; Chidambara *et al*., 2002; Gil *et al*., 2000; Poyrazoglu *et al*., 2002; Noda *et al*., 2002; Halvorsen *et al*., 2002; Li *et al*., 2006; Negi *et al*., 2003). Iran is a native land of the pomegranate which is grown in every region, both coastal and mountainous areas. The total pomegranate production in Iran was 665,000 tons in 2003 (Iran Statistical Year Book, 2003). No literature, to date, was found reporting the antioxidant activity of the Persian pomegranate in Iran and little attention has been paid to its health promoting values. Based on numerous evidences about the increase in oxidative stress related diseases in Iran and the scarcity of data of the antioxidant content in foods, the aim of the current study was to determine the antioxidant capacity of pomegranate. If shown to be a valuable source of antioxidants, various food industries can explore the possibility of developing a nutritional agent rich in natural antioxidants from the pomegranate.

MATERIALS AND METHODS

Materials: All solvents and chemicals used were of analytical grade and obtained from Merck Company (Darmstadt, Germany). Double-distilled deionized water was used for preparation of aqueous solutions.

Sample preparation: Pomegranates were obtained from the agricultural research centers of Saveh in the Markazi province (10 cultivars) in Iran. This province represents more than 15% of the total pomegranate production among 28 total provinces (Iran Statistical Year Book, 2003). Commercially ripe fresh fruits were harvested during September of 2005 from mature trees randomly selected to represent the population of the plantation. A total of 30 pomegranates (three from each cultivar) were collected. They were first flushed by tap water and then washed in distilled water three times before the peel and pulp fractions were carefully separated. For the preparation of pomegranate extract, fresh fruits were peeled and their edible portions (seed coats and juice) were separated. A portion of 5 g was weighed and ground in a grinder with 50 mL of distilled water. The homogenate was centrifuged at 10,000 g for 15 min. The supernatant was filtered, diluted 1:5 with distilled water and used directly for FRAP assay without storage. The peels were manually removed, sun dried and powdered to get 60 mesh size. The peel powder (1 g) was extracted with 100 mL water; the extract was filtered for removal of peel particles and then centrifuged at 10,000 g for 15 min. The supernatant was filtered, diluted 1:25 with distilled water and used directly for FRAP assay without storage. For stability testing, the pulp extracts of each cultivar were stored in refrigerator at 4°C for three weeks and the antioxidant assay was performed each week.

FRAP assay: The procedure described by Benzie and Strain was followed (Benzie and Strain, 1999). The principle of this method is based on the reduction of a ferric-tripyridyltriazine complex to its ferrous colored form in the presence of antioxidants. The FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe^{II}-tripyridyltriazine compound from the colorless oxidized Fe^{III} form by the action of electron donating antioxidants. The FRAP reagent consist of 300 mM acetate buffer (3.1 g sodium acetate + 16 mL glacial acetic acid, made up to 1 L with distilled water; pH = 3.6), 10 mM TPTZ in 40 mM HCl and 20 mM $FeCl_3.6H_2O$ in the ratio of 10:1:1. Briefly 50 μL of sample supernatant was added to 1.5 mL freshly prepared and pre warmed (37°C) FRAP reagent in a test tube and incubated at 37°C for 10 min. The absorbance of the blue colored complex was read against reagent blank (1.5 mL FRAP reagent+50 μL distilled water) at 593 nm. For construction of the calibration curve, five concentrations of $FeSO_4.7H_2O$ (1000, 750, 500, 250 and 125 μmol L^{-1}) were used and the absorbencies were measured as sample solution. The data was expressed as mmole ferric ions reduced to ferrous form per liter (FRAP value) and was given in Table 1.

Imprecision: The within-run coefficient of variation (C.V.) was estimated by assaying the FRAP value of each $FeSO_4$ standard and pomegranate cultivar three times in the same analytical run. The between run CV was obtained by estimating three replicates of each FRAP value in another analytical run.

Statistical analysis: Results are expressed as mean±SD. The statistical examination of the data was performed using the SPSS version 11.5 program. Mean values of antioxidant activity in different cultivars were compared by using analysis of the variance (ANOVA) test. When significant ($p<0.05$) difference was detected, the means were compared by using the post-hoc Dunnett's T3 test. The relationships between variables were assessed by linear regression analyses ($p<0.005$).

The FRAP value of peel and pulp fractions in ten different pomegranate cultivars (mmol Fe/100 g) was given in Table 1 and the results of imprecision test in Table 2.

Table 1: The FRAP value of peel and pulp fractions in ten different pomegranate cultivars (mmol Fe/100 g)

Cultivars	Pulp	Peel	Total
Sour alac	1.439±0.042	90.503±7.863	91.942
Sweet white peel	1.048±0.113	169.608±15.165	170.656
Agha Mohammad Ali	0.682±0.024	44.239±3.118	44.921
North white peel	0.711±0.030	72.972±2.117	73.683
Sour white peel	0.974±0.061	102.167±7.694	103.140
Sweet malas	0.607±0.051	95.111±10.150	95.717
Sour summer	0.923±0.027	63.389±3.404	64.312
Sweet saveh malas	0.885±0.043	122.711±9.208	123.597
Sweet alac	1.184±0.143	77.722±10.281	78.907
Black peel	1.319±0.060	33.639±1.389	34.959
Mean±SD	0.977±0.270	87.206±38.152	88.183

Table 2: The pomegranate imprecision test

	Within-run coefficient of variation (%)		Between-run coefficient of variation (%)	
Cultivars	Pulp	Peel	Pulp	Peel
Sour alac	0.384	0.149	3.430	8.689
Sweet white peel	0.341	0.067	10.748	8.941
Agha Mohammad Ali	0.223	0.586	3.452	7.048
North white peel	0.160	0.080	4.148	2.901
Sour white peel	0.749	0.135	6.318	7.531
Sweet malas	0.080	0.747	8.436	10.671
Sour summer	0.058	0.023	2.904	5.369
Sweet saveh malas	0.228	0.308	4.836	7.504
Sweet alac	0.785	0.059	12.055	13.227
Black peel	0.250	1.078	4.571	4.129

RESULTS

The present study was undertaken to evaluate the antioxidant power of pomegranate extract and to utilize it as a substitute for synthetic antioxidants. For assaying of imprecision, the within-run and between run coefficients of variation were estimated three times using each of the $FeSO_4$ standard solutions and pomegranate cultivars. The results of pomegranate imprecision test are given in Table 2 and demonstrate the reproducibility and repeatability of the method. The antioxidant activity of pomegranate extract in ten different cultivars was determined in an attempt to make a systematic comparison among their antioxidant activities and identify the cultivar with high antioxidant power for further studies. The FRAP value of peel and pulp fractions (mmol Fe^{II}/100 g sample) of ten different pomegranate cultivars is summarized in Table 1. The peel extract FRAP value was much higher than the pulp extract, indicating that peel extract has more potential antioxidant activity (Mean FRAP value±SD, Pulp: 0.977±0.270; Peel: 87.206±38.152). We further made a systematic comparison among the antioxidant activities of ten different cultivars with ANOVA test and Dunnett's T3 Post Hoc (Fig. 1, 2). The results showed that among pulp and peel fractions, the sour alac and sweet white peel had the most potential FRAP values respectively. The stability test was performed weekly on the each pomegranate cultivar extract stored in the refrigerator for three weeks. The results showed a significant reduction in FRAP value between the first and other weeks (Mean FRAP value ± SD, first week: 1.425±0.002; Second week: 1.269±0.003; Third week: 1.245±0.011, Fig. 3).

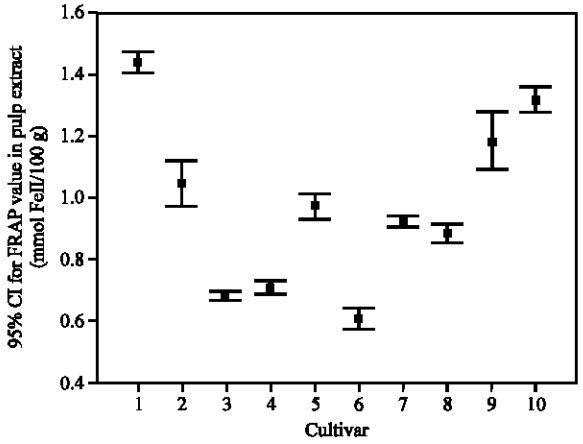

Fig. 1: The antioxidant activities of ten different pomegranate pulp cultivars grown in Iran (The ANOVA test shows the significant difference between the samples, $p<0.05$). [1]Sour alac; [2]Sweet white peel; [3]Agha Mohammad Ali; [4]North white peel; [5]Sour white peel; [6]Sweet malas; [7]Sour summer; [8]Sweet saveh malas; [9]Sweet alac; [10]Black peel

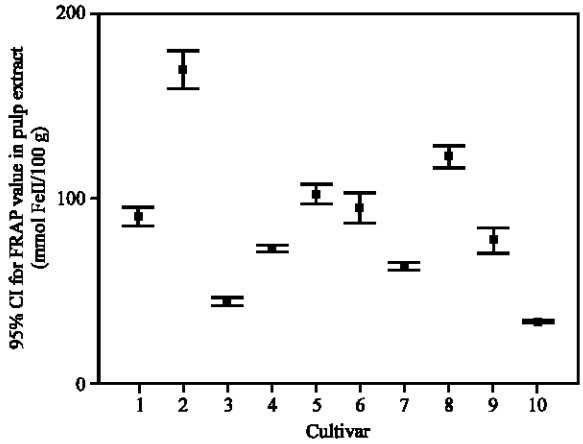

Fig. 2: The antioxidant activities of ten different pomegranate peel cultivars grown in Iran (The ANOVA test shows the significant difference between the samples, $p<0.05$). [1]Sour alac; [2]Sweet white peel; [3]Agha Mohammad Ali; [4]North white peel; [5]Sour white peel; [6]Sweet malas; [7]Sour summer; [8]Sweet saveh malas; [9]Sweet alac; [10]Black peel

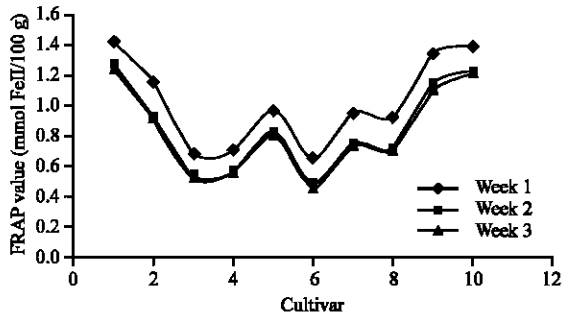

Fig. 3: FRAP value of ten different pomegranate water extract stored in refrigerator (4°C) for three successive weeks (The ANOVA test shows the significant difference between the first week with the second and third, $p<0.05$). [1]Sour alac; [2]Sweet white peel; [3]Agha Mohammad Ali; [4]North white peel; [5]Sour white peel; [6]Sweet malas; [7]Sour summer; [8]Sweet saveh malas; [9]Sweet alac; [10]Black peel

DISCUSSION

The within and between-run coefficient variation of method are shown in Table 2. Both pomegranate pulp and peel contains many different kinds of antioxidants, possibly including those not so well vcharacterized. Gil *et al.* (2000) identified several phenolic compounds from pomegranate juice, such as anthocyanins, punicalagins, ellagic acids and hydrolysable tannins. Noda *et al.* (2002) reported that three major anthocyanidins found in pomegranate juice were delphinidin, cyanidin and pelargonidin. Purification of each antioxidant for pomegranate peel and pulp is a time consuming process. From a practical point of view, a suitable method for determination of total antioxidant power can be used to assay antioxidant capacity in peel and pulp pomegranate fraction. The FRAP assay treats the antioxidants contained in the sample as reductants in a redox and links it to a colorimetric reaction. The value reflects the reducing power of the antioxidants. The procedure is relatively simple and easy to standardize. Thus, it has been used frequently in the assessment of antioxidant activity of various fruits and vegetables and some biological samples.

Pomegranate is a native plant in Iran, but there is no literature reporting it's antioxidant activity. In the present study, the FRAP value of peel and pulp fractions of ten cultivars of pomegranate was determined in an attempt to make a systematic comparison among their antioxidant activities and to identify the fractions with high antioxidant power for further studies. The average FRAP value of ten different pomegranates of Iran is less than other countries. We attempted to correlate the FRAP values obtained in this study with the data reported by Guo *et al.* (2003), Chidambara *et al.* (2002), Gil *et al.* (2000), Poyrazoglu *et al.* (2002), Noda *et al.* (2002), Halvorsen *et al.* (2002), Li *et al.* (2006) and Negi *et al.* (2003) and found no positive relationship between them. There are various methods for determination of antioxidant activity and it is not surprising that different methods would give different results because they are based on different principles as discussed above. Although Halverson *et al.* (2002) and Guo *et al.* (2003) used the same FRAP assay, discrepancies may still exist because the antioxidant activity could be influenced by the geographical origin, cultivar, harvest or storage time (Van der Sluis *et al.*, 2001).

There are many different antioxidants contained in fruits. Among them, vitamin C is the most unstable. Guo *et al.* (2003) calculated the contribution of vitamin C to the fruit pulp FRAP value varied among different cultivars, ranging from 107.75 to 2.43% and is 2.58% for white pomegranate. In this study, the FRAP value decreased about 10 to 30% over time which relates to loss of vitamin C and other unstable antioxidants. It is worth mentioning, that the remaining FRAP value of pomegranates is still high among the FRAP value of other fruits. There is no available stability indicating tests for comparison.

In conclusion, the antioxidant activities of peel and pulp fractions of ten pomegranate cultivar have been compared using the FRAP assay in this study. The most important finding was introducing the sour alac cultivar as a potent source of natural antioxidants for beverage industry and the peel of sweet white cultivar as a suitable origin for extraction and purification of natural antioxidants. To our knowledge, the study reported here is the most comprehensive comparison of the antioxidant activity among different pomegranate cultivars. Some pomegranate peel and pulp fractions have strong antioxidant activity and may be rich sources of antioxidants. 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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