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Hyperoside and Anthocyanin Content of Ten Different Pomegranate Cultivars

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Abstract: Pomegranate (*Punica granatum* L.) is native to the Mediterranean region and has been used extensively as a medicine in many counties. Hyperoside is known as an important flavonoid with antioxidant activity and anti hypertension effect. Anthocyanins are the active component in several herbal medicines, thus accurate measurement of hyperoside and anthocyanins, along with their degradation indices, is very useful to food technologists and horticulturists. The aim of the current study was to determine the antioxidant capacity as hyperoside and anthocyanin content of ten different Iranian pomegranate cultivars. Spectroscopic analyses of the pomegranate showed Black peel cultivar had the highest hyperoside content (25.93±2.87, 620.41±30.32 mg/100 g) in its pulp and peel, respectively. Based on this study, the amounts of anthocyanin in pulp ranged between 1.56±0.05 and 3.89±0.07 mg g^{-1} which related to Sweet white peel and Sweet alac cultivars, respectively. More over the highest and also the lowest peel anthocyanin contents related to these cultivars. The results revealed that the hyperoside and also anthocyanin peel content of each variety is higher than its pulp content. In addition the potency of black peel (Medicinal pomegranate) and Sweet alac cultivars for prevention of coronary heart disease and hypertension were presented.

Key words: *Punica granatum* L., hyperoside, anthocyanin, pulp, peel

INTRODUCTION

Punica granatum L., is one of the oldest known fruits. This nutrient dense, antioxidant rich fruit has been revered as a symbol of health, eternal life and fertility. It is one of the considerable horticultural plants of Iran which is cultivated in most of the regions throughout the country and grows well in arid and semiarid regions especially in Saveh and Yazd cities due to its adaptation to adverse ecological conditions.

Many studies have shown the use of pomegranate as antiviral (Neurath *et al*., 2005), antimicrobial (Braga *et al*., 2005), anticancer agent (Jeune *et al*., 2005; Malik *et al*., 2005) and its effect for prevention of coronary heart disease (Fuhrman *et al*., 2005; Sumner *et al*., 2005; Hajimahmoodi *et al* 2008a). There are some reports about the presence of tannins, alkaloids, glycosides, flavonoids and phenolic compounds as antioxidant factors in juice, peel, pulp and pomegranate seed fractions (Gil *et al*., 2000; Halvorsen *et al*., 2002; Murthy *et al*., 2002; Noda *et al*., 2002; Poyrazoglu *et al*., 2002; Guo *et al*., 2003; Negi *et al*., 2003; Li *et al*., 2006). It is an important source of anthocyanins, the 3-glucosides, 3, 5-diglucosides of delphinidin, cyanidin, pelargonidin, ellagitannins and gallotannins. It also contains 1 g L^{-1} citric acid and 7 mg L^{-1} ascorbic acid (Gil *et al*., 2000) which dramatically affect on various disorders including cardiovascular, neurological diseases and cancer (Halliwell, 1999).

On the other hand one of the agricultural wastes is pomegranate peel extract which is more potential than the pulp extract and can be regarded as a health supplement rich in natural antioxidants cause pomegranate peel performed significantly better than the pulp in flavonoids content. Also previous researches showed that pomegranate peel has anticancer and cardiovascular preventing properties, chemo-preventive and adjuvant therapeutic effect on breast cancer (Kim *et al*., 2002). More over high antioxidant content of pomegranate peel extract has a therapeutic effect on protecting the liver from fibrosis and oxidative injury due to biliary obstruction (Toklu *et al*., 2007).

One of the antioxidant which is known in this study is Hyperoside as an important flavonoid with anti hypertension effect (Benzie and Strain, 1996) it is present

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in many fruit and vegetables. It has been documented as having anti-inflammatory and diuretic properties (Ekenseair *et al*., 2006). Recently, interest in anthocyanin which is responsible for the color of the pomegranate has increased because of their benefits to human health as dietary antioxidants. These water-soluble pigments are intensely colored and are responsible for nearly all of the pink, scarlet, red, mauve, violet and blue colors found in the petals, leaves and fruits of higher plants (Khoshayand *et al*., 2012).

In the present study, the hyperoside and anthocyanin content in pulp and peel fractions of ten pomegranate cultivars were determined in an attempt to identify the major effective compounds for further studies. Although the effective compounds could be influenced by the geographical origin, cultivar, harvest or storage time (Van der Sluis *et al*., 2001), but we correlate the data obtained in this study with the data reported by the others.

Punica granatum L. could easily be used in pharmaceutical and food products. Also further studies are necessary to assess their potential components as effective natural remedies.

MATERIALS AND METHODS

All reagents were from Sigma-Aldrich Chemical Co. A UV visible Cintra 40 double-beam spectrophotometer equipped with a 1.0 cm path length cell which connected to an IBM compatible Pentium 100 computer was used.

Plant materials: *Punica granatum* L. was obtained from the Saveh Farmer Investigation Center in Markazi province, Iran (Sour alac; Sweet white peel; Agha Mohammad Ali; North white peel; Sour white peel; Sweet malas; Sour summer; Sweet saveh malas; Sweet alac and Black peel), they collected from different mature trees randomly selected to represent the total populate plantation. Voucher specimens were deposited in the Herbarium of the agricultural research centers of Saveh, Iran. Three individual 3 g samples of crushed dry pomegranates pulp and peel were used, respectively for hyperoside assay. The anthocyanins content were determined on pomegranate extract. To prepare pomegranate extract, fresh fruits were peeled and their edible portions (seed coats and juice) were separated. Thirty grams of pulps and peel were weighted and extracted separately for 4 h by Soxhlet apparatus with acetone, followed by ethyl acetate, methanol and water solvent, respectively (4 h 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. One g of extracts were dissolved and diluted with methanol 80% (V/V) to 25 mL for sample analysis.

Measurement of total flavonoid base on hyperoside content: To assay total flavonoid amount of pomegranate pulp and peel, hyperoside content was determined by spectrophotometry methods based on German Pharmacopoeia monograph (Van der Sluis *et al*., 2001). For preliminary extraction of flavonoids, three individual 3 g samples of each dry cultivar were separately heated in presence of HCl, methenamine and acetone. The subsequent flavonoid extracts were subjected to further purification using ethyl acetate and distilled water. $AlCl_3$ was added to the solutions and the absorbance of each mixture was determined based on hyperoside content using of UV-spectrophotometer. The percentage of total flavonoid was reported as (w/w) based on specific absorbance of hyperoside.

Total anthocyanin assay: Vanillin assay method (Sun *et al*., 1998) was used to determine anthocyanin content in the samples. Catechin is commonly used to standardize the vanillin reaction. One milliliter of samples and standard solutions of catechine (100, 150 200, 250, 300, 350 μg mL^{-1}) was added to 2.5 mL of vanillin (1%) and 2.5 mL of HCl (9 M). Then it was incubated in water bath for 30 min at 30°C and the absorbance was measured at 500 nm. The results were expressed in mg catechin equivalents to 1 g pomegranate extract.

Statistical analysis: All analyses were done in triplicate (n = 3). Results were reported as Mean±standard deviation. Statistical analysis was carried out using the software package SPSS v17.0 (SPSS Inc., Chicago, USA).

RESULTS AND DISCUSSION

Pomegranate is a native plant in Iran, but there is no literature reporting the hyperoside and anthocyanin content of mentioned cultivars and their relation with each other.

The results of the pulp flavonoid contents by hyperoside assay are shown in Table 1. All extracts contained a considerable amount of flavonoid metabolites from 1.77±0.31 mg of hyperoside/100 g pulp in Sour white peel to 25.93±2.87 in Black peel pomegranate while the ethanolic extract of pomegranate seeds previously revealed that Sour white peel and Black peel cultivars have the highest (3.88±1.31 μm) and lowest (1.62±0.47 μm) antioxidant activity, respectively (Sadeghi *et al*., 2009).

Table 1: Hyperoside and anthocyanin content of ten different pomegranate cultivars

	Hyperoside (mg/100 g)		Anthocyanin-Catechin (mg g^{-1})	
Cultivar	Pulp	Peel	Pulp	Peel
Sour alac	6.13±0.39	157.40±09	1.60±0.03	5.260±0.03
Sweet white peel	5.45±0.31	157.11±1.210	1.56±0.05	3.950±0.02
Agha Mohammad Ali	5.03±0.26	165.62±1.31	2.14±0.00	20.087±0.42
North white peel	2.53±0.26	66.13±2.0	1.93±0.06	26.601±0.07
Sour white peel	1.77±0.31	155.20±3.42	2.13±0.01	6.182±0.00
Sweet malas	5.24±0.49	157.70±1.91	3.17±0.09	7.602±0.00
Sour summer	11.63±1.42	45.33±2.30	2.38±0.00	16.545±0.01
Sweet saveh malas	2.25±0.15	136.00±3.81	1.70±0.05	14.901±0.05
Sweet alac	5.17±0.18	38.52±8.72	3.89±0.07	29.529±0.15
Black peel	25.93±2.87	620.41±30.32	1.65±0.03	17.038±0.28

The hyperoside in peels varied from 38.50±8.73 mg of hyperoside/100 g peel in Sweet alac to 620.41±30.32 in Black peel. The results showed that, the Black peel cultivar as a medicinal pomegranate had the most potential hyperoside content among 10 different cultivars. But there isn't any differences between other varieties consist of Sweet malas, Sour alac, Sweet white peel, Sour white peel. The result presented North white peel and Sour summer had the lowest content in both pulp and peel with significant difference ($p<0.001$) but the results of another study inversely showed that Sour summer pulp cultivar had the most total antioxidant activity with significant difference with the other cultivars (Ardekani *et al*., 2011). As indicated before, pomegranates total anthocyanin content strongly depends on the cultivars (Alighourchi *et al*., 2008; Turfan *et al*., 2011). In accordance with our results Sweet white peel cultivar with 1.563±0.05 mg of anthocyanin/g pulp and 3.93± 0.02 mg of anthocyanin g^{-1} peel had the lowest content of anthocyanin while Sweet alac with 3.89±0.07 mg of anthocyanin g^{-1} pulp and 29.52± 0.15 mg of anthocyanin g^{-1} peel showed the predominant amount of anthocyanin (Table 1). In preior pomegranate study Sweet alac had the major amount of total phenolic (19.93±0.42 mg GAE g^{-1} extract) after sour summer cultivar (Ardekani *et al*., 2011). More over hydro extract of Sweet alac had significant FRAP value in the past (Hajimahmoodi *et al*., 2008b).

Gil *et al.* (2000) studied Pomegranate juice introduced as an important source of anthocyanins, with hydrolysable tannins as the main antioxidant compounds but anthocyanins and ellagic acid derivatives also contribute to the total antioxidant capacity of the juice. Total anthocyanins which reported by Gil *et al.* (2000) are 306.0, 172.2, 387 and 4161.9 mg L^{-1} which related to Juice from fresh arils, juice from frozen arils, single-strength commercial juice and commercial juice from concentrate, respectively.

The fact that cyanidin-3, 5-diglucoside is the major anthocyanin in pomegranates revealed by Turfan *et al.* (2011). In mentioned research total anthocyanin contents obtained from HPLC and pH-differential methods were highly correlated with each other (Turfan *et al*., 2011).

Based on Alighourchi *et al.* (2008), the amount of total anthocyanins, in juice of some variety such as Mesri Torshe Kazeron, Jangali Pust Germeze Rodbare Torsh and Torshe Mamoli Lasjer were the highest more than 250 mg L^{-1} whilst this amount was lower than 25 mg L^{-1} in some others.

There wasn't any literature about pomegranate hyperoside to compare data with them but some herbal varieties are evaluated by the hyperoside method. The content of hyperoside in *L. fischeri* determined by HPLC was reported 0.38±0.00 mg g^{-1} (Piao *et al*., 2011). In *Abelmoschus manihot* (L.) the content of hyperoside decreased in the following order; flower, leaf, stem, root and seed (Chou *et al*., 2011).

The hyperoside contents in different parts of *Poacynum hendersonii* (Hook.f.) Woodson measured in the leaves was 0.24% higher while almost no hyperoside is found in the flowers, stems and roots. Similarly, the total flavonoid content in the leaves is also higher, about 3.54% while that is lower in the flowers, stems and roots (Shi *et al*., 2009).

Accordingly, the most important finding was introducing the Sweet alac and Black peel cultivar as a potent source of natural antioxidants for beverage industry and a suitable origin for extraction and purification of natural antioxidants. The experimental evidence revealed that the hyperoside and also anthocyanin peel content of each variety is higher than its pulp content. This would explain that peel of pomegranate has the high antioxidant capacity. This achievement is in good agreement with other study.

The latest results showed that the hyperoside content in the leaves of *Poacynum hendersonii* which used indiscriminately in some areas of China is higher, about 0.24% while almost no hyperoside is found in its flowers, stems and roots. The total flavonoid content in the leaves is also higher, about 3.54% while that is again

lower in its flowers, stems and roots similarly (Shi *et al*., 2009). Furthermore flavonoids determination content of strawberry leaves and fruits indicated that the leaves are richer in flavonoids (0.52-2.00%) than fruits (0.10-0.29%) (Males *et al*., 2006).

The relationship between hyperoside and anthocyanin content of each cultivar are illustrated in Fig. 1 and 2. Anthocyanin contents show more variations as compared with contents of hyperoside in both pulps and peels. It is worth to say that when Sweet alac peel have maximum amount of hyperoside the related anthocyanin is in the lowest amount (Fig. 2). Kim *et al*. (2002) claimed when hydroxyl substituents present on the B-ring for anthocyanidins increase the antioxidant activity increases directly whereas the converse was observed for catechins (Seeram and Nair, 2002).

The outcome of the assessment of some antioxidant studies described the relation between different antioxidants as an instance. Relationship between anthocyanin and trolox equivalent antioxidant capacity and ferric reducing antioxidant power content of *C. monogyna callus* extracts showed correlation coefficients of 0.74 and 0.63, respectively (Toklu *et al*., 2007). 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 effective antioxidants contained in these fruit fractions and mechanisms by which they protect against disease development are highly recommended.

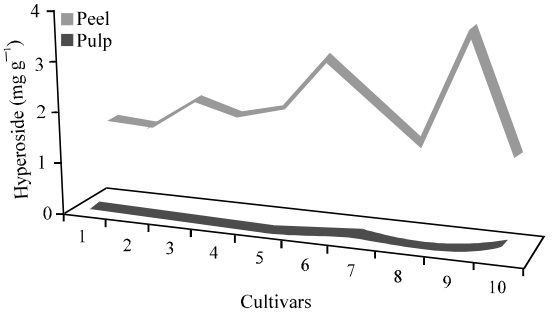

Fig. 1: Comparison between Hyperoside content of different pomegranate peels and pulps

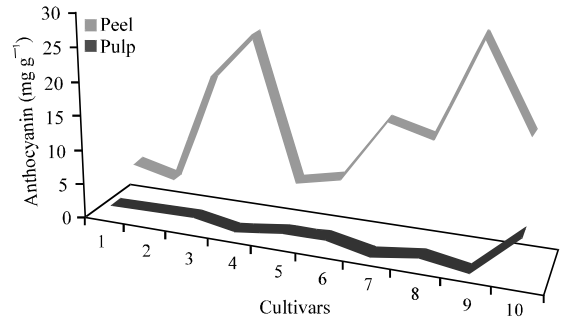

Fig. 2: Comparison between Anthocyanin content of different pomegranate peels and pulps

CONCLUSION

Taken together, the results can provide an extra income and may contribute to have good nutritional values of this product. Current study shows that the studied pomegranate cultivars are one of the most powerful, nutrient dense foods for overall good health. The pomegranate cultivars used in this study are commercially important for Iranian production. The peel extracts contain a considerable amount of hyperoside and anthocyanin, than the pulp. It is worthy to note that the antioxidant capacity of pomegranate peel extract is higher than the pulp extract. Also a large quantity of pomegranate peel could be easily collected from the pomegranate processing industries or from the waste products originating.

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