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Antioxidant Activity of Peel Fraction of Seven Pomegranate (Ripe and Unripe) Varieties Grown in Iran

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Antioxidant capacity and total phenolic compounds of peel fraction of seven pomegranate varieties grown in Iran were determined. The effect of maturation on antioxidant activity was also determined. The results showed that Pust Ghermeze Shirin (sweet taste with red peel) had the highest antioxidant value among all varieties and followed by Pust Sefeede Shirin (sweet taste with white peel), Yazdi (sub acid taste with pink peel), Pust Ghermeze Torsh (sour taste with red peel), Pust Sefeede Torsh (sour taste with white peel), Malas (sub acid taste) and Makhmali (sub acid taste with velvet peel). Maturation of pomegranate increased the antioxidant capacity.

Key Words: Pomegranate, Peel, Antioxidant activity, Ripe, Unripe.

INTRODUCTION

Antioxidant is defined as any substance that when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate[1]. Antioxidants have the ability to hunt down superoxide, hydroxyl and peroxyl radicals, which can be harmful to the body. An intake of antioxidants has shown a decrease in the oxidation of LDLs (low-density lipoprotein) as well as to support and improve tissue recovery. Flavanoid antioxidants have the ability to reduce the oxidative damage of DNA. Antioxidants can be found in different plant sources. Pomegranate is an important source of bioactive compounds and has been used for folk medicine for many centuries. Pomegranate likely originated in Iran and Afghanistan and is currently grown mainly in Iran, India and the United States. This fruit is now gaining importance because of its potent antioxidant activity. Pomegranate fruit juice, fruit and peel extracts have been found to possess a tremendous antioxidant activity[2-5]. All these activities may be related to diverse phenolic compounds present in pomegranate, including punicalagin isomers, ellagic acid derivatives and anthocyanins (delphinidin, cyanidin and pelargonidin 3-glucosides and 3,5-diglucosides). These compounds are known for their properties in scavenging

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free radicals and inhibiting lipid oxidation *in vitro*[2,3]. Pomegranate peel also contains substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid. It has been used in the preparation of tinctures, cosmetics, therapeutic formula and food recipies. Singh *et al.*[5] recently reported that methanol extract of pomegranate peel had much higher antioxidant capacity than that of seeds, as demonstrated by using the β-carotene-linoleate and DPPH model. However, no studies have so far been reported on effect of maturation and varieties on antioxidant activity of pomegranate extracts. The objective of the present study is to determine the variety and maturation effects of pomegranate peel extracts on antioxidant activity.

EXPERIMENTAL

The samples were obtained from ripe and unripe fruits growing in Isfahan province (seven varieties) in Iran. Commercially fresh fruits were harvested during September and November from different trees randomly selected the population of the plantation. The sweet varieties represent Pust Sefeede Shirin, Pust Ghermeze Shirin. The sub acid varieties are: Malas, Yazdi and Makhmali. The sour varieties are: Pust Sefeede Torsh, Pust Ghermeze Torsh.

All solvents/chemicals used were of analytical grade and obtained from Merck.

Preparation of pomegranate peel extracts: All the samples were first flushed by tap water and dried before the peel, pulp and seed fractions were carefully separated. The peels were manually removed, sun-dried and powdered. Powder was extracted with a Soxhlet extractor using EtOAc, acetone, MeOH and water for 6 h each. The extract was filtered through Whatman No. 41 filter paper for removal of peel particles and concentrated[6] under vacuum at 60 °C.

Total phenolics: Total phenolics in the alcoholic extract of pomegranate were determined by the method of Taga *et al.*[7]. One hundred milligrams of the extract was extracted with 250 mL of methanol/water (60:40, v/v, 0.3 % HCl) and filtered through a 0.45 mL Millipore filter. To 100 µL filtrate, 100 µL of Folin-Ciocalteu reagent (50 %, v/v) and 2.0 mL sodium carbonate (2 %, w/v) were added and mixed completely. After 2 h, the absorbance of the solution was measured at 750 nm. Quantification was based on the standard curve of gallic acid (0-1.0 mg mL^{-1}), dissolved in methanol/water (60:40, v/v; 0.3 % HCl). Phenolic content was expressed as mg/L of gallic acid equivalent (GAE).

Evaluation of antioxidant capacity by phosphomolybdenum method: The total antioxidant capacity of pomegranate peel extracts was evaluated by the method of Prieto *et al.*[8]. An aliquot of 0.1 mL of sample solution (25, 50, 75 and 100 mg mL^{-1}) was combined with 1 mL of reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). For the blank, 0.1 mL of methanol was used in place of sample. The tubes were capped and incubated in a boiling water bath at 95 °C for 1.5 h. After the samples had cooled to room temperature, the absorbance of the aqueous solution of each was measured at 695 nm against a blank in a Genesys-5-UV-Visible spectrophotometer (Apple, New York,

USA). For samples of unknown composition, water-soluble antioxidant capacity was expressed as equivalents of ascorbic acid (mg L^{-1} of extract).

Statistical analysis: The statistical examination of the data was performed using SAS software package.

RESULTS AND DISCUSSION

In this study, the antioxidants from pomegranate peel were extracted using a mixture of ethyl acetate, acetone, methanol and water and the antioxidant properties of the extract were further investigated in seven varieties of ripe and unripe pomegranate. The concentrated extracts were used to determine their antioxidant capacities by the formation of phosphomolybdenum complexes. This method is based on the reduction of Mo(VI) to Mo(V) by the antioxidant compounds and the formation of a green Mo(V) complex with a maximal absorption at 695 nm. The different pomegranate peel extracts exhibited various degrees of antioxidant capacities (Fig. 1) (antioxidant capacities expressed as ascorbic acid equivalent on the basis of 5 g dry weight/mmol g^{-1} of extract).

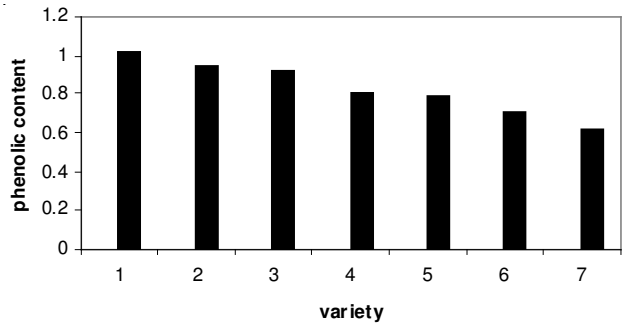

Fig. 1. Comparison of phenolic content of peel extract in different varieties. (1) Pust Sefeede Shirin; (2) Pust Ghermeze Shirin; (3) Yazdi; (4) Pust Ghermeze Torsh; (5) Pust Sefeede Torsh; (6) Malas; (7) Makhmali. Data were analyzed by one way analysis of variance, *p < 0.01

Among all varieties tested, the Pust Sefeede Shirin had the highest total phenolics value among all varieties and followed by Pust Ghermeze Shirin, Yazdi, Pust Ghermeze Torsh, Pust Sefeede Torsh, Malas and Makhmali. Total phenolic values shown by the pomegranate extracts maybe due to the presence of polyphenols, such as ellagic tannins, ellagic acid and gallic acid[2,6].

The effect of ripeness on total phenolic values of pomegranate was evaluated. Table-1 showed with ripeness of pomegranate, amount of phenolic compounds increased. Pust Sefeede Shirin and Pust Ghermeze Shirin had higher antioxidant values than Pust Sefeede Torsh and Pust Ghermeze Torsh so with ripeness of pomegranate, antioxidant capacity increased. Kulkarni and Aradhya[9] also showed an increase in antioxidant activity in the late-developmental stage.

TABLE-1
EFFECT OF RIPENING ON PHENOLIC CONTENT OF VARIOUS POMEGRANATE PEEL EXTRACTS AS GALLIC ACID EQUIVALENTS (mg L^{-1} OF EXTRACT)*

Variety	Unripe	Ripe
Pust Sefeede Shirin	0.9060 ± 0.0005	1.134 ± 0.0010
Pust Ghermeze Shirin	0.8010 ± 0.0005	1.083 ± 0.0010
Yazdi	0.7600 ± 0.0005	1.080 ± 0.0005
Pust Ghermeze Torsh	0.7101 ± 0.0005	0.887 ± 0.0010
Pust Sefeede Torsh	0.7150 ± 0.0010	0.866 ± 0.0005
Malas	0.6570 ± 0.0010	0.748 ± 0.0010
Makhmali	0.5790 ± 0.0010	0.653 ± 0.0005

*Values expressed are means ± SD of three experiments, $p < 0.01$.

As the results of the evaluation of antioxidant capacity show there was significant correlation between antioxidant activity and the contents of phenolic compounds of the samples (Table-2). The antioxidant activities of the analyzed extracts can be attributed to the result of the action of different phenolic compounds present in the pomegranates.

TABLE-2
EFFECT OF RIPENING ON ANTIOXIDANT CAPACITY OF VARIOUS POMEGRANATE PEEL EXTRACTS AS ASCORBIC ACID EQUIVALENTS (mg L^{-1} OF EXTRACT)*

Variety	Unripe	Ripe
Pust Sefeede Shirin	0.280 ± 0.0010	0.147 ± 0.0020
Pust Ghermeze Shirin	0.221 ± 0.0020	0.345 ± 0.0020
Yazdi	0.212 ± 0.0006	0.319 ± 0.0010
Pust Sefeede Torsh	0.173 ± 0.0006	0.182 ± 0.0006
Pust Ghermeze Torsh	0.153 ± 0.0020	0.321 ± 0.0010
Malas	0.119 ± 0.0010	0.121 ± 0.0020
Makhmali	0.104 ± 0.0010	0.114 ± 0.0030

*Values expressed are means ± SD of three experiments, $p < 0.01$.

The contents of total phenolics were also measured. The results showed that ripe pomegranate peel extract had markedly higher antioxidant capacity than the unripe peel extract. The contents of total phenolics were also higher in ripe peel extract than in unripe extract.

Conclusion

The high antioxidant activity of the peel extract appeared to be attributed to its high phenolics content as the evaluation of total phenolic compounds showed in this study. Intervarietal differences in antioxidant properties were also found. The lowest and highest were Makhmali and Pust Sefeede Shirin, respectively. Finally, it is found that ripeness of pomegranate can increase antioxidant activity and also sweet varieties had higher activity than other samples.

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