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Peel Extracts of Two Iranian Cultivars of Pomegranate (*Punica granatum*) have Antioxidant and Antimutagenic Activities

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Abstract: Peels of two Iranian pomegranate cultivars were dried, powdered and extracted in a Soxhlet extractor with methanol, ethanol, acetone and water for 4 h each. Extracts were pooled together and dried. The dried extracts were used to determine their antioxidant capacity by the formation of phosphomolybdenum complex and antimutagenicity against the mutagenicity of sodium azide by the Ames test. The peel extracts of both cultivars exhibited marked antioxidant capacity, but at each concentration, this capacity was higher in Aghamohammadali cultivar than that of Malas-Saveh. Peel extracts of both cultivars decreased sodium azide mutagenicity in *Salmonella typhimurium* strains (TA100 and TA1535) strongly, except for Malas-Saveh cultivar extract at 625 mg/plate concentration that decreased sodium azide mutagenicity moderately. The peel extracts of both cultivars showed an increase in total phenolics, antioxidant capacity and antimutagenicity with increase in concentration. The overall results showed that the pomegranate peel extracts have both antioxidant and antimutagenic properties and may be exploited as biopreservative in food applications and neutraceutical, specially when the extracts be obtained by use of a mixture of solvents.

Key words: Pomegranate, antioxidant capacity, antimutagenicity, Ames test, *Salmonella typhimurium*, anticarcinogens, polyphenols

INTRODUCTION

Many mutagens and carcinogens may act through the generation of free radicals such as reactive oxygen species (ROS). The generation of free radicals is associated with environmental pollution, UV radiation and several normal metabolic processes (Adegoke *et al*., 1998; Mccord, 1994). The role of free radicals in various human diseases is becoming increasingly recognized (Martinez-Cayuela, 1995; Ames *et al*., 1993; Halliwell *et al*., 1992) Free radicals attack the unsaturated fatty acids of biomembranes which results in lipid peroxidation and destruction of proteins and DNA, that may be related to cancer, heart disease and aging (Ames, 1983).

The consumption of antioxidants, play a vital role in protecting against free radicals, specially ROS. An antioxidant is a substance that when present at low concentration compared to that of an oxidisable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1990). Antioxidants play their role by scavenging free radicals. Many antioxidants also are being identified as anticarcinogens (Ames, 1983). The possible toxicity as well as general consumer rejection, has lead to decreased in the use of synthetic antioxidants (Namiki, 1990). Therefore, research in this area has focused on the detection of antioxidants in food. Fruits and vegetables have received particular attention because they contain high amounts of known antioxidants such as polyphenols, vitamin C, Vitamin E, β-carotene and lycopene.

Pomegranate (*Punica granatum* L., Punicaceae) has been known to considerable pharmacological properties with antimicrobial, antiviral, anticancer, potent antioxidant and antimutagenic effects (Seeram *et al*., 2005; Negi *et al*., 2003) and been used in the markets in the preparation of tinctures, juice, cosmetics and therapeutic formulae (Kim *et al*., 2002). Because pomegranate peel is a rich source of antioxidants, specially polyphenols, such as ellagic acid, querecetin and punicalagin (Li *et al*., 2006; Poyrazoglu *et al*., 2002; Gil *et al*., 2000) and many plant polyphenols as well as their dietary sources, have been shown to act as potent antimutagenic and anticarcinogenic agents (Bu-Abbas *et al*., 1994; Tanaka *et al*., 1993; Ayrton *et al*., 1992), we have investigated the antioxidant capacity and antimutagenic effects of pomegranate peel, a byproduct of juice and to exploit its potent as a natural preservative and nutraceutical.

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MATERIALS AND METHODS

Pomegranates (*Punica granatum* Malas-Saveh and Aghamohammadali cultivars) were obtained from the agricultural research center of Saveh (Markazi province) in Iran. Professor B.N.Ames (University of Berkeley, California, USA) Kindly supplied *Salmonella typhimurium* strains, TA100 and TA1535.

The peels of pomegranate fruits were manually removed, sun-dried and powdered. Powder was extracted with a Soxhlet extractor using methanol, ethanol, acetone and water for 4 h each (Negi *et al*., 2003). Extracts were filtered through Whatman No.41 filter paper for removal of peel particles. All extracts were pooled together and concentrated under vacuum at 60°C and the concentrate was powdered (Li *et al*., 2006). It was dissolved in methanol: Water (6:4 v/v) (1 mg mL^{-1}) for evaluation of antioxidant capacity and in propylene glycol (25 mg mL^{-1}) for antimutagenic activity (Negi *et al*., 2003).

The content of phenolic compounds in the extracts was determined according to the method of Jayaprakasha *et al*. (2001).The extracts were dissolved in water. Aliquots of 0.5 mL samples were mixed with 2.5 mL of 10-fold-diluted Folin-Ciocalteu reagent and 2 mL of 75% sodium carbonate. The mixture was allowed to stand for 30 min at room temperature before the absorbance was measured at 760 nm. The final results were expressed as tannic acid equivalents ±SD.

The total antioxidant capacity of pomegranate peel extracts was evaluated by the method of Prieto *et al*. (1999). An aliquot of 0.1 mL of sample solution (25, 50, 75 and 100 μg 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 90 min. After the samples had cooled to room temperature the absorbance of the aqueous solution of each was measured at 695 nm against a blank. Antioxidant capacity was expressed as equivalents of ascorbic acid (μmol g^{-1} of extract) (Negi *et al*., 2003).

In the antimutagenicity test, the inhibition of mutagenic activity of the sodium azide by the test samples was determined. The test samples (625, 1250 and 2500 μm/plate) were assayed by plating with molten top agar (2 mL) containing 24 h-old culture (0.1 mL) of strains of *Salmonella typhimurium* (TA100 and TA1535). Positive and negative controls were also included in each assay. Sodium azide was used as a diagnostic mutagen (1.5 μg per plate) in the positive control and plates without sodium azide and without test samples were considered as negative controls (Negi *et al*., 2003). In all plates 0.5 mL of metabolic activating system (S9) were added. S9 fractions were prepared from livers of male Wistar rats (weighing 200-250 g).

His^+ revertants were counted after incubation of the plates at 37°C for 48 h. Each sample was assayed using duplicate plates and the data presented as mean±SD of three independent assays. The mutagenicity of sodium azide in the absence of test samples was defined as 100% or 0% inhibition. The calculation of % inhibition was done according to the formula given by Ong *et al*. (1986): % inhibition = [1-T/M] × 100 where T is the number of revertants per plate in the presence of mutagen and the test sample and M is the number of revertants per plate in the positive control. The number of spontaneous revertants was subtracted from the numerator and the denominator.

The antimutagenic effects were considered moderate when the inhibitory effects were 25-40% and strong when more than 40% (Ikken *et al*., 1999).

RESULTS

The phenolic contents of peel extracts for Malas-Saveh and Aghamohammadali cultivars were found to be 232.8± 15.1 and 251.3 ±16.7 mg g^{-1}, respectively, which were comparable to reported values (Li *et al*., 2006).

The dried mixture 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 (Negi *et al*., 2003). Both extracts showed an increase in antioxidant capacity with increase in dose (Table 1).

Mutagenic assays, such as the Ames test have been widely used to assess the antimutagenic and anticarcinogenic activities of various compound (Ikken *et al*., 1999).

The antimutagenic activity of pomegranate peel extracts against sodium azide was evaluated by means of the Ames test, using two strains of *S. typhimurium* i.e., TA-100 and TA-1535. Both the pomegranate peel extracts at all concentration inhibited the mutagenicity of sodium azide in both strains of *Salmonella* strongly except for

Table 1: Antioxidant capacity of pomegranate peel extracts as ascorbic acid equivalent (μmol g^{-1} of extract).[a]

Concentration (μg mL^{-1})	Malas-Saveh peel extract	Aghamohammadali peel extract
25	903±141	1002±94.6
50	1510±72.3	1713±81.1
75	2019±85.1	2201±80.4
100	2743±98.3	2887±87.2

[a]Values expressed are mean ±SD of three experiments

Table 2: Inhibitory effect of pomegranate peel extracts against the mutagenicity of sodium azide to *Salmonella typhimurium* TA100

Concentration of extracts µg/plate	Malas-Saveh peel extract	Aghamohammadali peel extract
625	37.1±8.7	41.8±7.9
1250	65.8±9.6	70.7±6.8
2500	86.3±9.4	89.2±7.3

Data expressed are % inhibition ±SD

Table 3: Inhibitory effect of pomegranate peel extracts against the mutagenicity of sodium azide to *Salmonella typhimurium* TA1535

Concentration of extracts µg/plate	Malas-Saveh peel extract	Aghamohammadali peel extract
625	39.3±9.5	54.0±10.6
1250	69.7±7.7	72.3±8.9
2500	87.1±10.2	91.4±7.0

Data expressed are % inhibition ±SD

Malas-Saveh extract at 625 µg/plate. All the extracts showed an increase in inhibition of mutagenicity with increase in dose (Table 2 and 3).

DISCUSSION

Negi *et al.* (2003) extracted antioxidant from pomegranate peel with the use of methanol, acetone, ethylacetate and water and found that at 25 and 100 µg mL^{-1} concentrations the methanol extract showed strong antioxidant capacity, whereas acetone and ethylacetate extract showed strong antioxidant capacity at 50 and 75 µg mL^{-1} concentrations respectively.

Li *et al.* (2006) found that the pomegranate peel extract obtained by use of a mixture, composed of methanol, ethanol and water, was significantly higher in FRAP value (a value for showing antioxidant efficiency) than those obtained using individual solvent, namely using methanol, ethanol or acetone. They considered that a combination of different solvents may be more efficient for extracting antioxidants because antioxidants may differ in their solubility in different solvents. For the same reason, in present study, the peel extracts obtained according to Li *et al.* (2006). Our results (Table 1) compare with the results of Negi *et al.* (2003), also showed that the mixture of different solvents is more powerful in recovering antioxidants than are individual solvents.

Negi *et al.* (2003) also evaluated the inhibitory effect of pomegranate peel extracts against the mutagenicity of sodium azide to *S.typhimurium* TA100 and TA1535. The results of present research (Table 2 and 3) in comparison with those of Negi *et al.* (2003), indicates that at all concentrations, extracts obtained from mixture of different solvents exhibit higher antimutagenic effect than those obtained from individual solvents, but the differences are not significant.

It has been observed that many plant polyphenols such as ellagic acid, catechins and chlorogenic, caffeic and ferulic acids act as potent antimutagenic and anticarcinogenic agents (Bu-Abbas *et al.*, 1994; Ayrton *et al.*, 1992).

Nasr *et al.* (1996) have reported that pomegranate peel contains ellagic acid, ellagitannins and gallic acids. The presence of these polyphenols in the pomegranate peel may be responsible for antimutagenicity of peel extracts (Gil *et al.*, 2000).

Active oxygen and free radicals are related to various physiological and pathological events, such as inflammation, immunization, aging, mutagenicity and carcinogenicity (Namiki, 1990). Kim *et al.* (1991) and Ueno *et al.* (1991) indicated that active oxygen scavengers reduce mutation induced by various mutagens. It has been suggested that compounds which posses antioxidant activity can inhibit mutation and cancer because they can scavenge a free radical or induce antioxidant enzymes (Hochstein and Atallah, 1988).

In present study we found a direct correlation between the antioxidant capacity and antimutagenic activity of peel extracts of both cultivars (Table 1,2 and 3). Our results also showed that the antioxidant activity and antimutagenic effect of pomegranate peel extract from Aghamohammadali cultivar were higher than those of Malas-Saveh cultivar. This may be due to variation in the quality and quantity of polyphenols and other bioactive compounds present in different cultivars.

The overall results showed that the pomegranate peel extracts have both antioxidant and antimutagenic properties and may be exploited as biopreservatives in food applications and neutraceuticals and also the mixture of different solvents is more powerful in recovering antioxidants than are individual solvents but in the case of antimutagenicity the difference between mentioned extracts is not significant.

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