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Food Chemistry 80 (2003) 393–397

Food Chemistry

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Antioxidant and antimutagenic activities of pomegranate peel extracts

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Received 22 January 2002; received in revised form 17 June 2002; accepted 17 June 2002

Abstract

Dried pomegranate peels were powdered and extracted in a Soxhlet extractor with ethyl acetate (EtOAc), acetone, methanol and water for 4 h each. 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. All the peel extracts exhibited marked antioxidant capacity, but the water extract was the lowest. The order of antioxidant capacity varied because of differential responses at four concentrations (25, 50, 75 and 100 μg/ml) in each solvent. All the extracts decreased sodium azide mutagènicity in *Salmonella typhimurium* strains (TA100 and TA1535), either weakly or strongly. At 2500 μg/plate all the extracts showed strong antimutagenicity. The antimutagenicity of the water extract was followed by acetone, EtOAc and methanol extracts. 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.
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Keywords: Pomegranate peel extracts; Antioxidant capacity; Antimutagenicity

1. Introduction

Dietary habits are important in human health. The human diet contains a great variety of natural antimutagens and/or anticarcinogens, such as fibres, polyphenolic compounds, flavonoids, isoflavones, tocoferols, ascorbic acid etc. (Ames, 1983; Stavric, 1994). Many mutagens and carcinogens may act through the generation of reactive oxygen species (ROS). The generation of ROS is associated with environmental pollution, UV radiation and several normal metabolic processes. The role of ROS in various human diseases is becoming increasingly recognised (Ames, Shigenaga, & Hagen, 1993; Halliwell, Gutteridge, & Cross, 1992; Martinez-Cayuela, 1995). ROS may also play a major role as endogenous initiators of degenerative processes, such as DNA damage and mutation (and promotion), that may be related to cancer, heart disease and aging (Ames, 1983). Besides the endogenous defences, the consumption of dietary antioxidants, such as tocoferol, ascorbic acid, carotenoids, phenolic compounds, play a vital role in protecting against ROS (Hodnick, Kung, Roetger, Bohmont, & Pardini, 1986; Niki, 1991; Rimm et al., 1993; Willet, 1994). Dietary intake of natural antioxidants could be an important factor in the body's defence mechanism against these agents and, also, many antioxidants are being identified as anticarcinogens (Ames, 1983). Low dietary intake of fruits and vegetables doubles the risk of most types of cancer as compared to high intake (Ames et al., 1993).

Many plant polyphenols, such as ellagic acid, catechins, chlorogenic, caffeic and ferulic acids, as well as their dietary sources, such as tea, have been shown to act as potent antimutagenic and anticarcinogenic agents (Ayrton, Lewis, Walker, & Ioannides, 1992; Bu-Abbas, Clifford, Walker, & Ioannides, 1994; Tanaka et al., 1993; Yen & Chen, 1994). Various tea extracts have been reported to be both antioxidant and antimutagenic (Yen & Chen, 1995).

Pomegranate peel 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 formulae and food recipes (Nasr, Ayed, & Metche, 1996). The presence of antioxidants has

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0308-8146/03/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0308-8146(02)00279-0

been reported in pomegranate juice (Gil, Tomas-Barberan, Hess Pierce, Holcroft, & Kader, 2000). Aviram et al. (2000) reported that pomegranate juice has potent anti-atherogenic effects in healthy humans and atherosclerotic effects in mice that may be attributable to its antioxidative properties. The objective of the present study was to determine antioxidant capacity and antimutagenic effects of pomegranate peel, a byproduct of juice, and to exploit its potential as a natural preservative and nutraceutical.

2. Materials and methods

2.1. Materials

All solvents/chemicals used were of analytical grade and obtained from Merck, Mumbai, India. Professor B. N. Ames (University of Berkeley, California, USA) kindly supplied *Salmonella typhimurium* strains, TA-100 and TA-1535.

2.2. Preparation of pomegranate peel extracts

Pomegranates (*Punica granatum,* Ganesha variety) were obtained from local markets. The peels were manually removed, sun-dried and powdered. Powder was extracted with a Soxhlet extractor using EtOAc, acetone, MeOH and water for 4 h each. The extract was filtered through Whatman No. 41 filter paper for removal of peel particles and concentrated under vacuum at 40 °C (Singh, Jayaprakasha, & Sakariah, 2001). It was dissolved in methanol:water (6:4 v/v) (1 mg/ml) for evaluation of antioxidant capacity and in propylene glycol (25 mg/ml) for antimutagenic activity.

2.3. Determination of total phenolics

The concentration of phenolics in the extracts was determined by the method of (Singh, Murthy & Jayaprakasha, 2002) and results were expressed as (+) catechin equivalents. Five milligrams of each dried pomegranate peel extract was dissolved in a 10-ml mixture of acetone and water (6:4 v/v). Samples (0.2 ml) were mixed with 1.0 ml of 10-fold diluted Folin–Ciocalteu reagent and 0.8 ml of 7.5% sodium carbonate solution. After standing for 30 min at room temperature, the absorbance was measured at 765 nm using a Genesys-5 UV-visible spectrophotometer (Milton Roy, NY, USA). The estimation of phenolic compounds in the extracts was carried out in triplicate.

2.4. Evaluation of antioxidant capacity by phosphomolybdenum method

The total antioxidant capacity of pomegranate peel extracts was evaluated by the method of Prieto, Pineda, and Aguilar (1999). An aliquot of 0.1 ml of sample solution (25, 50, 75 and 100 μg/ml) 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 in a Genesys-5-UV-Visible spectrophotometer (Milton Roy, New York, USA). For samples of unknown composition, water-soluble antioxidant capacity was expressed as equivalents of ascorbic acid (μmol/g of extract)

2.5. Antimutagenicity assay

The standard plate incorporation test was carried out according to Maron and Ames (1983). In the antimutagenicity test, the inhibitions of mutagenic activity of the sodium azide by the test samples were determined. The test samples (625, 1250 and 2500 μg/plate) were assayed by plating with molten soft agar (2 ml) containing 10 h-old culture (0.1 ml) of strains of S. *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. 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$\pm$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, Wong, Stewart, and Brockman (1986); % inhibition$=[1-T/M] \times 100$ 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 effect was considered moderate when the inhibitory effect was 25–40% and strong when more than 40%. Inhibitory effects of less than 25% were considered as weak and was not recognised as a positive result (Ikken et al., 1999).

3. Results and discussion

The phenolic contents of EtOAc, acetone, MeOH and water extracts were found to be 16.5, 52, 46.2 and 4.8%, respectively, which were comparable to reported values (Singh, Murthy, & Jayaprakasha, 2002). The dried extracts were used to determine their antioxidant capacities by the formation of phosphomolybdenum complexes. This

Table 1
Antioxidant capacity of pomegranate peel extracts as ascorbic acid equivalents (μmol/g of extract)[a]

Concentration (μg/ml)	Ethyl acetate extract	Acetone extract	Methanol extract	Water extract
25	537.±49.7	648.±94.8	708±135	476±84.4
50	1331±54.0	1392±69.0	1298±40.0	663±56.3
75	1935.±99.4	1778±233	1749.±128	903±16.5
100	2396.±47.2	2173±79.8	2457±56.3	956±12.7

[a] Values expressed are mean±S.D. of three experiments.

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 capacity (Table 1). It is difficult to assign an order of antioxidant capacity to the extracts because of the differential responses at four concentrations. At 25 and 100 μg/ml concentration, the MeOH extract showed strong antioxidant capacities, whereas acetone and EtOAc extracts showed strong antioxidant capacities at 50 and 75 μg/ml concentrations, respectively. The water extract showed less antioxidant capacity at all the concentrations compared to other extracts. Variations in antioxidant capacity of methanol and water extracts may be attributed to differences in their phenolic contents. All the extracts showed an increase in antioxidant capacity with increase in dose, except the water extract, which showed almost the same antioxidant capacity at 75 and 100 μg/ml concentrations. A similar trend has been reported in the case of olive extracts (McDonald, Prenzler, Antolovich, & Robards, 2001). The antioxidant activity shown by the pomegranate extracts may be due to the presence of polyphenols, such as ellagic tannins, ellagic acid and gallic acid (Gil et al., 2000).

Mutagenic assays, such as the Ames test, have been widely used to assess the antimutagenic and anticarcinogenic activities of various compounds (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. All the pomegranate

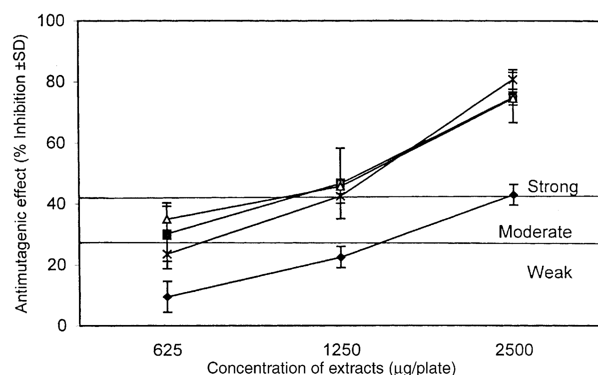

Fig. 1. Inhibitory effect of pomegranate peel extracts against the mutagenicity of sodium azide to *Salmonella typhimurium* TA 100. –◆– Methanol extract, –■– EtOAc extract, –△– acetone extract, –×– water extract.

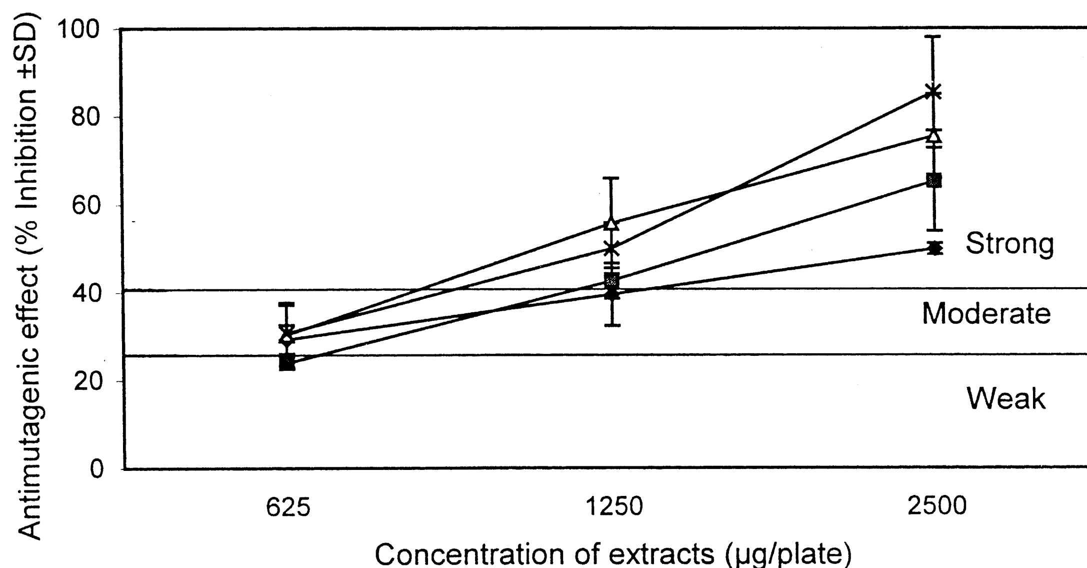

Fig. 2. Inhibitory effect of pomegranate peel extracts aganist the mutagenicity of sodium azide to *Salmonella typhimurium* TA 1535. –◆– Methanol extract, –■– EtOAc extract, –△– acetone extract, –×– water extract.

peel extracts inhibited the mutagenicity of sodium azide in both strains of *Salmonella,* and inhibition ranged from weak to strong, depending on the concentration of extract per plate (Figs. 1 and 2). In the case of TA-100, a strong inhibitory effect was shown by all extracts at or above 1250 μg/plate, except the methanol extract which had a strong inhibitory effect at 2500 μg/plate only. The methanol extract showed the least inhibition and it showed weak inhibitory effects at 625 and 1250 μg/plate. In the case of TA-1535, all the extracts showed moderate inhibition at 625 μg/plate except the EtOAc extract, which had a weak effect and strong inhibition at 1250 μg/plate except the methanol extract, which showed moderate inhibition. However, all the extracts showed strong antimutagenicity at 2500 μg/plate in both the strains of *Salmonella*. The antimutagenicity of the water extract was followed by acetone, EtOAc and methanol extracts. Ikken et al. (1998) observed that carrot aqueous extract exhibited a strong antimutagenic effect against *N*-nitrosodibutylamine, and moderate effects against *N*-nitrosodimethylamine and *N*-nitrosopiperidine. But ethanol extract of carrot showed slight antimutagenic activity against *N*-nitrosamine. The data may suggest the presence of variable antimutagenic factors in water and ethanol extracts (Ikken et al., 1999).

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 (Ayrton et al., 1992; Bu-Abbas et al., 1993). 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, Kim, Yeum, and Park (1991) and Ueno, Nakmuro, Sayato, and Okada (1991) indicated that active oxygen scavengers reduce mutation induced by various mutagens. It has been suggested that compounds which possess antioxidant activity can inhibit mutation and cancer because they can scavenge a free radical or induce antioxidant enzymes (Hochstein & Atallah, 1988).

Water extract showed low antioxidant activity but very strong antimutagenic activity. Similarly, methanolic extracts had high antioxidant activity but weak antimutagenic activity. Thus, in our study we could not find a direct correlation between the antioxidant capacity and antimutagenic activity of various peel extracts, even though the antioxidants are probable antimutagens. This may be due to variation in the quality and quantity of polyphenols and other bioactive compounds present in different extracts. Further work is required for the isolation and characterization of individual phenolic compounds present in various extracts and to determine the mechanisms involved in the antioxidant capacity and antimutagenic effects of pomegranate peel extracts.

Acknowledgements

We wish to thank Professor B. N. Ames, University of Berkely, California, for supplying *Salmonella* strains, and Dr. V. Prakash, Director and Dr. K. K. Sakariah, Head, Human Resource Development, Central Food Technological Research Institute, Mysore, for their constant encouragement.

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