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Nutritional Value and Antioxidant Activity of Various Extracts and Fractions of *Punica granatum* (Pomegranate) Peel

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Abstract. Evaluation of the nutritional value and antioxidant activity of *Punica granatum* peel extracts for their radical scavenging activity revealed that potent antioxidant activity, in the order of ethyl acetate fraction > chloroform fraction > petroleum ether fraction > water fraction. Ethyl acetate fraction had higher radical scavenging activity in comparison to a standard antioxidant like BHT.

Keywords: *Punica granatum*, nutritional profile, DPPH, reducing power activity, antioxidant activity

Introduction

Punica granatum (pomegranate) belongs to the family Punicaceae and has been used in a number of natural medicines since time immemorial (Murthy *et al*., 2004; 2002). It is widely cultivated throughout India, Pakistan, South East Asia and East Indies.

The fruits improve heart health, give protection against prostate cancer and slow down the cartilage loss in arthritis. The dried rind of *P. granatum* fruit is used in the treatment of amoebic dysentery, diarrhoea, low appetite, hyper-acidity, hemorrhages and tapeworm infestation (Nadkarni, 1982). Pomegranate peel is the major source of polyphenol (flavonoids, anthocyanin, proanthocyanidins) and tannins (Jaiswal *et al*., 2010; Guo *et al*., 2003) showing antiinflammatory, antibacterial, anticancer and antioxidant activities (Lansky and Newman, 2007; Daniells, 2006; Li *et al*., 2006; Prashanth *et al*., 2001).

It is well known that reactive oxygen species (ROS) formed *in vivo*, such as superoxide, hydrogen peroxide, hydroxyl radical and singlet oxygen are implicated in degenerative diseases such as cancer, inflammation, atherosclerosis and aging as also in food deterioration. In foods, rancidity is caused by formation of free radicals that lead to development of off-flavours and undesirable chemical compounds (Chatterjee *et al*., 2007). Antioxidants are phytochemicals, vitamins and other nutrients that protect our cells from damage caused by free radicals. Artificial antioxidants like butylated hydroxyanisole (BHA) and butylated hydroxy-toluene (BHT) which are often used in food industry, are suspected to be toxic and carcinogenic. Hence, the importance of research for finding naturally occurring antioxidants has been greatly increased in the recent years (Nadaroglu *et al*., 2007). The aim of the present study was to evaluate the nutritional profile and antioxidant activity of various fractions of H_2O, methanol, ethanol extracts of *P. granatum* peels.

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Materials and Methods

Collection of plant material. *P. granatum* fruit was purchased from local market and peels were removed from the fruit manually. Peels were dried at 60 °C for 36 h and ground into powder.

Reagents and solvents. 2,2'-diphenylpicrylhydrazyl (DPPH) (Sigma Co, USA), BHT (Merck), hexane, methanol, ethanol, petroleum ether, chloroform, ethyl acetate (Merck) and distilled water etc., were used.

Nutritional profile evaluation. Nutritional profile was evaluated according to the standard AOAC methods (AOAC, 2005).

Extraction procedure. Three extracts of peels were obtained using solvents: water, methanol and ethanol. Completely dried powdered peels (100 g) were extracted with 500 mL of water at 80-90 °C for about 7-8 h. Similar process was repeated with methanol and ethanol at 60 °C for 7-8 h. Procedure was repeated thrice with each solvent. The extracts were oven dried and their percentage yield was calculated. These extracts were dissolved in distilled water and partitioned sequentially with solvents such as petroleum ether, chloroform and ethyl acetate, These fractions were subjected to tests for free radical scavenging and reducing power activity.

DPPH free radical-scavenging assay. The free radical-scavenging activity of Pomegranate peel fractions on the DPPH radical was assessed using the method described by

Banerjee *et al*. (2005). When DPPH reacts with an antioxidant compound, which can donate hydrogen, it is reduced. The change in colour (from deep-violet to light-yellow) was measured at 517 nm on a UV/visible light spectrophotometer. 100 µL of 0.4 mg/mL of each fraction of water, methanol and ethanol extract and BHT were added to 3 mL of 0.004% (0.004 g/100 mL methanol) solution of DPPH. After 30 min incubation at room temperature, the absorbance was measured against a blank at 517 nm on UV-visible spectrophotometer (Thermo). The experiment was carried out in triplicate. Radical scavenging activity was calculated by the following formula:

$$\text{Inhibition \%} = (A_{blank}\text{-}A_{sample}/A_{blank}) \times 100$$

where:

A_{blank} is the absorbance of the control (containing all reagents except the test sample) and
A_{sample} is the absorbance of the test sample (different fractions).

Reducing power activity. Reducing power was measured by $K_3Fe(CN)_6$-$FeCl_3$ (Hou *et al*., 2003). The test sample was mixed with an equal volume of 0.2 M phosphate buffer (pH 6.6) and 1% potassium ferricyanide (K_3FeCN_6). The mixture was incubated at 37 °C for 20 min. An equal volume of 10% trichloroacetic acid was added to the mixture, then it was centrifuged at 3000 rpm for 10 min. The supernatant was mixed with distilled water and 0.1% $FeCl_3$ in the ratio of 1:1:2. After standing for 10 min, the absorbance was measured at 700 nm.

Statistical analysis. Data are presented as the mean ± standard deviation (SD) of each triplicate test.

Results and Discussion

Nutritional profile. The data obtained for nutritional profile of *P. granatum* peels are given in Table 1. These profiles are comparable to the profile given by Kingsly *et al*. (2006).

Table 1. Nutritional profile of *Punica granatum* peel

Parameters	Composition (%)
Moisture	9.52
Ash	0.93
Protein	6.95
Fat	0.99
Fiber	7.52
Carbohydrates	74.09

Yield. For finding an effective solvent for extracting a fraction showing high antioxidant activity, three solvents i.e. water, methanol and ethanol were tested (Fig. 1). The yields were approximately twice more when methanol was used as solvent as compared to other solvents. Similar results were obtained by Iqbal *et al.* (2008). These extracts were partitioned using different suitable solvents like petroleum ether, chloroform and ethyl acetate and their free radical scavenging/ reducing power activity was determined.

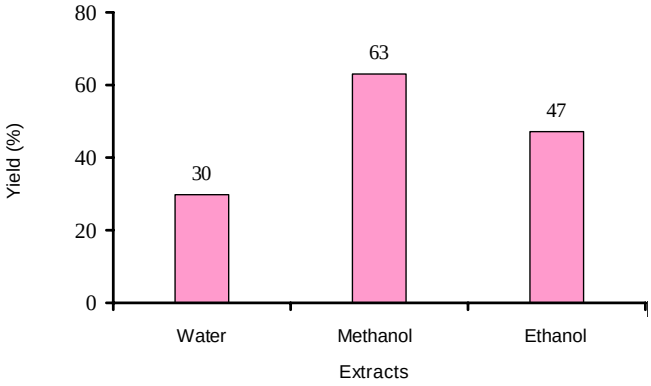

Fig. 1. Percentage yield of *Punica granatum* peel extracts.

Radical-scavenging activity. The hydrogen atom or electron donation ability of the corresponding extracts was measured through bleaching of purple-coloured methanol solution of DPPH. This spectrophotometric assay uses the stable radical 2,2'-diphenylpicrylhydrazyl (DPPH) as a reagent (Eminagaoglu *et al*., 2007). The DPPH free radical is a stable free radical, which has been widely accepted as a means of estimating free radical scavenging activities of antioxidants (Saeed *et al*., 2007).

Percentage inhibition of DPPH with 0.4 mg/mL concentration of different fractions of water, methanol and ethanol extracts is shown in Fig. 2. The levels of DPPH inhibition of petroleum ether fraction (PtF) and water fraction (WtF) were low as compared to the highest DPPH inhibition in chloroform fraction (CfF) and ethyl acetate fraction (EaF). When these values were compared with BHT, it was found that ethyl acetate fraction (EaF) had higher radical scavenging activity than that of BHT.

The antioxidant activity of methanol extract of Pomegranate peels has been reported as 92.7% by Iqbal *et al*. (2008) and 81.6% antioxidant activity of ethanol extract has been reported by Kaur *et al*. (2006) while water extract was reported to possess the least antioxidant activity (Negi and Jayaprakasha,

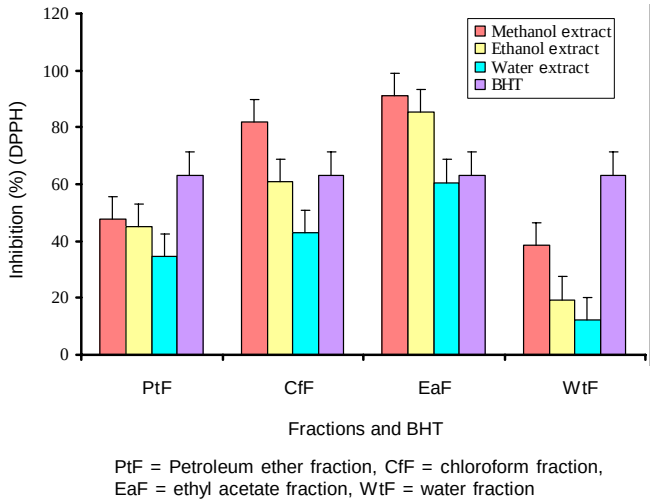

Fig. 2. Radical scavenging activity of various fractions and standard butylated hydroxytoluene (BHT).

2003). Free radical scavenging activity of the EaF was altered by increasing the active components/units through condensation effect during the solvent-solvent partitioning processes. The results suggested that EaF is more potent fraction than the other fractions. A similar study reports *P. granatum* to possess potent antioxidant activity by activity-guided fractionation (Ricci *et al.*, 2006).

Reducing power activity. In the reducing power assay, the antioxidant activity of samples was measured by their ability to reduce the Fe^{3+}/ferricyanide complex by forming ferrous products. Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Increased absorbance at 700 nm indicates a stronger reducing power. When these

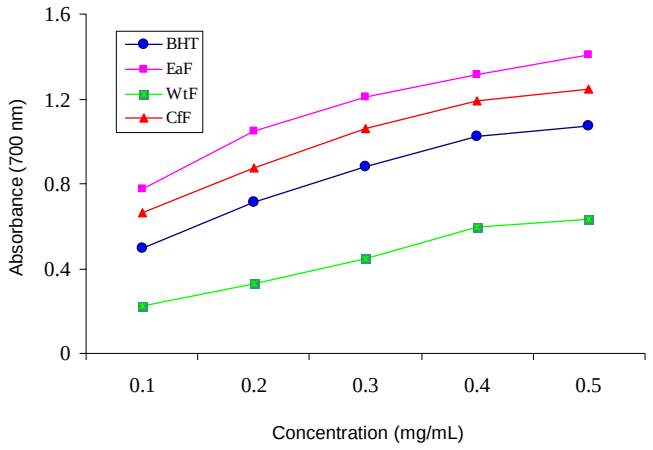

Fig. 3. Reducing power activity of various fractions of methanol extract and standard BHT.

tested extracts (with a concentration of 0.1-0.5 mg/mL) were added to the reaction mixture, ethyl acetate and chloroform fractions showed greater reducing powers relative to water fraction of pomegranate (*P. granatum*) extracts and standard antioxidant BHT (Fig. 3-5), at 700 nm absorbance. They also showed a dose-dependent effect. The EaF and CfF may contain more active materials which can donate electrons and react with free radicals, and then convert them into more stable metabolites and terminate the radical chain reaction (Hou *et al.*, 2003).

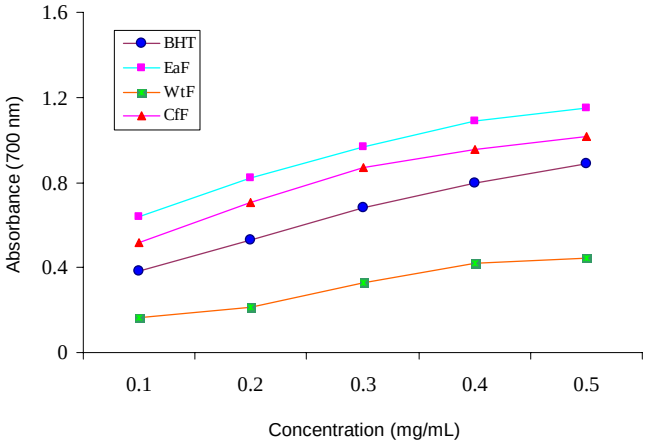

Fig. 4. Reducing power activity of various fractions of ethanol extract and standard BHT.

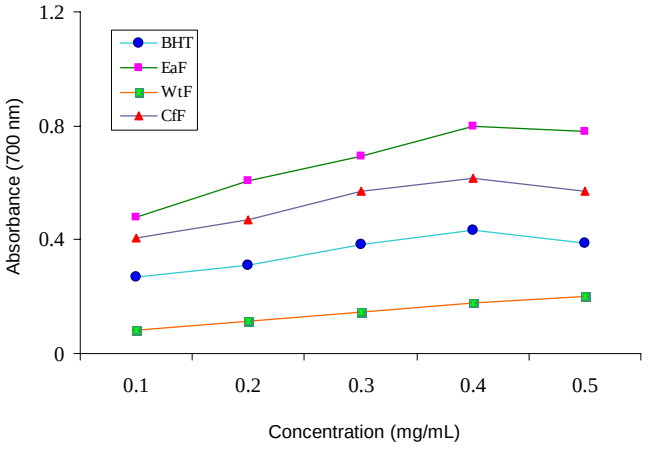

Fig. 5. Reducing power activity of various fractions of water extract and standard BHT.

Conclusion

It is concluded that *P. granatum* peels possess potent antioxidant activity as shown by the DPPH free radical scavenging

and reducing power activity assays. The results provide useful information on the pharmacological activities associated with free radicals. Further studies are in progress to determine the antioxidant activity *in vivo* and to identify the active compounds present in the plant.

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