



Evaluation of Total Phenolic Contents, Antioxidant and Antibacterial Capacity of Aqueous Methanolic Extracts obtained from *Punica granatum* Peel

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Accepted on: 18-01-2014; Finalized on: 31-03-2014.

ABSTRACT

Pomegranate has been used for thousands of years to cure a wide range of diseases. The recently research suggests that pomegranates might be useful in treating such serious conditions as prostate cancer, skin cancer, osteoarthritis, and diabetes. Pomegranate (*Punica granatum*) peel extracts have been shown to possess significant antioxidant activity in various in vitro models. This study revealed polyphenolic content, antioxidant and antibacterial activities aqueous methanolic extracts of *Punica granatum* peel. Dried pomegranate peels were powdered and extracted with water:methanol (1:1). *Punica granatum* extract material was tested for antimicrobial and antioxidant activities. *Punica granatum* extract was tested against gram +ve and gram -ve bacterial for their antibacterial activity.

Keywords: *Punica granatum* Peel, Phenolic Contents, Antioxidant and Antibacterial activities.

INTRODUCTION

Oxidative stress plays an important role in atherogenesis, its inhibition by nutritional antioxidants should retard the progression of the disease. Metabolism of oxygen in living cells also leads to the unavoidable production of oxygen-derived free radicals, commonly known as reactive oxygen species (ROS). These free radicals attack the unsaturated fatty acids of biomembranes, which results in lipid peroxidation and the destruction of proteins and DNA, which causes a series of deteriorative changes in the biological systems leading to cell inactivation. The pomegranate fruit contains several very potent antioxidants. Pomegranates (*Punica granatum*), a native shrub of occidental Asia and Mediterranean countries, have a high content of health-promoting compounds. The medicinal and nutritional value of *Punica granatum* peels has been heralded for thousands of years. Over the past decade, significant progress has been made in establishing the pharmacological mechanisms of different parts of pomegranate.

In India, it is used in the form of juice, concentrate, canned beverage, jam, and jelly. In fact, recent studies of health volunteers found that supplementation with *Punica granatum* peels extract substantially increased levels of antioxidants in the blood. Pomegranate juice and peel contain substantial amounts of polyphenols such as ellagic tannins, ellagic acid and gallic acid¹. Antioxidant and antibacterial properties of pomegranate peel in in-vitro model systems have been reported²⁻⁴. Several studies have reported the efficacy of extracts from different tree parts, such as bark, leaves, fruit, and fruit peel to inhibit the growth of Gram-positive and Gram-negative bacteria, which are food borne pathogens, spoilage bacteria, and human pathogens⁵⁻¹². Pomegranate

extracts also exhibit antifungal activities¹³. Therefore, the perception of this study is to evaluate the antioxidant activity of aqueous methanolic extract of *Punica granatum* peels.

MATERIALS AND METHODS

Chemical & Equipments

All the chemicals used in this investigation were of analytical reagent (AR) grade and were purchased from sigma, merck etc. Deionised water was used for the complete study. All the glassware and equipment used for the handling of bacterial cultures and plant extracts were sterilized prior to use. Sterilization procedures were performed by autoclaving at 120°C for 15 minutes.

Collection and Authentication of Plant Material

Punica granatum was purchased from local market the local market of Meerut (Uttar Pradesh). The peels were manually removed taken out and dried in shade. Then it powdered by using mortar and pestle. The powdered were then preserved in a tight container for experiment.

Preparations of aqueous methanolic extract

In a round bottom flask powdered material of *Punica granatum* peels (100 gm) was dissolved in 600 ml methanol and 600 ml water. The resulting mixture was refluxed for 24 hrs. The extract was filtered through Whatman filter paper for removal of peel particles. The extract was concentrated under vacuum at 40°C. The content was dark brown in color. Total yield of aqueous methanolic extract was 6.1 %. The extract was stored at 5°C until required.



Determination of Plant Extract Yield

The yield of evaporated dried Peels extracts of *Punica granatum* based on dry weight basis was calculated from the following equation:

$$\text{Yield} = (W_1 \times 100) / W_2$$

Where, W_1 and W_2 were the weight of the extract after the solvent evaporation and the weight of the dry peels, respectively.

Total Polyphenol Compound Determination

Total polyphenols were determined using a colorimetric method. The concentration of phenolic compounds in the extract was determined according to the method of Jayaprakasha et al¹⁴. 0.25 mg sample was dissolved in MeOH and mixed with 1.0 mL of diluted Folin-Ciocalteu reagent (1:10 in water), then added 0.75 mL of a 10 % sodium carbonate solution to it. The mixture was allowed to stand for 30 min at 35 °C. The absorbance of the cooled samples was measured at 765 nm using UV-visible Spectrophotometer. The estimation of phenolic compounds in the fractions was carried out in triplicate.

Antibacterial Assay

The agar well diffusion method was used to evaluate the antibacterial activity. The culture were grown in nutrient broth and incubated at 37°C for 24 hr. After incubation period is finished the absorbance of culture was adjusted to 0.5 according to Mc Farland turbidity standard with sterile nutrient broth. The 0.02 ml of the culture was seeded on the sterile petri plates containing sterile Muller Hinton Agar Media. The wells were bored with 9 mm borer in seeded agar. Then the particular concentration of the plant extract was added in each well. Plates were then incubated at 37°C for 24 hrs. After incubation period was finished the zone of inhibition was measured and recorded against *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Staphylococcus (local)*, *Klebsiella pneumoniae* and *Salmonella typhimurium*.

Assay of Reducing Power

The antioxidant activity was measured by Ferric Reducing Ability of Plasma or Plants (FRAP) method. Reducing power assay of *Punica granatum* was determined according to the method of Oyaizu¹⁵. One ml of extract at various concentrations (0 - 500 mg/l) was added to a test tube. One ml potassium phosphate buffer (0.2 M, pH= 6.6) and freshly prepared potassium ferricyanide [$K_3Fe(CN)_6$] (1 ml, 1%) were added to extracts. The mixture was incubated in a water bath (50°C for 20 min). One ml of trichloroacetic acid (10% TCA) was added to the mixture followed by centrifugation for 10 min. From the upper layer of mixture, 1 ml was taken and mixed with 1 ml distilled water followed by 100 µl of freshly prepared $FeCl_3$ (0.1 %) solution. The absorbance (A) of samples was measured at 700 nm against blank. Increased absorbance of the reaction mixture indicated increased reducing power. Ascorbic acid was used as a reference standard. Phosphate buffer (pH 6.6) was used as a blank solution.

The absorbance of the final reaction mixture of the two parallel experiments was taken and expressed as mean \pm standard deviation. Augmented absorbance of the mixture indicates stronger reducing influence of the extract.

RESULTS AND DISCUSSION

Yield and Total Phenolics of Peel Extracts

The percentage yield the *Punica granatum* peel extract was 6.1%. Phenolic compounds may contribute directly to the anti oxidative action. The total phenolic content was 36 % \pm 6.23 % (w/w) of peel extract.

Reducing Power of Extracts

The reductive capabilities of the peel extract compared with ascorbic acid have been depicted in Figure-1. Figure illustrates the reducing power of peel extracts using the ferricyanide reduction method. The increase in absorbance A at 700 nm indicated better reducing power of test materials. The reducing power of the extract of *Punica granatum* peel was found to be remarkable, which increased gradually with a rise in the concentration. Fe^{3+} was transformed to Fe^{2+} in the presence of the extract and the reference compound ascorbic acid to measure the reductive capability. The antioxidant activity has been reported to be concomitant with the reducing power of plant materials. The higher reducing power indicated presence of reductones which are able to break free radical chains by donating hydrogen atoms and thus converting them to a more stable non-reactive species. At 50 ppm the absorbance of the extract was 0.15, while at 500 ppm the absorbance of aqueous methanolic extract was found 2.08. The result has been summarized in table 1.

Table 1: Experimental data on Antioxidant power of *Punica granatum* peel extracts.

Aq. methanolic extract concentration (ppm)	Antioxidant power
50	0.15
100	0.25
200	0.74
300	1.11
400	1.43
500	2.08

Phytochemicals against Bacterial Infection

Peels extract was tested against gram +ve and gram -ve bacterial for their antimicrobial activities. All the bacteria *Bacillus subtilis*, *pseudomonas aeruginosa*, *staphylococcus*, *klebsiella pneumonia*, *salmonella typhimurium* found susceptible, showing the zones of inhibition in the order *pseudomonas* (27 mm) > *staphylococcus* (24 mm) > *klebsiella pneumonia* (23 mm) > *salmonella typhimurium* (17 mm) > *bacillus subtilis* (14 mm). The result of the phytochemical test has been summarized in table 2.



The bacterial infections against the individual pathogen are treated with the peels extract, the mechanism of action would possible hold the phytochemicals of grape seed responsible for the inactivation of enzymes which help bacterial biosynthesis of proteins etc.

Table 2: Antimicrobial activity of Aq. methanolic extract of *Punica granatum* peel against gram (+ve) and gram (-ve) bacteria.

Strain Used	Extract Concentration (ppm)	Zone of Inhibition (mm)	Control
Bacillus subtilis	500	14	nil
Pseudomonas aeruginosa	500	27	nil
Staphylococcus	500	24	nil
Klebsiella pneumoniae	500	23	nil
Salmonella typhimurium	500	17	nil

Zone of inhibition= Complete zone- Diameter of well; Diameter of well= 9 mm., Control= DMSO

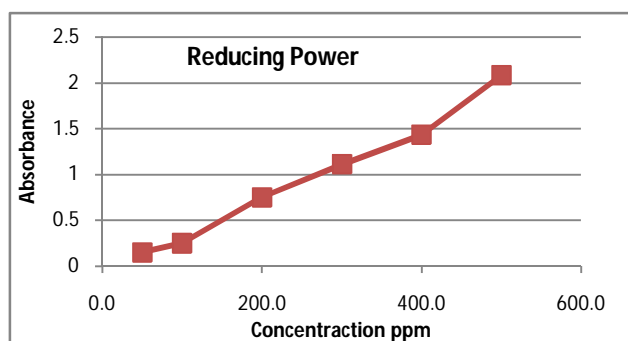


Figure 1: The reductive Reducing capabilities of the peel extract.

CONCLUSION

In conclusion, the *Punica granatum* peel was found to contain a noticeable amount of total phenols, which play a major role in controlling oxidation. The results of this study show that the *Punica granatum* peel can be used as an easily accessible source of natural antioxidant. The results obtained in this study clearly demonstrate broad spectrum antimicrobial activity of pomegranate against bacteria. The presence of phytochemicals in the extracts including phenols, tannins and flavonoids as major active constituents may be responsible for these activities.

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Source of Support: Nil, Conflict of Interest: None.

