Research Article

Phytochemical characterization and antioxidant properties of *Punica granatum* peel extract

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ABSTRACT

Background: The pomegranate (*Punica granatum*) is a fruit-bearing deciduous shrub or small tree in the family Lythraceae. As intact arils or juice, pomegranates are used in baking, cooking, juice blends, meal garnishes, smoothies, and alcoholic beverages such as cocktails and wine. In India's ancient Ayurveda system of traditional medicine, the pomegranate is frequently described as an ingredient in remedies. **Materials and Methods:** The peel from the fruit was removed carefully by knife and allowed to dry. The dried material was properly ground into powder and kept in refrigerator for further analysis. The peel powder was loaded into Soxhlet extractor and subjected to extraction with methanol. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator. Phytochemical screening: Phytochemical screening of *P. granatum* peel ethanolic extracts was assessed by standard method. **Results:** 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity: In the present study, *P. granatum* peel ethanolic extract showed a significant increased the DPPH radical scavenging activity. However, the maximum inhibition was observed at 500 µg/ml equal to that of Vitamin C, a standard drug used in this study. As like DPPH radical scavenging activity, the extract showed a significant increased the nitric oxide (NO) radical scavenging activity. However, the maximum inhibition was observed at 500 µg/ml equal to that of Vitamin C, a standard drug used in this study. **Conclusions:** The *in vitro* studies on DPPH and NO radical scavenging study showed *P. granatum* peel extract has a strong dose-dependent free radical scavenging ability may be due to the presence of phytochemicals such as saponins terpenoids, steroids, flavonoids, phenols and alkaloids could have contributed for such antioxidant potentials. Hence, the present findings indicate that *P. granatum* may be used as potential antioxidant which may help for the treatment of various diseases.

KEY WORDS: *Punica grantum* peel extract, DPPH, NO, Antioxidant potential

INTRODUCTION

The pomegranate (*Punica granatum*) is a fruit-bearing deciduous shrub or small tree in the family Lythraceae. As intact arils or juice, pomegranates are used in baking, cooking, juice blends, meal garnishes, smoothies, and alcoholic beverages such as cocktails and wine. In India's ancient Ayurveda system of traditional medicine, the pomegranate is frequently described as an ingredient in remedies.[1] The most abundant phytochemicals in pomegranate juice are polyphenols, including the hydrolyzable tannins called ellagitannins formed when ellagic acid and gallic acid bind with a carbohydrate to form pomegranate ellagitannins, also known as punicalagins.[2] The red color of the juice is attributed to anthocyanins such as delphinidin, cyanidin, and pelargonidin glycosides.[3] In general, an increase in juice pigmentation occurs during fruit ripening. The phenolic content of pomegranate juice is degraded by processing and pasteurization techniques.[4] Compared to the pulp, the inedible pomegranate peel contains as much as 3 times the total amount of polyphenols including condensed tannins, catechins, gallocatechins, and prodelphinidins.[5] The higher phenolic content of the peel yields extracts for use in dietary supplements and food preservatives.[2] The potential therapeutic properties of pomegranate are wide ranging and include treatment and prevention of cancers, cardiovascular disease, diabetes, dental conditions, erectile dysfunction, and prevention from ultraviolet radiation. The pericarp of *P. granatum* is used to treat infections found in human sexual

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organs as well as mastitis, acne, folliculitis, piles, allergic dermatitis, tympanitis, scalds, diarrhea, and dysentery.[2] Medicinal plants play a major role in meeting the medicinal and health needs of about 70% of populations in developed and developing countries, which serve as an important resource for the treatment of various maladies and illnesses.[6] Secondary metabolites are synthesized during secondary metabolism of plants. They are the basic source for the establishment of several pharmaceutical industries since they have enormous medicinal properties.[7] The most important secondary metabolites are alkaloids, tannins, flavonoids, phlobatannins, saponins, and cardiac glycosides. All secondary metabolites have specific function such as saponins that have antifungal activity,[8] some alkaloid may be useful against HIV infection.[9] Flavonoids have strong anticancer activity[10] and tannin has antimicrobial activity. The potent antioxidant capacity of pomegranate and its components has been reported by numerous scientists using multiple *in vitro* assay systems.[11]

MATERIALS AND METHODS

Collection of Plant Material

Fresh fruits of *P. granatum* were collected from local market of Chennai and transported to laboratory. The fruits were washed with running tap water, rinsed well in distilled water, and exposed to drying at room temperature for about 5 min in open air. The peel from the fruit was removed carefully by knife and allowed to dry. The dried material was properly ground into powder and kept in refrigerator for further analysis.

Preparation of Extracts

The peel powder was loaded into Soxhlet extractor and subjected to extraction with methanol. After extraction, the solvent was distilled off and the extracts were concentrated on water bath to a dry residue and kept in a desiccator.

Phytochemical Screening

Phytochemical screening of *P. granatum* peel ethanolic extracts was assessed by the standard method as described by Savithramma *et al*. (2011) and Selvaraj *et al*. (2014).

Test for tannins

About 1 mL of the peel extract was added to 1 mL 5% ferric chloride. Formation of dark blue or greenish-black indicates the presence of tannins.

Test for saponins

About 1 mL of the peel extract was added to 1 mL distilled water and shaken in graduated cylinder for 15 min; lengthwise formation of 1 cm layer of foam indicates the presence of saponins.

Test for alkaloids

About 1 mL of the peel extract was added to 2 mL conc. HCl. Then, few drops of Mayer's reagent were added. The presence of green color or white precipitate indicates the presence of alkaloids.

Test for glycosides

About 1 mL of the peel extract was added to 3 mL chloroform and 10% ammonium solution. Formation of pink color indicates the presence of glycosides.

Test for terpenoids

About 1 mL of the peel extract was added to 2 mL chloroform along with conc. sulfuric acid. Formation of red-brown color at the interface indicates the presence of terpenoids.

Test for phenols

About 1 mL of the peel extract was added to 2 mL distilled water followed by few drops of 10% ferric chloride. Formation of blue/green color indicates the presence of phenols.

Test for steroids

About 1 mL of the peel extract was added to 2 mL chloroform and 1 mL sulfuric acid. Formation of reddish-brown ring at interface indicates the presence of steroids.

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) Free Radical Scavenging Activity of *P. granatum*

Scavenging of DPPH radical was assessed by the method of Hatano *et al.* (1989). Briefly, DPPH solution (1.0 ml) was added to 1.0 ml of different extracts of *P. granatum* in ethanol at different concentrations (100, 200, 300, 400, and 500 µg/ml). The mixture was kept at room temperature for 50 min and the activity was measured at 517 nm. Ascorbic acid at various concentrations (100, 200, 300, 400, and 500 µg/ml) was used as standard. The capability to scavenge the DPPH radical was calculated using the following formula:

DPPH radicals scavenged (%) = Control OD − Sample OD/Control OD×100

Nitric Oxide (NO) Radical Scavenging Activity of *P. granatum*

Scavenging of NO radical was assayed by the method of Garrat (1964). Briefly, the reaction mixture (3 ml) containing sodium nitroprusside (10 mM, 2 ml), phosphate buffer saline (0.5 ml), and different concentrations (100, 200, 300, 400, and 500 µg/ml) of extracts of *P. granatum* (0.5 ml) was incubated at 25°C for 150 min. After incubation, 0.5 ml of the reaction mixture containing nitrite was pipetted out and mixed with 1 ml of sulfanilic acid reagent (0.33%

in 20% acetic acid) and allowed to stand for 5 min for completing diazotization. Then, 1 ml of naphthyl ethylenediamine dihydrochloride was added, mixed, and allowed to stand for 30 min at 25°C. A pink-colored chromophore is formed in diffused light. Ascorbic acid at various concentrations (100, 200, 300, 400, and 500 µg/ml) was used as standard. The activity was measured at 550 nm and the results were expressed as percentage of scavenging using the following formula:

DPPH radicals scavenged (%) = Control OD − Sample OD/Control OD×100

Statistical Analysis

Results are expressed as mean ± SD. The difference between experimental groups was compared by one-way analysis of variance followed by Dunnett's multiple comparison test (control vs. test) using the software GraphPad Prism software.

RESULTS

Phytochemical Screening of *P. granatum* Peel Ethanolic Extracts

Table 1 shows the phytochemical active constituent of *P. grantum* peel extract. This study reveals that presence of tannins, saponins, terpenoids, steroids, flavonoids, phenols, alkaloids and glycosides.

DPPH Radical Scavenging Activity

In the present study, *P. granatum* peel ethanolic extract showed a significant increased the DPPH radical scavenging activity. However, the maximum inhibition was observed at 500 µg/ml equal to that of Vitamin C, a standard drug used in this study [Figure 1].

NO Radical Scavenging Activity

In the present study, *P. granatum* peel ethanolic extract showed a significant increased the NO radical scavenging activity. However, the maximum inhibition was observed at 500 µg/ml equal to that of vitamin C, a standard drug used in this study [Figure 2].

DISCUSSION

The preliminary phytochemical screening tests may be useful in the detection of the bioactive principles and subsequently may lead to the drug discovery and development. Further, these tests facilitate their quantitative estimation and qualitative separation of pharmacologically active chemical compounds.[12]

The phytochemical screening, in the present study, has revealed the presence of triterpenoids, steroids, glycosides, flavonoids, tannins, carbohydrate, and Vitamin C in the peel extract; triterpenoids, steroids, glycosides, saponins, alkaloids, flavonoids, tannins,

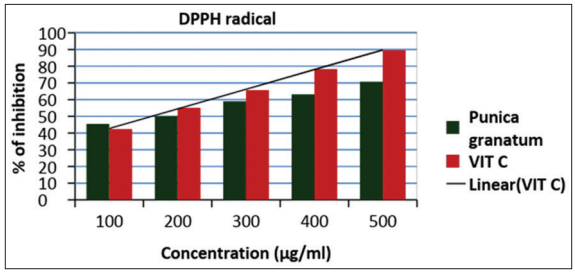

Figure 1: Effect of *Punica grantum* peel extract on DPPH radical scavenging activity. Each bar represents mean ± of 5 observations

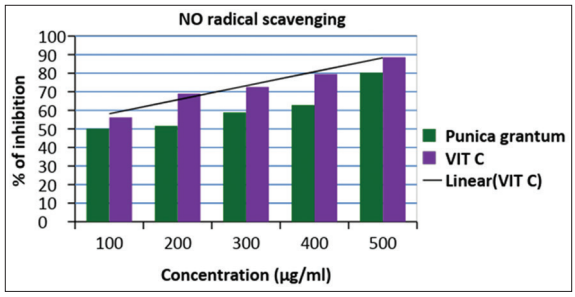

Figure 2: Effect of *Punica grantum* peel extract on NO radical scavenging activity. Each bar represents mean ± of 5 observations

Table 1: Phytochemical screening of *P. granatum* peel ethanolic extracts

Phytochemical tested	Ethanolic peel extract
Tannins	-
Saponins	+
Terpenoids	+
Steroids	+
Flavonoids	+
Phenols	+
Alkaloids	+
Glycosides	−

+: Present, −: Absent. *P. granatum: Punica granatum*

carbohydrate, and Vitamin C in the whole fruit extract; and triterpenoids, steroids, glycosides, saponins, alkaloids, tannins, carbohydrate, and Vitamin C in the seeds extract. Further, the presence of different phytoconstituents in the three different extracts may be responsible for the therapeutic properties of pomegranate. Flavonoids and tannins are phenolic compounds and plant phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers. Since these compounds were found to be present in the extracts, it might be responsible for the potent antioxidant capacity of pomegranate. The secondary metabolites (phytochemicals) and other chemical constituents of medicinal plants account for their medicinal value. For example, saponins have hypotensive and cardiodepressant properties.[13] Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia.[14] The presence of saponins in whole fruit and seeds extract and glycosides in all the extracts

might play a role in the cardioprotective potential of pomegranate. Flavonoids have been used against the cancer-causing tumors and it inhibits the promotion of growth and progression of tumors.[15] Phenols when mixed with the flavonoids compounds in plants are reported to show multiple activities such as antioxidant, anticarcinogenic, and anti-inflammatory.[16] Plants with tannins are used for healing of wounds, varicose ulcers, hemorrhoids, frostbite, and burns.[17,18] Terpenoids are reported to have anti-inflammatory, antiviral, antimalarial, inhibition of cholesterol synthesis, and antibacterial activity.[19,20]

CONCLUSIONS

The *in vitro* studies on DPPH and NO radical scavenging study showed *P. granatum* peel extract has a strong dose-dependent free radical scavenging ability may be due to the presence of phytochemicals such as saponins terpenoids, steroids, flavonoids, phenols and alkaloids could have contributed for such antioxidant potentials. Hence, the present findings indicate that *P. granatum* may be used as potential antioxidant is similar to other studies[21] and may help for the treatment of various diseases.

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