

SCREENING OF PHYTOCHEMICALS AND BIOACTIVE COMPOUNDS IN *PUNICA GRANATUM* PEEL TO EVALUATE ITS HEMATOLOGICAL POTENTIAL

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Received: 08 March 2018, Revised and Accepted: 04 May 2018

ABSTRACT

Pomegranate has been found to have ethnomedical history and is a rich reservoir of phytochemicals. The present study was carried out for phytochemical analysis in the hydroalcoholic pomegranate peel extracts. At present, phytochemical analysis and analysis of bioactive compounds are done, the pomegranate peel extracts are found to have carbohydrates, alkaloids, flavonoids, steroids, tannic acids, and phenolic compounds. The antioxidant activity, 1, 1-diphenyl-2-picrylhydrazyl free radical scavenging activity, and nitric oxide radical scavenging activity were analyzed. For good health, the quality of blood should be maintained and for this antioxidants may be the key so as to protect the blood from oxidative stress. Thus, from the present study, it is concluded that pomegranate peel is a rich source of phytochemicals and bioactive compounds such as antioxidants and so can be used for various pharmaceutical and therapeutic purposes like increase in hemoglobin count.

Keywords: Pomegranate peel, Antioxidant activity, 1, 1-diphenyl-2-picrylhydrazyl free radical scavenging, Nitric oxide radical scavenging, Hemoglobin booster.

INTRODUCTION

Phytochemicals are found in plants and are non-nutritive in nature but have certain disease preventing properties. They offer protection against pathogens and are not required by human beings for perpetuating life [1]. Phytochemicals work in many ways such as antioxidants which protect the cells from damage. These can stimulate certain enzymes and can reduce the risk of various ailments. These can also act as antibacterial and hormonal stimulant.

There are various pharmacological and epidemiological evidences which prove that plants contain biologically active components which offer various health benefits and also protect against degenerative ailments [2]. The health benefits are often associated with plant phytochemicals as of consumption of higher levels of fruits and vegetables. The phenolic compounds found in plants are found to exhibit various properties such as antiallergic, anti-inflammatory, antimicrobial, and antioxidant [3-5].

Pomegranate is the most significant tree which has been domesticated for incalculable human benefits and serves as a food medicine of great importance. It has been widely used in Middle East, Iran, India, for antimicrobial activities. Many researchers have conducted various experiments for studying phytochemicals present in pomegranate. These are found to possess several chemicals and have immense economic value. Its various parts are exploited by pharmaceuticals and are considered as a powerhouse of energy due to its nutritive value. Due to the presence of many phytochemicals, it is also known as the "superfruit" [6].

The fruit and its products are used anciently for diseases such as stomachic, inflammation, fever, bronchitis, diarrhea, dysentery, vaginitis, urinary tract infections, and others. Pomegranate peels are found to be effective against malaria [5,7,8]. Various pomegranate supplements are found in the market [9]. Ellagic tannins and ellagic acid are among the most potent antioxidants found in peel [8,10]. Antioxidants play an important role in the quality of our blood. Strong blood cells and vessels with adequate antioxidant capacity help maintain regular cholesterol levels essential for heart health helping keep heart diseases such as heart attacks at bay [11]. Pomegranate extracts are found to increase the hemoglobin count [12].

Plant profile

Botanical name: *Punica granatum* L.

Taxonomical classification

Kingdom: Plantae
Division: Magnoliophyta
Class: Magnoliopsida
Subclass: Rosidae
Order: Myrtales
Family: Punicaceae

The major basic antioxidants or free radical scavengers from plant phenolics constitute as a major group of compounds. These include statins and flavonoids [13]. Whereas, saponins behave as cardiodepressants and show hypotensive properties [14]. Glycosides are naturally cardioactive drug and are used to treat heart failures [15]. Thus, the purpose for the present investigation was to evaluate the phytochemicals present in pomegranate peel using *in vitro* methods. The screening results are tabulated.

MATERIALS AND METHODS

The fresh pomegranate fruits were collected from the fruit market, Bittan Market, Bhopal. The fruits were washed and then disinfected. The peel was carefully removed from the seeds. These peels were shade dried for 10 days. Then, the pomegranate peels were grinded in the grinder. Then, these are taken for extraction process (Fig. 1).

Preparation of hydroalcoholic extract

The powdered material is extracted with the help of Soxhlet apparatus. 200 ml of the solvent is used in the ratio of 70:30 (methanol/water). A Soxhlet extraction is used when the desired compound has a limited solubility in a solvent, and the impurity is insoluble in that solvent. It allows for unmonitored and unmanaged operation while efficiently recycling a small amount of solvent to dissolve a larger amount of material (Fig. 2).

- Initial weight of pomegranate peels=320 g
- Weight after drying=65.984 g
- Solvent system used=Hydroalcoholic system (200 ml)
- Percentage yield=30.04%.

Phytochemical analysis

The hydroalcoholic extract of Punica granatum fruit peel was studied for their phytoconstituents using different phytochemical tests [16].

Qualitative phytochemical analysis

The crucial starting point for assessing the pharmacological aspect of pomegranate is the identification of the phytochemicals. The pomegranate peel extract was screened to identify the key families of phytochemicals. Following tests were performed for identification.

1. Molisch test
 - This test is performed to identify carbohydrates. In this, 2.3 ml of the extract was taken and four drops of naphthol in alcohol are added. This is then shaken well and conc. H_2SO_4 is then poured from the sides of the test tube.
2. Test for steroids
 - This test is known as the Salkowski test. In this, 2 ml of chloroform and 2 ml of conc. H_2SO_4 are added to 2 ml of the extract. This is then shaken well.
3. Test for flavonoids
 - Take 2 ml of test solution, then add small quantity of reduced lead acetate solution (10%).
4. Millions test
 - 2 ml solution and Million reagent will mix properly
5. Mayer's test
 - Take 2-3 ml filtrate extract solution with few drops of Mayer reagent.
6. Wagner's test
 - 2-3 ml filtrate extract solution with few drops of Wagner's reagent.
7. Tannic acid and phenolic tests
 - Take 2-3 ml of aqueous or alcoholic extract add few drops for following reagent.



Fig. 1:



Fig. 2: Extraction process

8. Test for sulfates
 - With lead acetate reagent gives white ppt. will form that soluble in NaOH solution.
9. Test for chloride
 - Take 5-7 ml filtrate plant extract solution and add 3-5 ml lead acetate solution.
10. Test for saponins
 - Foam test - the extract and powder were mixed vigorously with water.

Reagent	Observation
5% $FeCl_3$ solution	Solution will appear deep blue-black color
Lead acetate solution (10%)	Solution will form white ppt.
Gelatin solution (10%) solution	Solution will form white ppt.
Acetic acid solution	Solution will appear red color
Dilute iodine solution	Solution will appear red color
Dilute potassium permanganate solution	Solution will decolorizes its color

Analysis of bioactive compounds

Total flavonoid content

A clean test tube was taken and 0.5 ml of the sample (extract) is added, containing 1.25 ml of distilled water. Then, 0.075 ml of 5% sodium nitrite solution is added and allowed to stand for 5 min. 0.15 ml of 10% aluminum chloride is added, after 6 min, 0.5 ml of 1.0 M sodium hydroxide was added and the mixture was diluted with another 0.275 ml of distilled water. The absorbance of the mixture at 510 nm was measured immediately. The flavonoid content was expressed as mg quercetin equivalents/g sample.

Total phenolic content

About 1.0 ml of sample was mixed with 1.0 ml of Folin and Ciocalteu's phenol reagent. After 3 min, 1.0 ml of saturated Na_2CO_3 (~35%) was added to 23 the mixture and made up to 10 ml by adding distilled water. The reaction was kept in the dark for 90 min, observed under ultraviolet-visible (UV-Vis) spectrophotometer at 760 nm absorbance. Gallic acid was used as a standard with varied concentration from 200 ppm to 1000 ppm. A calibration curve was constructed with different concentrations of tannic acid (0.02-0.1 mM) as standard. The results were expressed as mg of catechol equivalents/g of extract [17].

1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity

The free radical scavenging activities were tested by their ability to bleach the stable radical DPPH. The antioxidant activity using the DPPH assay was assessed by this method. The sample extracts and standards (BHA and ascorbic acid) were prepared at various concentrations (200-1000 ppm) and mixed with ethanolic solution of DPPH with a concentration of 0.04 mg/ml. After stand for 20 min in the dark, the mixtures were measured at 517 nm against ethanol as blank using UV-vis spectrophotometer.

The result obtained was calculated using formula:

$$\text{Scavenging activity (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control}} \times 100 \quad (1)$$

Nitric oxide radical scavenging activity

The procedure is based on the principle that sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions that can be estimated using Griess reagent. Scavengers of nitric oxide complete with oxygen, leading to reduced production of nitrite ions. Plant extract was dissolved in distilled water for this quantification. Sodium nitroprusside (5 mM) in standard phosphate buffer saline (0.025 M, pH 7.4) was

incubated with different concentrations (100–400 µg/ml) of methanol extract, and tubes were incubated at 29°C for 3 h. Control experiment without the test compounds but with equivalent amount of buffer was conducted in an identical manner. After 3 h, incubated samples were diluted with 1 ml of Griess reagents. The absorbance of the color developed during diazotization of nitrite with sulfanilamide and its subsequent coupling with naphthyl ethylenediamine hydrochloride was observed at 550 nm on spectrophotometer. Same procedure was done with ascorbic acid which was standard in comparison to methanol extract. Calculated the percentage inhibition by formula and plot graph in compared to standard.

$$\text{Nitric Oxide (\%)} = \frac{(A_{\text{control}} - A_{\text{test}})}{A_{\text{control}}} \times 100 \quad (2)$$

Where, A_{control} = Absorbance of control reaction

A_{test} = Absorbance in the presence of the samples of extract.

Total antioxidant activity

Determination of total antioxidant capacity was evaluated by the phosphomolybdenum method. 0.3 ml of extract and subfraction in ethanol, ascorbic acid used as standard (5–200 g/ml) and blank (ethanol) were combined with 3 ml of reagent mixture separately and incubated at 95°C for 90 min. After cooling to room temperature, the absorbance of each sample was measured at 695 nm against the blank. Ascorbic acid was used as standard, and the total antioxidant capacity is expressed as equivalents of ascorbic acid or gallic acid. The antioxidant activity is expressed as the number of equivalents of ascorbic acid and was calculated by the following equation:

$$A = \frac{(c \times v)}{m} \quad (3)$$

Where,

A = total content of antioxidant compounds, mg/g plant extract, in ascorbic acid equivalent,

c = the concentration of ascorbic acid established from the calibration curve, mg/ml,

V = the volume of extract (ml), and m = the weight of crude plant extract (g).

RESULTS

Medicinal plants have been exploited continuously by researchers to produce the potent drugs of medicinal properties with reduced toxicity [18]. The phytochemicals present in the hydroalcoholic extract of pomegranate peel shows the results as shown in Table 1.

Phytochemical analysis

Table 1: The phytochemical tests confirm the presence of carbohydrates which are confirmed by Molisch test as a violet ring formed at the ring of the two junctions of the liquids. The other tests such as tests for steroids, proteins, alkaloids, tannic acids, and phenolic compounds, and flavonoids are found to be positive in the peel extracts. Whereas, sulfates and chlorides are found absent in this extract.

DPPH free radical scavenging activity

The DPPH radical scavenging activity of pomegranate peel extract is depicted by Fig. 3. Pomegranate fruit peel extract potently scavenged DPPH radicals similar to catechin, it is likely that peel extract possessed proton-donating ability and in association with a number of hydroxyl groups to stabilize free radicals [19,20].

The respective values for pomegranate peel are also shown in Fig. 3 and Table 2.

Nitric oxide radical generated from sodium nitroprusside at physiological pH was found to be inhibited by the peel extracts. The nitric oxide scavenging activity is shown in Fig. 4. The scavenging activity is higher in pomegranate peels as compared to the standard ascorbic acid. From

the figure, it is clear that with increasing concentration the activity also increases.

Total phenolic content

The total phenolic content was found to be 273.26 catechol equivalents/g. The results are in agreement to Singh *et al.* [21] who studied this process for extraction of antioxidants from pomegranate peel extracts, but this result differs slightly due to environmental and experimental conditions. The best solvent for the extraction of the phenolic compound is methanol as it has the ability to inhibit the reaction of PPO which leads to the oxidation of phenolics [22]. The results are shown in Fig. 4 and Table 3.

The total flavonoid contents in pomegranate peel extracts were found to be 87.122 mg of quercetin equivalent/g. The regression coefficient taken here is $R^2=0.990$, and the equation for standard curve is taken as $y=0.244x+0.697$ (Fig. 6 and Table 4).

The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto (1999). This method is based on the reduction of phosphomolybdic acid to phosphomolybdenum blue complex by sodium sulfide. The obtained phosphomolybdenum blue complex is oxidized by the addition of nitrite, and this causes a reduction in intensity of the blue color. The results show that at lower concentration the percentage inhibition of pomegranate peel is more as compared to standard ascorbic acid. This is shown in Fig. 7. *P. granatum* peel extracts can be used as a natural source of antioxidants.

Table 1:

S. No	Phytochemical	Name of test	Result
1.	Carbohydrates	Molisch test	+
2	Steroids	Salkowski test	+
3	Proteins	Millions test	+
4	Alkaloids	Mayer's test	+
5	Alkaloids	Wagner's test	+
6	Tannic acid and phenolic compounds	Tannic acid and phenolic compounds test	+
7	Sulfates	Sulfate test	-
8	Chlorides	Chloride test	-
9	Flavonoids	Flavonoid test	+

Table 2: Nitric oxide radical scavenging activity

Concentration	Standard ascorbic acid	Pomegranate
0.1µg	1.031	0.702
0.2 µg	1.072	0.628
0.3 µg	1.073	0.769
0.4 µg	0.865	0.704
0.5 µg	0.966	0.693

Table 3: Total flavonoid content

Concentration (µg/ml)	Absorbance
20	0.181
40	0.293
60	0.422
80	0.574
100	0.743

Table 4: Total antioxidant activity

Concentration (µg/ml)	Absorbance
20	0.9
40	1.207
60	1.477
80	1.682
100	1.883

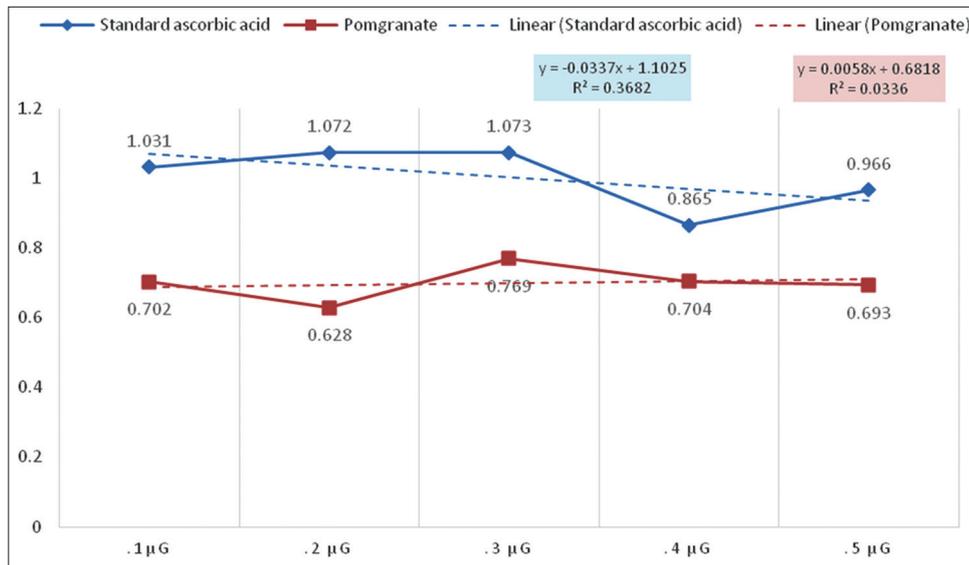


Fig. 3: 1, 1-diphenyl-2-picrylhydrazyl assay pomegranate peel extract

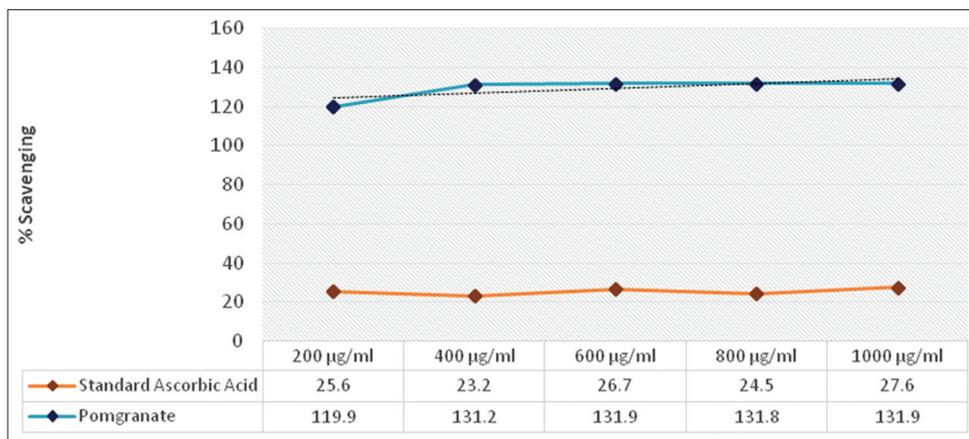


Fig. 4: Nitrous oxide scavenging activity of pomegranate peel extract

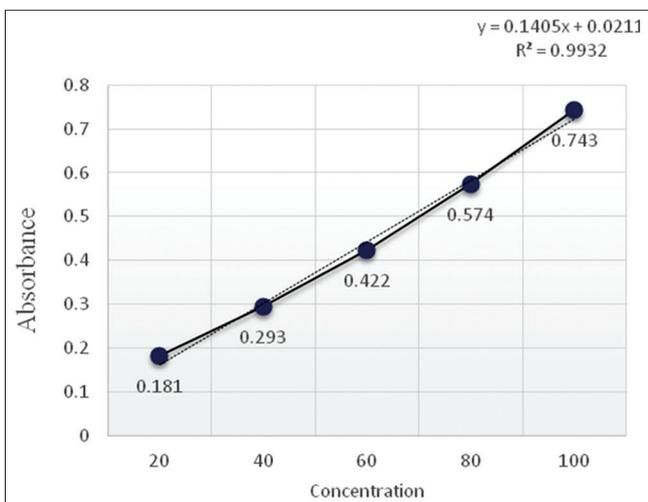


Fig. 5: Total phenolic contents of pomegranate peel extract

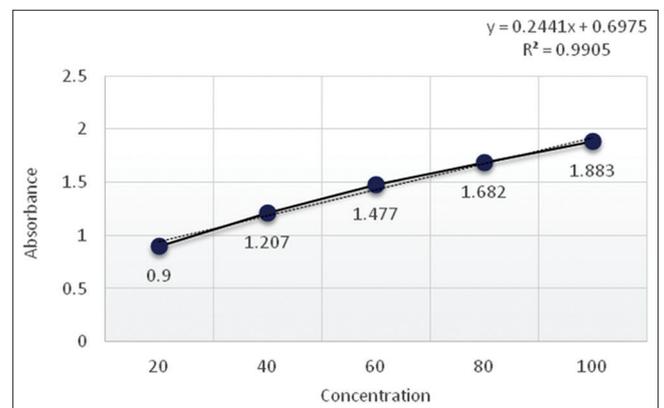


Fig. 6: Total flavonoid content of pomegranate peel extract

CONCLUSION

Pomegranate has an important role in folk medicine. It is known as a rich source of pharmacological properties. The present study showed

interesting preliminary phytochemical constituents in hydroalcoholic peel extracts of *P. granatum*. As the pomegranate peel extracts have promising antioxidant properties, so these can act as a powerful ingredient in increasing the hemoglobin count. Further, characterization and quantitative assay may be carried out to test the peel extracts for various therapeutic and pharmacological activities.

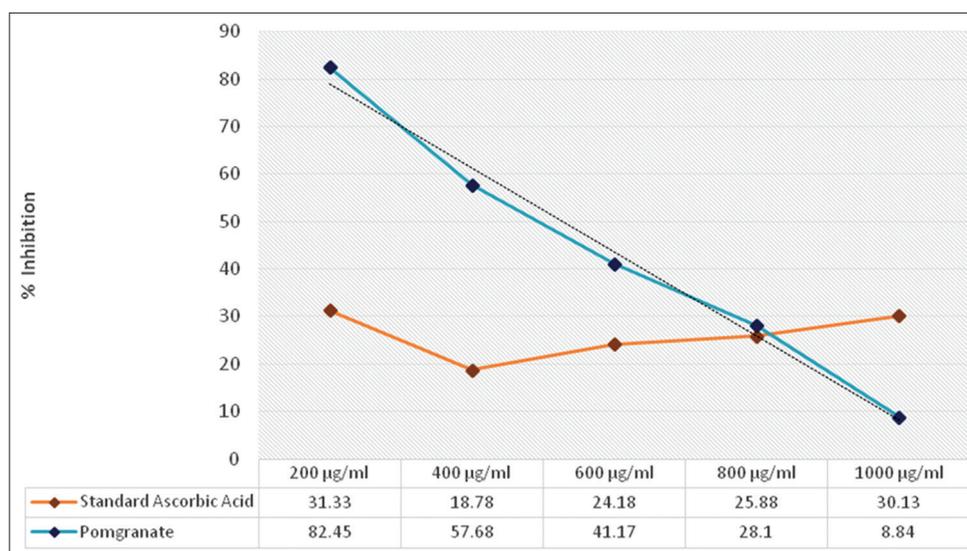


Fig. 7: Total antioxidant activity of pomegranate peel extract

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