



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

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ABSTRACT

Background: Pomegranate is the most significant tree which has been domesticated for incalculable human benefits and serves as a food medicine of great importance. Pomegranate has been found to have ethnomedical history and is a rich reservoir of phytochemicals. 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.

Methods: The present study was carried out for phytochemical analysis in the hydroalcoholic pomegranate peel extracts. The 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, DPPH free radical scavenging activity, nitric oxide radical scavenging activity was analyzed.

Results: For good health, the quality of blood should be maintained and for this antioxidant may be the key so as to protect the blood from oxidative stress. The results of various tests performed are compiled in various graphs and tables.

Conclusions: 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.

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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 [Kokate CK et al 2006]. 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 anti-bacterial 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 protects against degenerative ailments [Mutahar S, Shibani et al, 2012]. 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 anti-allergic, anti-inflammatory,

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anti-microbial, antioxidant etc [Huang DO et al 2005, Balasundram N et al 2006, Reddy M et al 2007]. 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 and 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 'super fruit.' [Sharma J et al. 2010]

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 [Li Y et al 2006, Reddy M et al 2007, Iqbal S et al 2008]. Various pomegranate supplements are found in the market [Negi P and Jayaprakasha J. 2003]. Ellagic tannins and ellagic acid are among the most potent antioxidants found in peel [Murthy KC et al 2002, Iqbal S et al 2008]. Antioxidants play an important role in the quality of our blood. Strong blood

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cells and vessels with adequate antioxidant capacity help maintain regular cholesterol levels essential for heart health helping keep heart diseases such as heart attacks etc at bay [http://healthforeversupplements.blogspot.in/2010/02/improve-blood-quality-lower-blood.html.Wednesday, February 10, 2010]. Pomegranate extracts are found to increase the hemoglobin count [Hemoglobin increase naturally Ayurveda Tips, 2016].

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 constitutes as a major group of compounds. These include stanins and flavonoids [Surya Prabha M *et al* 2013]. Whereas saponins behaves as cardio depressants and shows hypotensive properties [Olaleye MT.2007]. Glycosides are naturally cardio active drug and is used to treat heart failures [Brain FH *et al* 1985]. 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 then shade dried for 10 days .Then the pomegranate peels were grinded in the grinder. Then these are taken for extraction process



Pomegranate Peel



Grinded Pomegranate Peel

Preparation of hydro alcoholic extract

The powdered material is extracted with the help of Soxhlet apparatus. 200ml 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.

Initial weight of Pomegranate peels	=	320 grams
Weight after drying	=	65.984 grams
Solvent system used	=	Hydro-alcoholic
system (200 ml)		
Percentage yield	=	30.04%



Figure 1 Extraction Process

Phytochemical analysis

The hydroalcoholic extract of Punicagranatum fruit peel were studied for their phytoconstituents using different phytochemical tests [Ayoola GA *et.al* 2008].

Qualitative Phytochemical analysis

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

Molisch test

This test is performed to identify carbohydrates. In this 2.3 ml of the extract was taken and 4 drops of naphthol in alcohol is added. This is then shaken well and conc. H₂SO₄ is then poured from the sides of the test tube.

Test for Steroids

This test is known as the Salkowski test. In this 2 ml of chloroform and 2 ml of conc.H₂SO₄ are added to 2ml of the extract. This is then shaken well.

Test for Flavanoids

Take 2 ml of test solution then add Small quantity of reduced lead acetate solution (10%).

Millions Test

2ml. solution and Million Reagent will mix properly

Maeyer's Test

Take 2- 3 ml. filtrate extract solution with few drops mayer reagent.

Wagner's test

2-3 ml. filtrate extract solution with few drops wagners reagent.

Tannic acid and Phenolic tests

Take 2-3ml. of aqueous or alcoholic extract add few drops for following reagent.

Test for Sulphates

With lead acetate reagent gives white ppt. will form that soluble in NaOH solution.

Test for Chloride

Take 5-7 ml. filtrate plant extract solution and add 3-5 ml. lead acetate solution.

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 colour.
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 colour
Dilute iodine solution.	Solution will appear red colour
Dilute potassium permagnate solution	Solution will decolourizes its colour.

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 % aluminium chloride is added, after 6 min 0.5 ml of 1.0 M sodium hydroxide was added and the mixture were 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

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 Na₂CO₃ (~35 %) was added to 2 3 the mixture and made up to 10 ml by adding distilled water. The reaction was kept in the dark for 90 min, observed under 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 [Morsi RMY et al 2002].

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 (1, 1-diphenyl-2-Picrylhydrazyl) 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:

$$\text{Scavenging Activity (\%)} = \frac{(\text{Abs Control} - \text{Abs Sample})}{\text{Abs Control} \times 100} \text{ ----- (2.2.2.1)}$$

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 (0.025m, pH 7.4) was incubated with different concentration (100-400µg/ml) of methanol extract and tubes were incubated at 29°C for 3 hours. Control experiment without the test compounds but with equivalent amount of buffer was conducted in an identical manner. After 3 hours incubated samples were diluted with 1 ml of Griess reagents. The absorbance of the colour developed during diazotization of Nitrite with sulphanilamide and its subsequent coupling with Naphthylethylenediamine hydro-chloride was observed at 550nm on spectrophotometer. Same procedure was done with ascorbic acid which was standard in comparison to methanol extract. Calculated the % inhibition by formula and plot graph in compared to standard.

$$\text{Nitric Oxide (\%)} = \frac{(A_{\text{control}} - A_{\text{test}}) \times 100}{A_{\text{control}}} \text{ ----- (2.2.2.2)}$$

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 sub-fraction in ethanol, ascorbic acid used as standard (5 to 200g/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:

$$A = \frac{(c \times V)}{m} \text{ ----- (2.2.2.3)}$$

Where,

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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 [Kaliyan Barathikannan *et al.* 2016] 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 is 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 sulphates and chlorides are found absent in this extract.

Table 1

S.N.	Phytochemical	Name of Test	Result
1.	Carbohydrates	Molisch test	+
2	Steroids	Salkowski test	+
3	Proteins	Millions Test	+
4	Alkaloids	Maeyer's test	+
5	Alkaloids	Wagner's Test	+
6	Tannic Acid and Phenolic compounds	Tannic Acid and Phenolic Compounds test	+
7	Sulphates	Sulphate Test	-
8	Chlorides	Chloride Test	-
9	Flavanoids	Flavanoid Test	+

DPPH Free Radical Scavenging Activity

The DPPH radical scavenging activity of Pomegranate peel extract is depicted by the figure 1. 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 [Al-Zoreky NS. *et al* 2009, Hajoori M *et al.* 2014].

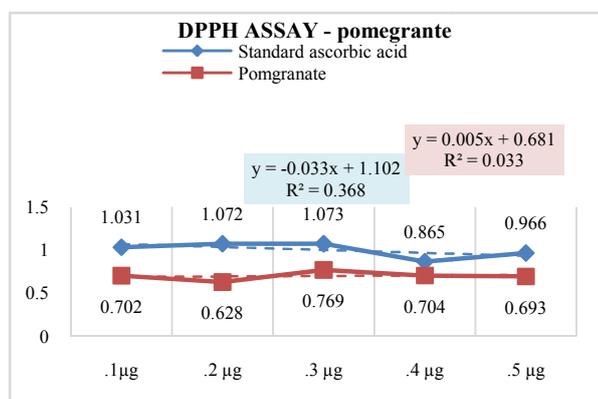


Figure 1 Dpph Assay Pomegranate Peel Extract

The respective values for pomegranate peel are also shown in figure 1 and table 2.

Table 2 Nitric oxide radical scavenging activity.

Concentration	Standard ascorbic acid	Pomegranate
0.1 µg	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

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 figure 2. 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.

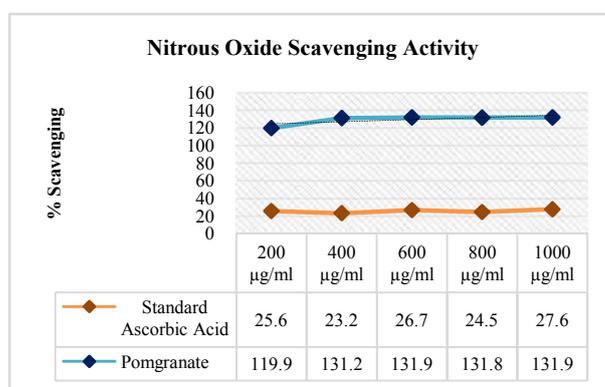


Figure 2 No Scavenging Activity of Pomegranate Peel Extract

Total phenolic content

The Total Phenolic Content was found to be 273.26 catechol equivalents/g. The results are in agreement to [Singh RP *et al* 2002] 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 [Yen GC *et al* 1994]. The results are shown in figure 2 and table 3.

Table 3 Total Flavonoid content

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

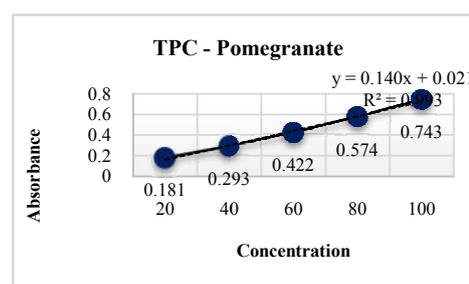


Figure 3 Total Phenolic Contents of Pomegranate Peel Extract

The total flavanoid content 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 $y = 0.244x + 0.697$ (figure 4) (table 4).

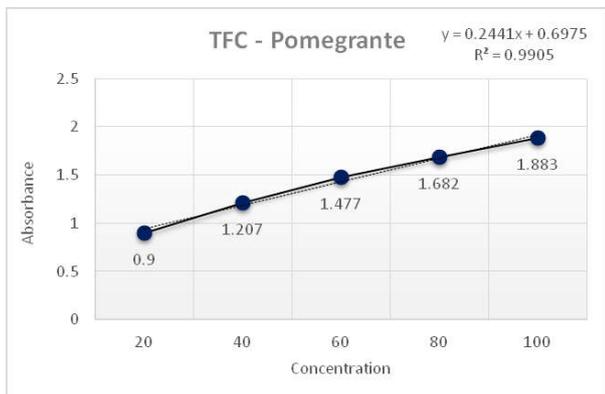


Figure 4 Total Flavonoid Content of Pomegranate Peel Extract

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 colour. The results shows that at lower concentration the percentage inhibition of pomegranate peel is more as compared to standard ascorbic acid. This is shown in figure 5. P. Granatum peel extracts can be used as a natural source of antioxidants.

Table 4 Total Antioxidant Activity

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

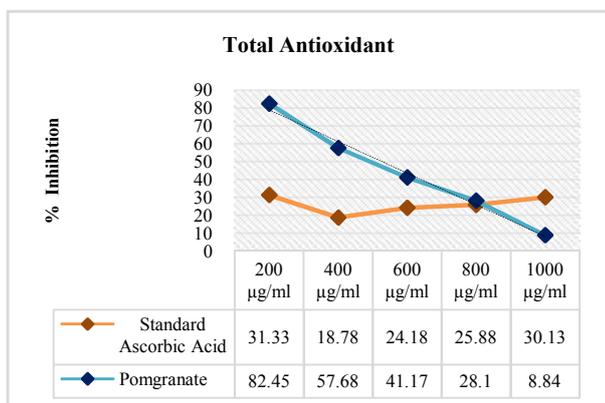


Figure 5 Total Antioxidant Activity of Pomegranate Peel Extract

CONCLUSIONS

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 Punica 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 activity.

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