International Journal of Pharmaceutical Science and Research
ISSN: 2455-4685
Impact Factor: RJIF 5.28
www.pharmacyjournal.net
Volume 2; Issue 6; November 2017; Page No. 24-27

Phytochemical screening and antioxidant scavenging activity of *Punica garanatum* L. fruit peel

[1] Sakthivel Vellaikkannu, [2] Vignesh Prasannan, [3] Saralabai Viswanathan Chathlingathe, [4] Dhanalakshmi Subramani
[1, 2] Department of Biotechnology, P.G. Extension Centre, Bharathidasan University, Perambalur, Tamil Nadu, India
[3] P.G. Research, Department of Botany, Government Arts College, (Autonomous) Coimbatore, Tamil Nadu, India
[4] Primary Health Centre, Inamkulathur, Tiruchirappalli, Tamil Nadu, India

Abstract
The pomegranate, botanical name *Punica granatum*, L. is a fruit-bearing deciduous shrub or small tree in the family Lythraceae that grows between 5 and 8 m (16 and 26 ft) tall. Pomegranate is rich in Vitamin C and act as effective detoxifying agent and various forms of pomegranate is being used to combat cough, sore throat, dental problems and heart diseases. The aim of the present study is to screen the phytochemical components of pomegranate peel extracts and also to investigate the free radical scavenging activity. The results showed that the total phenolics, flavonoids and polysaccharides were found to be more in methanolic than aqueous extract. DPPH scavenging activity of methanolic extract of pomegranate peel was found to be maximum (91.9%) than aqueous extract (69.46%). Hydrogen peroxide scavenging activity of methanolic extract was 28.32% whereas 20.29% was observed in aqueous extract. Among both extracts, methanolic extract was found to be most effective in scavenging free radicals and also found to be good antioxidant and therefore play an important role to protect damages caused by oxidative stress.

Keywords: pomegranate peel, phytochemicals, antioxidants, DPPH, free radical scavenger

1. Introduction
Human beings as well as some animals are consuming plant and plant parts as a source of nutrients as well as a medicine from time immemorial. Medicinal plants are important for their pharmaceutically valuable secondary metabolites like alkaloids, amino acids, antibiotics, various enzymes, steroids, flavonoids, terpenoids, glycosides, etc. Research on the plant derived products has been initiated to evaluate the feasibility of using herbal medicines in disease management (Kuganathan and Ganesh lingam, 2011).

Medicinal plants are a natural source of producing wide number of phytoconstituents. Since the middle of 19th century, Phenolic compounds, including flavonoids, anthocyanins and tannins are the main group of phytochemicals with interesting biological effects and have deep value due to their antioxidant and free radical scavenging activities (Elfalleh *et al*., 2011) [5].

Pomegranate peel extract have been found to possess wide applications in the food industry as they are an important source of phenolics, flavonoids and tannins occurring as natural ingredients and co-products of related preparation. From different parts of pomegranate plant with various bioactive compounds in pharmaceutical, pharmacological and medicinal potential have been isolated.

Free radicals are atoms or groups of atoms with an odd (unpaired) number of electrons and can be formed when oxygen interacts with certain molecules. Once formed these highly reactive radicals can start a chain reaction, like dominoes. To prevent free radical damage the body has a defense system of antioxidants. An antioxidant is a molecule that inhibits the oxidation of other molecules. Oxidation is a chemical reaction that can produce free radicals, leading to chain reactions that may damage cells. Antioxidants such as thiols or ascorbic acid (vitamin C) terminate these chain reactions. Total antioxidant activity, metal chelation, radical scavenging (DPPH) effects and reducing power as well as activities destructive to active oxygen species such as the superoxide anion radical, hydroxyl radical, and hydrogen peroxide are widely used for this purpose (Shimada *et al*., 1992) [12]. DPPH has two major applications, both in laboratory research: one is a monitor of chemical reactions involving radicals, most notably it is a common antioxidant assay and another is a standard of the position and intensity of electron paramagnetic resonance signals. The aim of the present study is to screen the phytochemical components of pomegranate peel extracts and also to investigate the free radical scavenging activity.

2. Materials and Methods
The *Punica granatum* L. fruits were procured from a Gandhi market in Tiruchirappalli city, Tamil nadu, India. The peel of the fruit was removed, shade dried and powdered with the help of a hand grinding mill. About 100g of the powder was extracted with methanol and distilled water for 4 hrs using soxhlet apparatus. The extract was filtered by using Whatmann No.1 filter paper and the filtrate was collected, then solvent was removed by a rotary evaporator at 50°C (Keser *et al*., 2011) [8].

2.1 Sample Preparation
The sample was prepared by dissolving 10mg of crude powder

in 100ml of both methanol and distilled water separately and this solution was used for phytochemical screening and study of antioxidant properties.

2.2 Qualitative Analysis of Phytochemicals

The phytochemicals were screened by following standard methods of Total Flavonoids (Ghias Uddin, 2011) [6], Tannins (Ayoola *et al.*, 2008) [1], Reducing sugar (Iyengar, 1995) [7], Phlobatannins (Ghias Uddin, 2011) [6], Terpenoids (salkowski test), Steroids (Mehta kavit, 2013) [10] and Glycosides (Egwaikhide, 2007) [4].

2.3 Quantitative Analysis of Phytochemicals

The phytochemicals were quantified by following the methods of Slinkard and Singleton, 1977 (total phenols), Djeridane *et. al*., 2006 (total flavonoids) and Keypour Sangesari, 2009 (total polysaccharides). Total antioxidant capacity (TAC) was determined by following the methodology of Uma maheshwari (2008). DPPH Radical Scavenging Assay: The antioxidant activity of the extracts was measured on the basis of the scavenging activity of the stable 1, 1- diphenyl 2-picrylhyorazyl (DPPH) free radical according to the method described by Brand-Williams *et al*., (1995) [2] with slight modifications. 1ml of 0.1mM DPPH solution in methanol was mixed with 1ml of plant extract solution of varying concentrations (50, 100, 150, 200 and 250 µg/ml). Corresponding blank sample were prepared and L-Ascorbic acid (1-100 µg/ml) was used as reference standard. Mixer of 1ml methanol and 1ml DPPH solution was used as control. The reaction was carried out in triplicate and the decrease in absorbance was measured at 517nm after 30 minutes in dark using UV-Vis spectrophotometer. The inhibition % was calculated using the following formula. Inhibition % = Ac-As/Ac×100 Where Ac is the absorbance of the control and As is the absorbance of the sample.

2.4 Hydrogen peroxide scavenging effect

To each extract in different concentrations (25µl, 50µl, 75µl, and 100µl/ml) 0.6ml of hydrogen peroxide solution was added. Blank tube contains only hydrogen peroxide without sample. All the test tubes were incubated at room temperature for 5min. After incubation, absorbance was read at 230nm (Ruch, 1989**)**. The analysis was performed in triplicate. H_2O_2 scavenging effect (%) was calculated by the following formula : A_0-A_1/ A_0 × 100 where A_0 = absorbance of control; A_1 = absorbance of sample/standard.

3. Results & Discussion

3.1 Screening of Phytochemicals

In the present study, the phytochemicals were screened in the crude powder of pomegranate peel extract and the results are given in Table 1.

Table 1: Phytochemical screening of both methanolic and aqueous extracts of pomegranate peel. The Data are mean values of three different experiments.

S. No	Phytochemical Components	Methanolic extract	Aqueous extract
1	Flavonoids	+	+
2	Saponins	+	+
3	Reducing sugars	+	+
4	Steroids	+	+
5	Phlobatannins	-	-
6	Tannins	+	+
7	Glycosides	+	-
8	Terpenoids	+	+

(+) - Present; (-) - Absent

From Table 1, it is inferred that different phytochemicals were present in both methanolic and aqueous extracts of pomegranate peel powder. Aqueous extract does not contain glycosides and phlobatannins. The phytochemical compounds are known to support bioactivities in medicinal plants **(**Prasad, 2012) and thus responsible for the antioxidant activities.

3.2 Quantitative assay of phytochemicals

Total phenolics, flavonoids, polysaccharides and total antioxidant capacity (TAC) of both methanolic and aqueous extracts of pomegranate peel and the results are represented in Fig. 1.

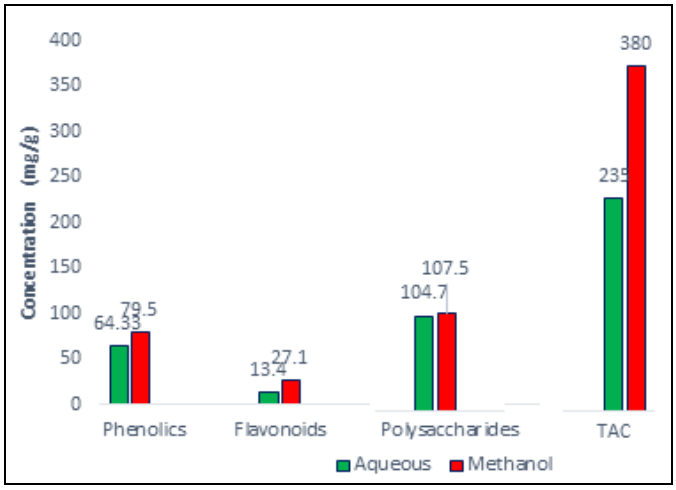

Fig 1: Total phenolics, flavonoids, polysaccharides and total antioxidant capacity (TAC) of both methanolic and aqueous extracts of pomegranate peel. The Data are mean of three different experiments

Total phenolic content of the methanolic extract was found to be maximum (79.54 mg/g) than aqueous fraction (64.33 mg/g). The flavonoid content of aqueous extract was

13.4 mg/g and of methanolic extract was 27.1 mg/g. The aqueous extract of pomegranate peel contains 104.7 mg/g of total polysaccharides and in methanolic extract 107.5 mg/g. It is inferred from Fig. 1, the methanolic extract contains more secondary metabolites and polysaccharides. Our findings are in agreement with prior studies (Mustafa, 2008). Methanol is efficient solvent due to its less polar nature than water; hence it helps in releasing polyphenols from the plant cell easily.

3.3 Antioxidant Scavenging Assays

3.3.1 DPPH Assay

DPPH was usually used as a reagent to evaluate free radical scavenging activity of antioxidants. The reduction capability of DPPH radical was determined by the decrease in absorbance at 517nm induced by antioxidants. Ascorbic acid was used as a standard and the results area given in Table 2. The scavenging effect of pomegranate peel methanolic extract was 91.90 % whereas aqueous extract was only 69.46 % compared to the standard ascorbic acid 78.79%.

3.3.2 Total Antioxidant Capacity (TAC)

Total antioxidant capacity of pomegranate peel extracts was expressed as the number of equivalents of ascorbic acid. The adopted phospho molybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate Mo (V) complex with a maximal absorption at 695nm. The methanolic extract was found to have a higher capacity than aqueous extract.

Table 2: Antioxidant assay of both methanolic and aqueous extracts of pomegranate peel. The Data are mean values of three different experiments.

Hydrogen peroxide (inhibition %)			DPPH scavenging (%)		
Concentration (µl)	Aqueous	Methanol	Aqueous	Methanol	Ascorbic acid
25	7.3	9.3	69.46	91.90	78.79
50	14	16.23			
75	18.12	21.18			
100	20.29	28.32			

3.3.3 Hydrogen Peroxide Scavenging Activity

The scavenging effect of methanolic and aqueous extracts of pomegranate peel on hydrogen peroxide was concentration-dependent (Table 2). The highest inhibition was shown by methanolic extract (28.32%) compared with aqueous extract (20.29%).

3.3.4 Reducing Power

The reducing power of a compound, which may sever as a significant reflection of antioxidant activity, was determined using a modified Fe (III) to Fe (II) reduction assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reducing power of samples. The presence of antioxidants in the samples causes the reduction of the Fe^{3+}/Ferric cyanide complex to the ferrous form. Therefore, the Fe^{2+} can be monitored by measurement of the formation of Perl's Prussian blue at 700nm. The reducing power of the extracts might be due to its hydrogen donating ability (Shimada, 1992) [12]. The reducing power was found to be increased with the increasing concentration of the extract, indicating the presence of bioactive compounds in the extract. The reducing power of both extracts and standard ascorbic acid antioxidant was revealed in Fig.2. The results indicated that methanolic extract have highest reducing power than the aqueous extract.

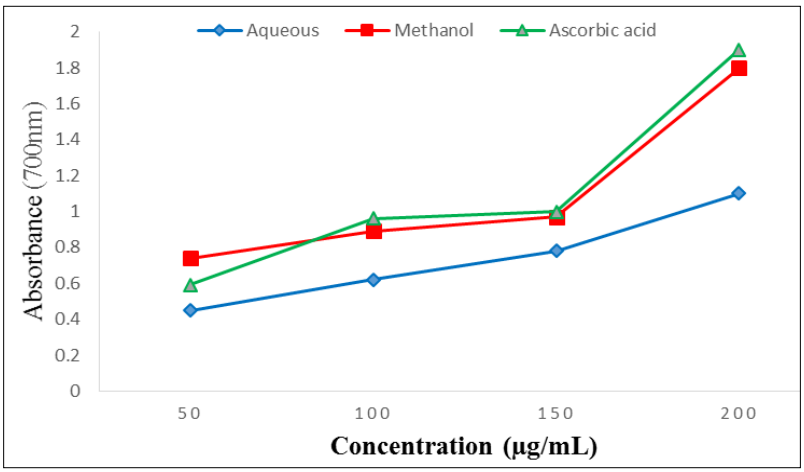

Fig 2: Ascorbic acid reducing power assay of different concentrations of methanolic and aqueous extracts of pomegranate peel. Ascorbic acid was used as standard. The Data are mean values of three different experiments.

The reductive capabilities of the plant extracts compared with ascorbic acid have been showed in Fig-2. The reducing power of the aqueous extract and methanol extract were found to be notable, which increased gradually with a rise in the concentration.

4. Conclusion

It may be surmised from the present study, the peel of pomegranate contains many secondary metabolites and also polysaccharides. Further, methanol is found to be more efficient than water in releasing polyphenols from the plant cell easily. It is obvious that methanolic extracts have high antioxidant capacity and can be used as natural antioxidants. Highest amount of phenols were found in the peel of pomegranate. The results are indicated that the peel of pomegranate are rich in primary and secondary compounds and therefore pharmaceutically very important and applicable in the health and food industries.

5. References

1. Ayoola GA, Coker HA, Adesegun SA, Adepoju-Bello AA, Obaweya K, Ezennia EC, *et al*. Phytochemical screening and antioxidant activities of some selected medicinal plants used for malaria therapy in Southwestern Nigeria. Tropical Journal of Pharmaceutical Research. 2008; 7(3):1019-1024.
2. Brand-Williams W, Cuvelier ME, Berset CLWT. Use of free radical method to evaluate antioxidant activity. LWT-Food Science and Technology. 1995; 28(1):25-30.
3. Djeridane A, Yousfi M, Nadjemi B, Boutassouna D, Stocker P, Vidal N. Antioxidant activity of some Algerian medicinal plants extracts containing phenolic compounds. Food Chemistry. 2006; 97(4):654-660.
4. Egwaikhide PA, Gimba CE. Analysis of the phytochemical content and anti-microbial activity of *Plectranthus glandulosis* whole plant. Middle-East Journal of Scientific Research. 2007; 2(3-4):135-138.
5. Elfalleh W, Ying M, Nasri N, Sheng-Hua H, Guasmi F, & Ferchichi A. Fatty acids from Tunisian and Chinese pomegranate (*Punica granatum* L.) seeds. International Journal of Food Sciences and Nutrition. 2011; 62(03):200-206.
6. Ghias uddin, Abdur Rauf, Muhammad Qaisar, Taj Ur Rehman, Abdul, Mumtaz Ali. Preliminary phytochemical screening and antimicrobial activity of *Hedera helix* L. Middle East Journal of Scientific Research. 2011; 8(1):198-202.
7. Iyengar MA. Study of Crude Drugs. 8th ed., Manipal Power Press, 1995.
8. Keser S, Celik S, Turkoglu S, Yilmaz O, Turkoglu I. Determination of antioxidant capacities of ethanol and water extracts of *Achillea millefolium* L. (Yarrow). Asian Journal of Chemistry. 2011; 23:3172-3176.
9. Keypour Sangesari S, Riahi H, Rafati H. Antibacterial activity of aqueous extract of Ganoderma lucidum Karst. J Med. Plant. 2009; 8:53-59.
10. Mehta kavit, Patel BN, Jain BK. Phytochemical analysis of lead extract of *Phyllantus fraternus*, Research journal of recent sciences. 2013; 2:12-15.
11. Prasad MP, Shekhar S. Amit B. Phytochemical analysis and antioxidant potential of Piper species and its molecular characterization by RAPD markers. International Journal of Fundamental and Applied Science. 2012; 1(1):7-10.
12. Shimada K, Fujikawa K, Yahara K, Nakamura T. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. J Agric. Food Chem. 1992; 40:945-948.
13. Slinkard K, Singleton VL. Total phenol analysis: automation and comparison with manual methods. American Journal of Enology and Viticulture. 1997; 28(1):49-55.
14. Umamaheswari M, Chatterjee TK. In vitro antioxidant activities of the fractions of *Coccinnia grandis* L. leaf extract. Afr. J Trad. Compl. Altern. Med. 2008; 5:61-73