



RESEARCH ARTICLE

PHYTOCHEMICAL ANALYSIS AND ANTI-OXIDANT ACTIVITY OF PUNICA GRANATUM L. FRUIT PEEL EXTRACT

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ABSTRACT

Pomegranate has been used for thousands of years to cure a wide range of diseases. It has been used in natural medicine to treat sore throats, coughs, digestive disorders, skin disorders and diabetes. Clinical research shows that pomegranate might help prevent heart disease, heart attacks. Pomegranate (*Punica granatum*) is a nutrient dense food rich in beneficial phytochemicals. Preliminary phytochemical constituents of *punica granatum* peel extracts were evaluated in the present study. The peel powder of *p. granatum* was extracted with ethanol. The ethanolic peel extract showed the presence of phytochemical constituents. The extract show anti-oxidant activity.

Key words: Antioxidant activity, phytochemical, *Punica granatum*.

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INTRODUCTION

The plants are always a rich source of compounds that do not appear essential for primary metabolism, including thousands of secondary metabolites and several macromolecules, such as peptides, proteins, enzymes, lignin and cellulose. Phytochemical are often referred to non-nutritive compounds thought to be produced by plants as means of protection against such dangers as harmful ultraviolet radiation, pathogens and herbivorous predators. The pomegranate, Botanical name is *Punica granatum* is a fruit bearing deciduous shrub or small tree in the family Punicaceae that grows between 5 to 8 meters (16 to 26 ft) tall. The fruit is typically in season in the Northern hemisphere from September to February and in the Southern hemisphere from March to May. pomegranate is a medicinal plant which belongs to punicaceae family. (subashini k 2016). As intact arils or juice, pomegranate are used in baking, cooking, juices, meal garnishes, smoothies, and alcoholic beverages such as cocktails and wine pomegranate is common name derived from Latin words Ponus and granatus, a seeded or granular Apple is a delicious fruit consumed worldwide. The fruit of pomegranate is rounded and red, catagorised as berry .The skin of fruit is thick and unedible but there are hundreds of ediable seeds called arils. (Dhinesh Babu 2010, Ahmad k et al 2015).

The fruit is a native to Afghanistan, Iran, China, and Indian subcontinent. The ancient sources of pomegranate inked Iran to Pakistan, China and Eastern India , where pomegranate had been under cultivation for thousands of years. In India Maharashtra is the leading producer of pomegranate followed by Karnataka, Andhra Pradesh. (Aher and Rahane 2016). *Punica granatum* is nutrient dense fruit rich in phytochemical compounds. phytochemical are of highest importance for evaluation of their potential health benefits to humans. Phenolic compounds, including Flavonoids, anthocyanins and tannins, are the main group of antioxidant phytochemical with interesting properties and have deeply value due to their biological and free radical scavenging activities. (Sampath et al 2016). There are two unique substances in the pomegranates that are responsible for most of their health benefits i.e. ; a) Punicalagins b) Punicic Acid, Punicalagins are extremely powerful antioxidants. Punicic Acid is also called as pomegranate seed oil, is the main fatty acid in the arils. Traditional medicine practitioners consider pomegranate as a provider of natural antiviral, antiviral, antifungal and antimicrobial benefits. Since ancient times, pomegranate juice has been used as a natural ancient astringent for treating diarrhea and harmful internal parasites. (Miguel et al 2010). The plant habit, flowering physiology and fruiting change is different with the habitat most commercially grown verities in India have an edge over the rest due to their flowering nature (Dhinesh Babu 2010).

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MATERIALS AND METHODS

Collection of plant material: Fresh fruits of *Punica granatum* were collected from local market of Nanded, Maharashtra. Then fruits are transported to laboratory, the fruits were washed with running tap water, rinsed well in distilled water and drying at room temperature for 10 min. the peel from the fruits was removed carefully and allowed to dry. the dried material was properly ground in to powder form and kept in refrigerator for further analysis.

Solvent extraction

Preparation of Ethanolic extract: 20 gm of dry powder of peel of *Punica granatum* was used for the extraction. The extraction was carried out by soxhlet method. For the extraction of peel ethanol solvent is used. The process was carried out for 6 hrs. The obtained extracts were evaporated at room temperature to get a dried solid product then stored in airtight bottles. The residual extracts were stored in refrigerator for further use.

Qualitative tests for phytochemical analysis: Phytochemical test were carried out on extract using for identification of various compound. The extract was tested for the presence of bioactive compounds by using following standard method of Niratker *et al.*, 2014 and Sangeetha and Jayaprakash 2015.

Test for protein: Ninhydrin test :-Crude extract when boiled with 1ml of 0.2% solution of ninhydrin, violet colour is appeared suggesting the presence of amino acids and protein.

Test for carbohydrates

Fehling's test: Equal of Fehling A and Fehling B reagents were mixed together and 1ml of sample was added to extracts and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicate the presence of reducing sugars.

Benedict's test: Extract when mixed with 1 ml of Benedict's reagent and boiled, reddish brown precipitate formed which indicate the presence of carbohydrates.

Iodine test: Extract were mixed 1ml of iodine solution respectively. A dark blue or purple coloration indicates the presence of carbohydrate.

Test for phenols and tannins: The extract was mixed with 1 ml of 1% solution of FeCl₃ respectively. A blue-green or black coloration indicates the presence of phenols and tannins.

Test for flavonoids: Shinoda test: Each extract was mixed with few fragments of magnesium ribbon and concentrated HCl was added drop wise. Pink scarlet color appeared after few minutes which indicated the presence of flavonoids.

Alkaline reagent test: Each extracts were with 1ml of 1%of solution of NaOH respectively. An intense yellow color was formed which turned colorless on addition of few drops of dilute acid which indicated the presence of flavonoids.

Test for saponins: Extract were mixed with 5ml of distilled water in respective test tubes and were shaken vigorously. The

formation of stable foam was taken as an indication for the presence of saponins

Test for glycosides

Liebermann's test: Extract were mixed with each of 1ml of chloroform and 1ml of acetic acid . The mixture was cooled in ice. Carefully concentrated H₂SO₄ was added. A colour changes from violet to blue to green indicated the presence of steroidal nucleus, i.e, and glycone portion of glycoside.

Salkowaski's test: Extract were mixed with 1ml of chloroform. Then 1ml of concentrated H₂SO₄ was added carefully and shaken gently. A reddish brown colour indicated presence of steroidal ring i.e., glycone portion of the glycoside.

Test for steroids: Extract were mixed each of 1ml of chloroform and concentrated H₂SO₄ was added sidewise. A red color produced in the lower chloroform layer indicates the presence of steroids. Another test was performed by mixing extracts with 1 ml of chloroform. Then 1ml of each concentrated H₂SO₄ was added and acetic acid was poured into the mixture. The development of a greenish coloration indicates the presence of steroids.

Test of terpenoids: Extract were dissolved in 1ml of chloroform and evaporated to dryness. To this 1ml concentrated H₂SO₄ was added and heated for couple of minute's. A grayish colour indicates the presence of terpenoids.

Test for alkaloids: Extract were mixed with 1ml of 1% HCl and gently heat. Mayer's and Wagner's reagents were then added to the mixture. Turbidity of resulting precipitate was taken as evidence for the presence of alkaloids.

Test for phlobatanins: 1ml of aqueous extract was added to 1ml of HCL and the mixture was boiled. Deposition of red precipitate was taken as an evidence for the presence of phlobatanins.

Antioxidant Activity of sample

DPPH radical scavenging assay (Shaikh *et al.*, 2014): DPPH radical scavenging assay was carried out as per the method reported earlier, with slight modifications. Briefly, 1 ml of the test solution (individual plant extracts) was added to an equal quantity of 0.1 mm solution of DPPH in ethanol. After 20 min of incubation at room temperature, the DPPH reduction was measured by reading the absorbance at 517 nm. Ascorbic acid (1 mM) was used as the reference compound.

RESULTS

Phytochemical analysis was done to determine the various phyto-components present in the extract of *Punica granatum* fruit peel. In the present study, phytochemical screening of the methanol Fruit extract showed the presence of Flavonoids, phenols, saponin, Tannin, Carbohydrates and alkaloid, but the absence of terpenoids and Glycoside was recorded as shown in the Table 1.

Antioxidant study: DPPH radical scavenging activity: The results of this assay it was observed that the ethanolic extract of the selected plant l fruit peel was more potent in stabilizing

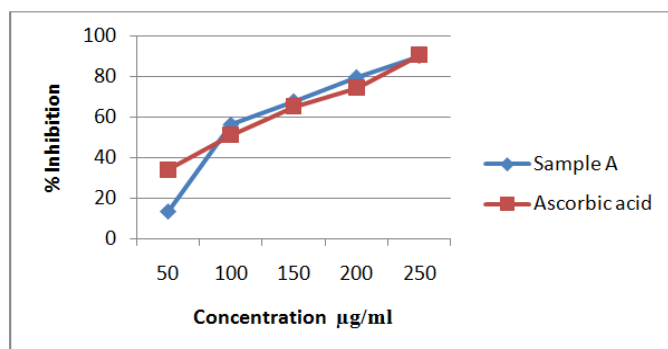
DPPH radicals. The ethanol extract of *Pomegranate (Punica granatum)* possessed the highest DPPH radical scavenging ability at 250µg/ml concentration.

Table 1. Phytochemical analysis of Ethanolic Extract of *Punica granatum* fruit peel

Sr. No.	TEST	RESULTS
1	Alkaloid	+
2	Flavonoids	+
3	Phenol	+
4	Glycoside	-
5	Saponin	+
6	Tannin	+
7	Terpenoids	-
8	Carbohydrates	+
9	Protein	-
10	Steroids	-
11	Phlobatanins	-

Table 2. Free radical scavenging activity of extract of *Punica granatum* fruit peel by DPPH radical inhibition

Concentration µg/ml	Sample	Ascorbic acid
50	13.72	34.21
100	56.36	51.18
150	67.76	65.13
200	79.56	74.47
250	90.08	90.65



Graph 1. Free radical scavenging activity of extract of *Punica granatum* fruit peel by DPPH radical inhibition.

DISCUSSION

The preliminary phytochemical analysis may be useful in the detection of bioactive compounds for the new drug discovery. Ethanolic extracts of fruit and peel of *P. granatum* were reported to have presences of flavanoids, carbohydrates, tannins, terpenoids, glycosides, vitamins, saponins and steroids (Sampath *et al* 2016, subashini 2016) similar results obtain in present study presence of same bioactive compounds. The biological screening of punica granatum extracts and compound have shown antioxidant, antiperoxidative, antibacterial, antitumor and antidiarrhoeal (Yogesh and Rahane, 2016) in our study the peel extract of *P. granatum* shows the antioxidant properties.

Conclusion

Plants are the best source of chemical compounds that show resistance against the number of micro organisms that cause disease. In the present research work the phytochemical analysis of fruit peel show the presence of secondary metabolites like Flavonoids, phenols, saponin and glycoside, alkaloid. The seed peel extract of *Punica granatum* show the destructive ability towards the free radicals as an antioxidant.

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