



# Antioxidant Potential Of Punica Granatum Peel And Seed Extracts

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## Abstract

The therapeutic efficacy of many indigenous plants for various diseases has been described by traditional herbal medicinal practitioners. The *Punica granatum* an ancient, mystical and highly distinctive fruit, is the predominant member of *Punicaceae* family.

Potential therapeutic properties of *Punica granatum* are wide ranging and include treatment and prevention of cancer, cardiovascular disease, diabetes, dental condition and protection from ultraviolet (UV) radiation. The medicinal importance of a plant is due to the presence of some special substance like alkaloids, glycosides, flavonoids etc. The active principle usually remains concentrated in the storage organ the plants. Considering all these, fact the present study was designed to investigate the presence of various phytoconstituents in selected part including seed and peel of plant *Punica granatum*.

## Introduction

Pomegranate (*Punica granatum* L.) is one of the oldest known edible fruit tree species, originating in Central Asia, but with a wide geographical global distribution, reflecting its adaptation to a wide range of climatic conditions <sup>[1]</sup>.

Pomegranate (*Punica granatum*) have prominent medical history and possess remarkable medicinal properties such as anti-inflammation, anti-cancer, anti-diarrhea, treat dental plaque and to combat intestinal infectious and malarial parasites. Recent studies also revealed the efficacy of the pomegranate fruit against cancer, atherosclerosis, infectious and coronary heart diseases<sup>[2]</sup>. Pomegranate peel is a thick reddish skin of pomegranate fruit, often discarded as a processing waste. It is a rich source for antioxidant and bio-preservatives such as polyphenols mainly ellagitannins and ellagic acid are identified in pomegranate peel

extract. Plant based anti-oxidants are generally extracted from raw materials or waste products of food industry by organic solvents such methanol, diethyl ether, and acetone. To avoid the decomposition, extracted pomegranate peel components are mix with bulking agents during drying as they are highly hygroscopic in nature. In recent years, studies have been conducted on utilization of pomegranate peel extract to improve the functional properties and shelf life of food products [3]. Pomegranate seeds are also rich trusted source in antioxidants, which can help protect the body against inflammation and free radical damage. Antioxidant content varies between pomegranate varieties, but all contain high levels of these healthful bioactive components [4].

## **Antioxidant**

Antioxidants are the substance that protects cells from the damage caused by free radicals (unstable molecules made by the process of oxidation during normal metabolism). Free radicals may play a part in prevention of disease like cancer, cardiovascular disease, diabetes, dental condition and protection from ultraviolet (UV) radiations etc. Antioxidant include beta- carotene, alkaloids, flavonoids, tannins, vitamins and other natural and manufactured substances. Pomegranate fruits with the scientific name of *Punica granatum L.*(belong to the family of Punicaceae) are excellent source of bioactive compounds mainly polyphenols. They are a rich source of polyunsaturated fatty acids, mostly linoleic acids and contain estrogenic compounds like estrone and estradiol, which are chemically identical to those synthesized in the human body.

## **Experimental Work**

### **Preparation of pomegranate peel powder (PPP)**

Pomegranate fruits shall be washed with distilled water and cut manually to separate the Seed and peel. The rind (peel) thus obtained shall be cut into small pieces using a sharp knife and dried in an air circulatory tray drier at  $60 \pm 5^{\circ}\text{C}$  for ~12 hrs. or till a moisture content of ~12-14% is reached. Dried pieces shall be cooled, powdered to be able to pass through a 20mesh sieve, packed in high density polyethylene bags and stored at room temperature ( $25 \pm 5^{\circ}\text{C}$ ) until use [5].

### **Preparation of pomegranate seed powder (PSP)**

Pomegranate fruits shall be washed with distilled water and cut manually to separate the seed and peel. The pomegranate arils shall be pressed manually to extract pomegranate juice. Pomegranate seeds (PS) thus obtained shall be washed with distilled water to remove any adhering pomegranate flesh and dried in an air circulatory tray drier at  $60 \pm 5^{\circ}\text{C}$  for 6 hrs or till its moisture content reaches ~5-6 %. Dried seeds shall be cooled, powdered to be able to pass through 40 mesh screen, packed in high density polyethylene bags and stored at room temperature ( $25 \pm 5^{\circ}\text{C}$ ) until use.[5]

## Decoction

The pomegranate peel & seed both powders are weighted 25g, then extracted with 100ml distilled water for 30min at 90° C in heating mentle. Decoction was filtered & diluted to mark with distilled water in 100ml volumetric flask. The method described by Tiwari et al. was used for phytochemical screening of the secondary metabolites in simplicia & decoction. The specific reagent were used to detect flavonoids, polyphenol, tannis, alkaloids & saponins. The decoction extract was concentrated with the assistance of water bath thermostatic. [6]



**Fig PPD – Pomegranate Peel Decoction**

**PSD – Pomegranate Seed Decoction**

## Maceration

Maceration is a simple extraction method that involves soaking the plant prepared raw material in a coarse or powder form in a solvent of interest at room conditions for at least three days with intermittent agitation. After the extraction is completed, the mixture is strained either through sieves or a net with tiny holes. Subsequently, the marc is pressed, and the liquid extract is cleaned using either filtration or decantation after standing. Maceration is preferably carried out in a stoppered container to minimize solvent loss through evaporation. It is undesirable to obtain an already concentrated extract through evaporation of the solvent during the extraction process. The product is concentrated frequently by the use of vacuum evaporation. It is crucial to select an appropriate solvent in the maceration as the solvent will delineate the phytochemicals classes salvaged from the samples. The solvent could also enable the extraction of thermolabile phytochemicals. [7]



**Fig. PPM – Pomegranate Peel Maceration**

**PSM - Pomegranate Seed Maceration**

**Phytochemical Investigation <sup>[8]</sup>**

**Table No 1: Test For Alkaloids**

<b>Sr. No.</b>	<b>Test</b>	<b>Observation</b>	<b>Inference</b>
01.	<b>Dragendorff's test:</b> Few mL filtrate + 1- 2 ml Dragendorff's reagents.	Formation of orange brown color precipitate.	Alkaloids are present.
02.	<b>Mayer's test:</b> Add few drops of Mayer's reagent to the 3ml of extract.	Formation of cream color precipitate.	Alkaloids are present.

Table No 2. Test for Flavonoids and Phenolic compound

Sr. No.	Test	Observation	Inference
01.	<b>Lead acetate test:</b> To small quantity of extract, add lead acetate solution.	Yellow colored precipitate formation.	Flavonoids & Phenolic compound are present.
02.	<b>Sodium Hydroxide test:</b> 1mL extract + 2mL of 2% NaOH solution (+ few drops dil. HCl)	An intense yellow colour, becomes colourless on addition of diluted acid.	Flavonoids & Phenolic compound are present.
03.	<b>Ferric chloride test:</b> Extract aqueous solution + few drops 10% ferric chloride solution.	A green precipitate.	Flavonoids & Phenolic compound are present.

Table No 3. Test for Saponins

Sr. No.	Test	Observation	Inference
01.	<b>Foam test:</b> 0.2gm plant extract + 5mL distilled water; shaken well; heated to boiling	Appearance of creamy mass of small bubbles	Saponins are present
02.	<b>NaHCO<sub>3</sub> test:</b> Plant extract + few mL sodium bicarbonate solution + distilled water	Stable honeycomb like froth	Saponins are present

Table No.4 Test for Tannis

Sr. No.	Test	Observation	Inference
01.	<b>Braymer's test:</b> Extract + 3mL distilled water + 1drops 10% Ferric chloride solution.	Blue-green colour.	Tannins are present.
02.	<b>10% NaOH test:</b> 0.4mL plant extract + 4mL 10% NaOH + shaken well	Formation of emulsion (Hydrolysable tannins)	Tannins are present.
03.	<b>Bromine water test:</b> 10 ml of bromine water + 0.5gm plant extract	Decoloration of bromine	Tannins are present.

### Determination of Anti-Oxidant Activity

Antioxidant activity in the sample compound was estimated for their free radical scavenging activity by using DPPH (1,1-Diphenyl-2-picryl-Hydrazyl) free radicals (George et al., 1996). 100 microL of 0.1% methanolic DPPH was added over the samples at different concentration (1000micro gm/ml) & incubated for 30 min in dark condition. The samples were then observed for discoloration; from purple to yellow & pale pink were considered as strong & weak positive respectively & read the plate on Elisa plate reader at 490nm Radical scavenging activity was calculated by the following equation. <sup>[9,10,11]</sup>

**DPPH radical scavenging activity (%) =**

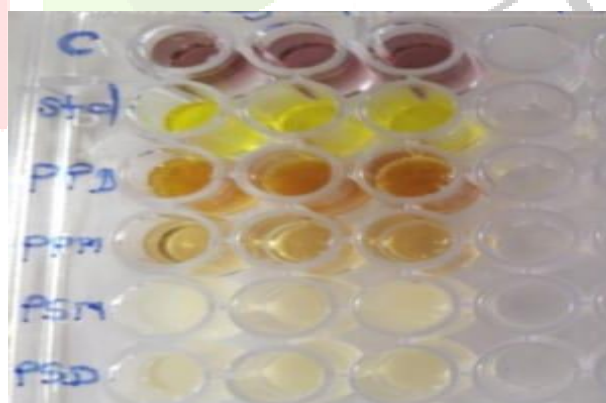
$$[(\text{Absorbance of control} - \text{Absorbance of test sample}) / (\text{Absorbance of control})] \times 100$$

### RESULT AND DISCUSSION

Extract of raw material was prepared by using different extraction method were prepared & evaluated for its antioxidant potential using DPPH scavenging assay.

**Table No. 5: Antioxidant activity result**

Sample code	Concentration	Antioxidant activity by DPPH (96 well method)		
		Absorbance	Mean	% Inhibition
Control	-	1.101	1.608	
		1.912		
		1.813		
Standard Ascorbic acid	1000 microgram	0.150	0.125	92.22
		0.106		
		0.120		
Sample - PPD	1000 microliter	0.546	0.572	64.11
		0.589		
		0.582		
Sample - PSD	1000 microliter	0.485	0.475	70.46
		0.470		
		0.472		
Sample - PPM	1000 microliter	0.486	0.464	71.14
		0.494		
		0.412		
Sample - PSM	1000 microliter	0.352	0.346	78.48
		0.340		
		0.346		

**Fig. Antioxidant activity**

The delocalization of electron also gives rise to deep violet color, characterized by adsorption band in ethanol solution centered at about 517nm. When a solution of DPPH is mixed with that of a substrate that can donate a hydrogen atom, then this gives rise to reduced form with the loss of this violet color. The direct scavenging activity of samples was evaluated against DPPH scavenging assays. In this, all compounds exhibited good inhibition against standard ascorbic acid.

## Conclusion

Extraction methods has been used since decade to extract the active components with pharmacological activity. Various extraction methods are reported like maceration, decoction, digestion, percolation, continuous hot extraction for extraction of the phytoconstituents and selection of all these methods depends on various factors like components to be extracted or type of crude drug etc.

In the present study antioxidant potential of *Punica granatum* was determined by using different extracts which were prepared by different extraction methods like decoction & maceration. Phytochemical investigation of each extract was carried out which shows presence of different phytoconstituents like alkaloid, flavonoids, flavonoids, phenolic compound, saponins, tannins etc.

Each extract was further evaluated for antioxidant potential & after comparing both the extract for antioxidant potential, it was found that the percent inhibition was 64.11% for sample PPD, 70.46% for sample PSD, 71.14% for sample PSM and 78.48% for sample PSM. While comparing with the peel and seed, the percentage inhibition was maximum found in the seed of *Punica granatum*.

## Conflict of Intrest

The authors have no conflicts of interest regarding this investigation.

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