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Evaluation of Phytochemicals and Antioxidant Potential of Pomegranate Peel

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A.Jeevarathinam*[1] and P.Muthulakshmi[2]

[1]*Assistant Professor of Food Processing and Quality Control, V.V.Vanniaperumal College for Women, Virudhunagar, Tamilnadu, India.*

[2]*Student, Department of Food Processing and Quality Control, V.V.Vanniaperumal College for Women, Virudhunagar, Tamilnadu, India.*

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ABSTRACT

The aim of the present study was to evaluate the phytochemical and antioxidant properties of Pomegranate peel aqueous extract. Qualitative phytochemical screening of pomegranate peel extracts was assessed by standard methods. The phytochemical constituents present in aqueous extract of *Punica granatum* peel were terpenoids, cardiac Glycosides, Saponins, Alkaloids, Flavonoids and Tannins. DPPH assay, reducing power assay were used to study antioxidant potentials of extracts. Results indicated that the pomegranates peel shows 43.24% of antioxidant activity. The results from our studies support the fact that pomegranate fruit is good source of phytochemicals which could be used for isolation of therapeutic compounds and to develop infusions, nutraceuticals and pharmaceuticals.

INTRODUCTION

Phytochemicals 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 consumption of a plant-based or phytochemical-rich diet has been associated with a reduced risk of chronic human illnesses such as certain types of cancers, inflammation, cardiovascular and neurodegenerative diseases (Beretta *et al.*, 2009). Phenolic compounds, including flavonoids, anthocyanins and tannins, are the main group of antioxidant phytochemicals with interesting properties and have deep value due to their biological and free radical scavenging activities (Elfalleh *et al*., 2011). Pomegranate (*Punica granatum L*.) belongs to the Punicaceae family. The fruit can be divided into several anatomical origins: peel, seeds, and arils. The juice is edible but seed (without juice) and peel are considered as inedible portion. This inedible portion is found to be with higher medicinal value. Pomegranate peels are exploited in traditional medicine because of their strong astringency, making them a popular remedy throughout the world. Pomegranate peel attracts attention due to its apparent wound healing properties, immune modulatory activity and antibacterial activity antiatherosclerotic and antioxidative capacities (Tzulker *et al.*, 2007). Comparatively the antioxidant property of pomegranate juice is higher than other fruit juices, red wine and green tea. Tannins and flavonoids are phenolic compounds and plant phenolic is a major group of compounds that act as basic antioxidants or free radical scavengers. Saponins have hypotensive and cardio depressant properties. Glycosides are naturally cardioactive drugs used in the treatment of congestive heart failure and cardiac arrhythmia (Radwan *et al., 2014).* Considering all these facts, the present study was designed to reveal the presence of phytochemicals and to quantify the total phenols and flavonoids beside the antioxidant activity of pomegranate of peel.

MATERIALS AND METHODS

Collection of Plant Material

The fruits of *Punica granatum* were collected from the local market, Virudhunagar and the specimens were identified.

Preparation of pomegranate peel powder

Pomegranate fruits were washed and cut manually to separate the seeds and peel. The rind (peels) thus obtained, cut into small pieces using a sharp knife and dried in an air circulatory tray drier at 60±5ºC for 6hrs. The pomegranate fruits were handy peeled and the required fruit rind was cut and removed from the fruits. The fruits rind (pomegranate peel) was dried in an oven at 4^0C for 24h, then mechanically powdered and the find powder was sieved through 24-mesh, then it was packed in high density polyethylene bags and stored at ambient temperature (25±5ºC) until use (Devatkal *et al.*, 2010).

Extraction of pomegranate peels

10 g of the dried powder was taken in 100 ml of water in conical flask, plugged with cotton wool and then kept in an orbital shaker at 120 rpm for 24h. After 24h the extract was filtered through Whatman filter paper no 1 for removal of peel particles. The dry extract was stored at 4°C.

Determination of extraction yield (Balasundram*et al, 2006)*

The residues obtained after filtration were weighed to obtain the extraction yield.
Extraction yield (%) = (weight of the residue) / (total weight of the peel powder)×100

Qualitative analysis of Phytochemicals

The test sample was subjected to phytochemical analysis in order to find out the presence of phytochemical constituents. The phytochemical tests employed for Carbohydrate, Vitamin C, alkaloids, tannins, cardiac glycosides, saponins, flavonoids and terpenoids (Sarah *et al.*, 2015).

Test for Carbohydrate:

Benedict's test – Test solution was mixed with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and boiled in water bath, observed for the formation of reddish brown precipitate to show a positive result for the presence of carbohydrate.

Test for Vitamin C:

DNPH Test– Test solution was treated with Dinitrophenylhydrazine dissolved in concentrated sulphuric acid. The formation of yellow precipitate would suggest the presence of vitamin C.

Test for tannins

Lead test

1-3 drops of ferric chloride were added to the 1ml pomegranate peel extract. Then the mixture was observed for blue and green color.

Test for cardiac glycosides

Dissolved in 1ml of glacial acetic acid and 1-2drop of ferric chloride solution was added to the 1ml pomegranate peel extract. 0.5ml of concentrated sulphuric acid was slowly added along the sides of the test tube. A brown ring at the interface indicated a deoxysugar characteristic of cardenolides.

Test for saponins

Foam test

1ml pomegranate peel extract dissolved in 5ml of distilled water and shaken vigorously till a stable persistent froth was obtained. The froth was mixed with 3drops of olive oil and shaken vigorously and then observed emulsion.

Test for flavonoids

Ferric chloride test

1ml pomegranate peel extract mixed to 0.5ml of dilute ammonia solution was added to 1 ml of conc. sulphuric acid was added later. A yellow color indicated the presence of flavonoids. The yellow color disappeared on allowing the solution to stand.

Test for terpenoids

Salkowski's test

Dissolved in 1ml of chloroform and 1ml of concentrated sulphuric acid was added to the 1ml pomegranate peel extract. A reddish brown discoloration at the interface showed the presence of terpenoids.

Test for lactones

Baljet test

Treat the pomegranate peel extract with sodium picrate solution. Appearance of yellow to orange color indicates presence of lactone ring.

Fixed oils and fatty acid

Spot test

Prepared spot on the filter paper with the test solution and oil staining on the filter paper indicated the presence of fixed oil and fats.

Quantification of phytochemicals in pomegranate peel

Determination of Total Phenol Content (Gutfinger, 1981).

The amount of total phenol content was determined by Folin-Ciocalteau reagent method. 1 ml of 50% Folin-Ciocalteau reagent was mixed with 1ml of pomegranate peel extract and the mixture was incubated at room temperature for 15 mins. Then 2.5 ml of sodium carbonate solution was added and further incubated for 30 mins at room temperature and the absorbance was measured at 760nm. Total phenol values are expressed in terms of catechin equivalent (mg/g of extracted compound).

Flavonoid determination (Bohan *et al.,* 1974)

2g of the sample was extracted repeatedly with 20ml of 80% aqueous methanol at room temperature. The whole solution was filtered through what man filter paper no 42 (125mm). The filtrate was later transferred into a crucible and evaporated to dryness over a water-bath and weighed to a constant weight. Percentage of crude flavonoids was calculation as,

$$\text{Percentage of total flavonoid} = \frac{\text{Weight of residue}}{\text{Weight of sample taken}} \times 100$$

Saponin determination *(*Obdoni *et al*., *2001)*

The samples were ground and 2g of each were put into a conical flask and 20 cm of 20% aqueous ethanol were added. The samples were heated over a hot water bath for 4h with continued stirring at about 55°C. The mixture was filtered and the residue re-extracted with another 200 ml 20% ethanol. The combined extracts were reduced to 40 ml over water bath at about 90°C. The concentrate was transferred into a 250ml separatory funnel and 20 ml of diethyl ether was added and shaken vigorously. The aqueous layer was recovered while the ether layer was discarded. The purification process was repeated. 60 ml of n-butanol was added. The combined n-butanol extracts were washed twice with 10 ml of 5% aqueous sodium chloride. The remaining solution was heated in a water bath. After evaporation the sample was dried in the oven to a constant weight: The saponin content was calculated as

$$\text{Percentage of total saponin} = \frac{\text{weight of residue}}{\text{Weight of sample taken}} \times 100$$

Determination of Proanthocyanidin (Li *et al.*, 2006)

A quantity of 0.05 g of dried extracts was dissolved in 5 ml methanol or the filtrates made up to 50 ml were used directly. A volume of 1 ml solution was mixed with 3 ml of 4% vanillin–methanol solution and 1.5 ml hydrochloric acid and the mixture was allowed to stand for 15 min at room temperature. The absorbance at 500 nm was measured and the Proanthocyanidins was expressed as catechin equivalents (CE, g/100g dry mass) using a catechin (0~0.08 mg/ml) standard curve.

Study on antioxidant activity of pomegranate peel

Reducing power assay (Behera *et al.* **2006)**

Reducing power was determined by the method prescribed by the sample in 1ml of methanol at various concentrations was mixed with a phosphate buffer (5 ml, 0.2 M, pH 6.6) and potassium ferricyanide (5 ml, 1%), and the mixture was incubated at 50°C for 20 min. Next,

5ml of trichloroacetic acid (10%) were added to the reaction mixture, which was then centrifuged at 3000 RPM for 10 min. The upper layer of the solution (5 ml) was mixed with distilled water (5ml) and ferric chloride (1 ml, 1%), and the absorbance was measured at 700 nm. A stronger absorbance will indicate increased reducing power.

Determination of DPPH radicals scavenging activity (Terao *et al.*, 1988)

The free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH. Determination of DPPH radicals scavenging activity was estimated with the method used by Kato. 1mM solution of DPPH in ethanol and also 1mg/1 ml extract solution in ethanol was prepared and 1.5ml of this solution was added to 1.5 ml of DPPH. The absorbance was measured at 517 m against the corresponding blank solution which is prepared by taking 3ml ethanol and control O.D. was prepared by taking 3ml of DPPH. The assay was performed in triplicates. Percentage inhibition of free radical DPPH was calculated based on control reading by following equation.

$$\text{DPPH scavenged (\%)} = \frac{(\text{A con} - \text{A test})}{\text{A con}} \times 100$$

A con - is the absorbance of the control reaction

A test - is the absorbance in the presence of the sample of the extracts.

RESULTS

For the photochemical study of pomegranate peel, the water is used as the solvent for extraction. The pomegranate peel yield of extract was 27%. The aqueous peel extract was used for the further studies. The extracts of pomegranate peel have revealed the presence of terpenoids, cardiac Glycosides, Saponins, Alkaloids, Flavonoids, and Tannins only in the peel extract. The results of preliminary phytochemical analysis shown in Table 1.

Table 1: Preliminary phytochemical analysis of pomegranate peel powder

Sr. No.	Name of the phytochemicals	Result
1.	Carbohydrate	Positive
2.	Vitamin C	Positive
3.	Alkaloids	Positive
4.	Terpenoids	Positive
5.	Flavonoids	Positive
6.	Saponins	Positive
7.	Tannins	Positive
8.	Cardiac glycosides	positive
9.	Fixed oil	positive

The phytochemical screening of present study results agreement with Hajoori*et al*, 2014 who found that phytochemical screening of *Punica granatum* peels aqueous extract show presence of flavonoids, steroids, cardiac glycosides and terpenoids.

Figure 1 illustrates the reducing power of peel extracts using the ferricyanide reduction method. The increase in absorbance at 700 nm indicated better reducing power of test materials. The reducing power of the extract of pomegranate peel was found to be remarkable, which increased gradually with a rise in the concentration. The maximum reducing property was found at 400 mg/ml of extract of pomegranate peel. Findings of the present study regarding antioxidant activity and reducing power assay result are similar to Pan *et al* (2007) whose result indicated a positive correlation between the reducing power and the antioxidant activity of *Polygonum cuspidatum* extract.

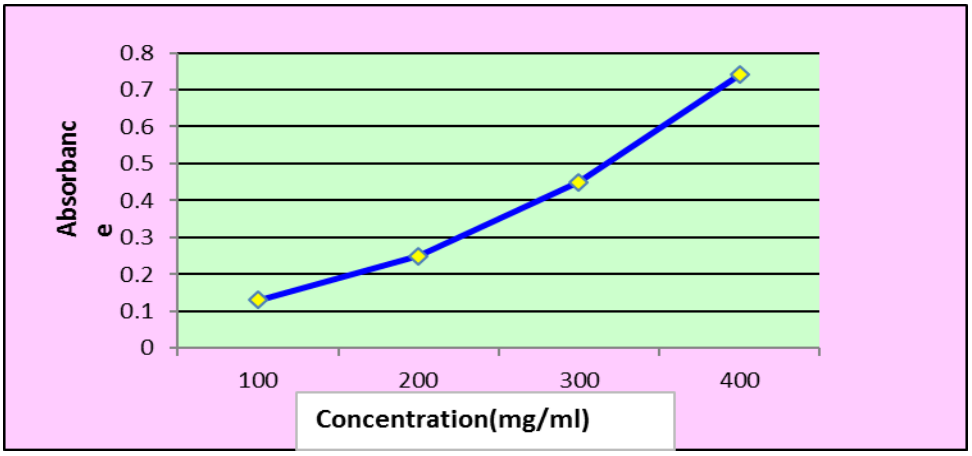

Figure 1: Reducing power of Pomegranate peel aqueous extract

Table 2: Antioxidant compounds and Total Antioxidant Activity of Pomegranate peel

Constituents	Per 100g
Yield of Extract	27%
Total Polyphenols	500mg
Flavonoids	2.5%
Proanthocyanidins	28mg
Antioxidant activity	43.24%

The antioxidant compounds and total antioxidant activity of pomegranate peel aqueous extract was determined by standard techniques results are represented in table 2. The peel of pomegranate was found to contain 0.5 g of total phenol, 2.5% of Flavonoids and 28µg/g of proanthocyanidins. The peel extract showed 43.24% of antioxidant activity. Zhenbin Wang *et al* 2011 compared methanol with water as the solvent in pomegranate peel antioxidant extraction, the total extract yield were 43.18% - 46.51% and the DPPH antioxidant activities were 53.74% - 65.30%, respectively. These studies gave strength to our findings.

CONCLUSION

The present study results concluded that the phytochemicals were present in the aqueous extracts of *Punica granatum* peel. The presence of phytoconstituents makes the plant useful for treating different ailments and have a potential of providing useful drugs of human use. It also concluded that inedible portion of pomegranate contains higher phytochemical constituents which can be used as the functional food.

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