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Research Article

ANTIOXIDANT ACTIVITY OF POMEGRANATE PEEL POWDER

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ABSTRACT

The aim of present study was to evaluate in-vitro anti-oxidant properties of *Punica granatum* fruit (Pomegranate fruit) peel. Antioxidants are molecules involved in defense mechanisms against the deleterious effects of free radicals in most organisms. Antioxidants are the agents responsible for scavenging free radicals. A number of methods are currently being used for the evaluation of the antioxidant and free-radical scavenging properties of natural and synthetic antioxidants, including the DPPH method. The *Punica granatum* fruit (Pomegranate fruit) peel powder suspension was prepared and the DPPH radical scavenging assay was the method adopted to determine antioxidant potentials of aqueous suspension of pomegranate peel powder. Results revealed that DPPH aqueous solution gave comparable free-radical activity 24 hours post preparation compared with the freshly prepared solution. After 24 hours, activity was greatly reduced. It is, therefore recommended that freshly prepared DPPH solution should be used at all times; however for prolonged experimental schedules, the DPPH solution should be used within 24 hours post preparation, so as to give comparable results with the freshly prepared solution and avoid ambiguity in results interpretation. Aqueous suspension of peel powder showed good antioxidant effect. Phenolic compounds, tannins and flavonoids are the major phytochemicals present in the pomegranate peel. Percentage of inhibition increased with the increased concentration of extracts. The present study provides evidence that the *Punica granatum* fruit peels is a potential source of natural antioxidant.

Keywords: Antioxidant, Pomegranate peel powder suspension, DPPH, Free radical scavenging.

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INTRODUCTION:

Punica granatum (Punicaceae) fruit peels [commonly called pomegranate] is rich in antioxidant of polyphenolic class which includes tannins and flavonoids.[1] Antioxidant activity has been proposed to play vital role in various pharmacological activities such as anti-aging, anti-inflammatory and anti-atherosclerotic activities. Inhibition of free radical induced damage by supplementation of antioxidants has become an attractive therapeutic strategy for reducing the risk of diseases. Oxidative stress is initiated by free radicals, which seek stability through electron pairing with biological macromolecules such as proteins, lipids and DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. These changes contribute to cancer, atherosclerosis, cardiovascular diseases, ageing and inflammatory diseases. All human cells protect themselves against free radical damage by enzyme such as SOD and catalase or compounds such as ascorbic acid, tocopherol and glutathione. Sometimes these protein mechanisms are disrupted by various pathological process and antioxidant supplements are vital to cambat oxidative damage.

Recently, much attention has been directed towards the development of ethno medicines with strong antioxidant

properties but low cytotoxicities. Several synthetic antioxidants are available, but are quite unsafe and their toxicity is of concern[2]. Natural product with antioxidant activity may be used for human consumption because of their safety. In the present study antioxidant activity of Pomegranate Fruite Peel Powder was determined. Plants are rich sources of natural antioxidants, the best known are tocopherols, carotenoids, vitamin C, flavonoids and different other phenolic compounds .Recently, among natural antioxidants, dietary flavanoids are considered to be more powerful antioxidants and received increasing attention as potential protectors against a variety of human diseases, in particular cardiovascular disease and cancer[3]. Flavanoids are found higher in plant fruit peel, and are proposed as food supplements. Flavanoids are known to be highly effective antioxidants by scavenging oxygen radicals. Moreover, the protective effects of flavanoids in biological systems are attributed to their capacity to scavenge free radicals, chelates metal catalysis, activate antioxidant enzymes, reduce alpha tocopherol radicals and inhibit oxidases. The pomegranate (*Punica granatum*) belonging to punicaceae family widely distributed all over the world and have highly distinctive nutritional value. In the ayurveda system of medicine, the pomegranate has extensively been used as a source of traditional remedies for housands of years. The rind of the fruit and the bark of the pomegranate tree are used as a traditional remedy against diarrhea, dysentery and intestinal parasites. Pomegranate juice is also used as eye drops as it is believed to slow the development of cataracts. Antioxidants contents were as follows: peel > flower > leaf > seed[4]. It was reported that pomegranate peel extracts exert anti-oxidant and anti-mutant activities in vitro due to their content of polyphenols (tannins, ellagic and gallic acids). These substances have been used in the preparation of cosmetics and tinctures as well as in therapeutic formulas and food recipes[5]. On the other hand, it was also reported an anti-oxidant and anti-sclerotic effects of pomegranate syrup on animal models in vitro. Furthermore, human studies have shown that daily consumption of pomegranate juice lowers blood pressure in hypertensive subjects, delays the atherosclerotic process and increases the total antioxidant status of blood[5, 6, 7]. Pomegranate juice has a remarkable ability to decrease oxidative stress by 40–80% and to increase the antioxidant enzymes-serum PON1 and macrophage PON2 by 50–100%[8].

Table 1: Nutrient content of pomegranate peel per 100g[8]

Composition	Content
Total solid	94.50
Moisture	5.40
Total sugars	17.70
Reducing sugars	4.34
Protein	4.90
Crude fiber	16.30
Fat content	1.26
Ash	3.40

Phytochemicals/Active Constituents: [9]

During the ancient era, significant efforts and progress were made in establishing the pharmacological mechanisms of Pomegranate peel (PP) and the individual constituents responsible for them. PP is known to possess diverse phytochemicals, most of which are observed to have therapeutic properties. Punicalin and punicalagin are the major constituents of pericarp ranging up to 0.2% of the total amount. The brilliant red color of peel is attributed to anthocyanidins and flavan-3-ols. Flavones and flavonols constitute the major flavonoids of peel. The methanolic extract of dried PP showed the presence of high content of phenolic compounds (44.0%) along with other constituents. Phenolic acids such as caffeic acid, fumaric acid, chlorogenic acid and p-coumaric acid are present in the pericarp. The amount of ellagic acid in fruit peel of 12 varieties examined and fluctuates considerably with a maximum of 50mg/100g and a minimum of 10mg/100g. It was reported that the amount of total phenolics in peel was evidently higher than arils of pomegranate fruit. It has been described that PP has ellagic acid (EA), ellagitannins and gallic acid (GA). PP contains hydroxyben-zoic acids such as GA, EA, and EA glycosides. Anthocyanidins are principally cyanidin, pelargonidin and delphinidin and flavonoids such as kaempferol, luteolin, and quercetin quantified methanolic extract of PP using HPLC and reported the presence of gallic acid (34.03%) and catechin (3.31%). Studies on PP were undertaken to investigate the changes in the major chemical composition during fruit maturation in two Israeli commercial varieties, "Wonderful" and "Rosh- Hapered". The result of the study revealed the levels of total phenolic antioxidant activity and hydrolysable tannins were reduced during maturation, while the anthocyanin level increased. This knowledge could help establish the optimum harvest date ensuring the maximum nutritional properties of PP. The results of Turkish researchers showed that the levels of total phenolic compounds changed depending on cultivars and fruit parts. In all cultivars, the highest levels of total phenolic content were obtained from the peel extracts[10].

MATERIAL AND METHOD:

Material:

Pomegranate peel (Pomegranate fruit (*Punica granatum*) were collected from the fresh fruits. 1, 1-diphenyl-2-picryl hydrazyl (DPPH), were issued from Pharmaceutics research laboratory, Allana College of Pharmacy. Spectrophotometric measurements were performed on Jasco Double-beam ultraviolet-visible (UV-VIS) Spectrometer (Model V530, Jasco International Co. Ltd, Tokyo, Japan).

Preparation of Pomegranate fruit peels powder suspension (PPPS):

The collected Fruit peel of *P. granatum* was, cleaned dried in a hot air oven at 50◦C for 48hours. Dried peels were powdered to get 60-mesh size using a grinder then dissolved in particular amount of distilled water to make

required concentrated suspensions of 1mg/ml, 5mg/ml and 10mg/ml and then sonicated for 30 minutes[10].

For preparation of 1mg/ml concentrated suspension 10mg of pomegranate peel powder (PPP) was dispersed in 10ml of distilled water in a 10ml volumetric flask.

For preparation of 5mg/ml concentrated suspension 10mg of PPP was dispersed in 50ml of distilled water in a 10ml volumetric flask.

For preparation of 10mg/ml concentrated suspension 100mg of PPP was dispersed in 10ml of distilled water in a 10ml volumetric flask.

Preparation of 0.1mM solution of DPPH:

DPPH solution (0.1mM) was prepared in distilled water by dissolving 1.9 mg of DPPH in methanol and the remaining volume was made up to 100ml with distilled water. The solution was kept in darkness for 30 minutes to complete the reaction[11].

In-vitro antioxidant assay:

Free radical-scavenging activity is done by the use of a stable DPPH radical.

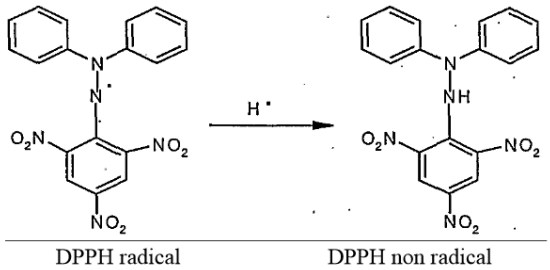
Principle

The scavenging reaction between (DPPH) and an antioxi dant (H-A) can be written as

(DPPH) + (H-A) → DPPH-H + (A)

(Purple) (Yellow)

Antioxidants react with DPPH, a stable free radical which was reduced to DPPH-H and as consequence the absorbance were decreased from the DPPH radical to the DPPH-H form. The degree of discoloration indicates the scavenging potential of the antioxidant compounds or extracts in terms of hydrogen donating ability [12].



Procedure

The free radical scavenging property of *Punica granatum* peels suspension was evaluated by spectrophotometric method using the stable DPPH. The percent antioxidant activities (AA %) of three concentrations of PPPS (1, 5, 10 mg/ml; prepared in distilled water) were used to evaluate the free radical activity of the DPPH samples. To 1ml of each PPPS concentration, 3 ml of 0.1 mM DPPH aqueous solution was added; reaction mixtures were allowed to stand for 30 minutes in the dark. Change in the color from deep violet to pale yellow was read at an absorbance of 517 nm using double beam UV/VIS Spectrophotometer (Model V530, Jasco International Co. Ltd, Tokyo, Japan) at the time interval of 0.5 (freshly prepared solution), 24, 48 and 120 hours. The blank consisted of pure distilled water without ascorbic acid or DPPH, while the control was made of 0.1 mM DPPH aqueous solution without PPPS. The antioxidant activity was determined using following formula [13].

$$AA\% = 100 - \frac{(Abs_{sample} - Abs_{blank})}{Abs_{control}} \times 100$$

RESULT AND DISCUSSION:

The DPPH· scavenging activity has been widely used to detect antiradical activity of different samples, due to its sensitivity to lower concentrations of active principles from natural sources. The stable radical, DPPH, has a λ_{max} at 517 nm[12] and could readily undergo scavenging by antioxidants. Higher free radical scavenging activities of samples is indicated by lower absorbance at 517 nm. PPPS exhibited a significant scavenging activity. In fact, the DPPH scavenging activity of PPPS substantially elevated as the concentration increased from 1mg/ml to 10 mg/ml but it was observed to decrease for each concentration with increase in time which is depicted in figure 1 and 2.

Table 2: Absorbance of Different Concentrations of PPPS at Different Time Interval

Concentration	Absorbance AT 517nm			
mg/ml	0.5 hour	24 hours	48 hours	120 hours
1	0.1899	0.2200	0.1830	0.1803
5	0.2862	0.3388	0.2725	0.2392
10	0.4991	0.4962	0.4813	0.4613
Blank	0.0002	0.0002	0.0002	0.0002
Control	1.7239	1.5339	1.2297	0.9221

Table 3: % AA of Different Concentrations of PPPS at Different Time Interval

Concentration	%AA				
mg/ml	0.5 hour	24 hours	48 hours	120 hours	Avg
1	71.05	67.64	60.87	49.99	62.38
5	83.4	77.92	77.81	74.08	78.55
10	88.99	85.67	85.1	80.46	85.05

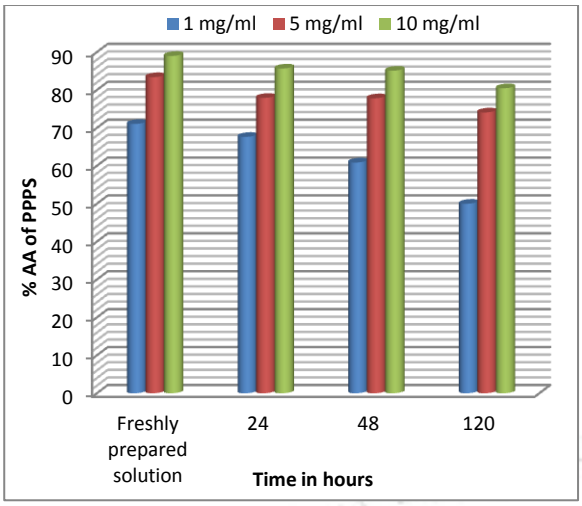


Figure 1: The percent antioxidant activities (AA %) of PPPS at different concentrations (1, 5, 10 mg/ml) used in evaluating the free radical activity of 0.1 mM DPPH aqueous solution.

Aliquots from freshly prepared, as well as 24, 48 and 120 hours post preparation stored at ambient temperature in the dark were assayed[14].

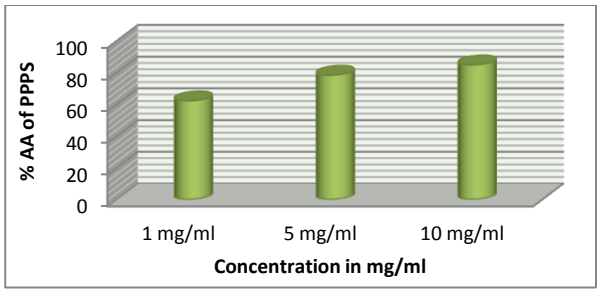


Figure 2: Average Radical scavenging activity of the PPPS at different concentrations.

CONCLUSION:

Pomegranate fruit peels are by products of the food industry. Added-value products could be made from those wastes. Aqueous suspension of pomegranate peel powder has shown good anti-oxidant activity evaluated by DPPH radical scavenging method. Phenolic content of pomegranate peel are responsible for potent anti-oxidant activity. Crude extracts and purified fraction from pomegranate peels could provide health benefits to humans and may be employed in food preservation and pharmaceutical purposes.

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