Research Paper

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Comparative Study of Chemical Constituents & Antioxidant Activity in Shade-Dried and Sun- Dried Fruit Peel of Punica granatum LINN.

Biochemistry

KEYWORDS : Antioxidant, scavenging activity, active principles, ultra violet.

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ABSTRACT

The present study was aimed to compare chemical constituents and antioxidant activity in shade dried and sun dried methanolic fruit peel extract of Punica granatum. The chemical constituents and % scavenging activity of shade dried and sun dried extracts was determined by standard qualitative tests and DPPH method, respectively. Shade dried and sun dried extract of P. granatum, standard exhibited highest free radical scavenging activity, i.e., 99.26%, 97.85 %, and 93.65% respectively at a concentration of 1000µg/ml. The findings showed that saponin was present only in shade dried peel extract of P. granatum. Rest of the chemical constituents was found to be present in both the extracts of P. granatum. However, determination of antioxidant activity of peel as well as other parts of P. granatum by other sensitive methods is warranted in order to explore full therapeutic potential of P. granatum peel.

Introduction

The consumption of antioxidants plays an important role in protecting against free radicals, especially reactive oxygen species (ROS). The generation of free radicals is associated with environmental pollution, u. v. radiation and several normal metabolic processes (Adegoke *et al.*, 1998, Mocord, 1994). Free radicals attack the unsaturated fatty acids of biomembranes which results in lipid peroxidation, destruction of proteins and DNA that may be related to cancer, heart disease and ageing (Ames, 1983). An antioxidant is a substance that when present at low concentration compared to that of oxidisable substrate, significantly delays or prevents oxidation of that substrate (Halliwell, 1990).

The possible toxicity as well as general consumer rejection, has led to decrease in the use of synthetic antioxidants (Namiki, 1990). Therefore, research in this area has focused on the detection of antioxidants in food. Fruits and vegetables have received particular attention because they contain high amounts of known antioxidants such as polyphenols, vitamin C, vitamin E, β-carotene and lycopene (Harman *et al.* 2001, Ames *et al. 1993*). The Pomegranate peel owes its antioxidant activity due to the presence of certain compounds like ellagic acid, ellagitannins, gallic acids (Ben *et al.*, 1996), phenolics and flavonoids (Ahmed, 2012) in it.

In the Ayurveda system of medicine, the pomegranate has extensively been used as a source of traditional remedies for thousands of years (Sumathy *et al.* 2013). Many reports are available on potent antioxidant property of *Punica granatum* peel (Singh *et al.*, 2001; Jaspreet, 2012; Ahmed, 2012) and this plant has been used in the markets in the preparation of tinctures, juice, cosmetics and therapeutic formulae (Kim *et al.*, 2002) but no information is published on change in chemical constituents and antioxidant potential of *P. granatum* peel by drying method i.e., shade-drying and sun-drying. Therefore, the present study was conducted with the objective of comparing chemical constituents and antioxidant potential in shade-dried and sun-dried methanolic extract of *P. granatum* fruit peel employing DPPH method.

Materials and Methods

Collection of plant material- The peel of *Punica granatum* was collected from the local market of Gonda, Aligarh in the month of February. The Pomegranate peel was washed with tap water followed by distilled water. The first group was dried away from direct sunlight (shade-drying) and the second group was sun-dried for 15 days each and then stored in airtight glass jars until extraction. The crude drug was authenticated by Dr. R. K. Jain, Head of the department, Botany, B.S.A., Degree College, Mathura, India.

Materials- 1, 1-diphenyl-2-picrylhydrazyl (DPPH) and ascorbic acid were purchased from Hi Media laboratories, Mumbai for the present study. Other chemicals and reagents used were of analytical grade. UV-visible spectrophotometer, Labonics was used for recording the absorbance.

Preparation of Extracts- The dried material was ground in to moderately coarse powder ("22") in a mixy grinder. About 50 gm. drug powder was uniformly packed into a thimble and run in soxhlet extractor using methanol as solvent at 25-30 ^{0}C temperature for 24 hrs. The extracts were filtered and left at room temperature for drying. The extracts were further dried under vacuum.

Phytochemical Analysis- The prepared extracts of Punica granatum peel were tested for the presence of secondary metabolites using standard qualitative tests (Ahirrao & Surywanshi *et al.* 2013).

In-vitro antioxidant studies- The methanolic extracts of Punica granatum fruit peel were tested for their free radical scavenging property using DPPH method. In-vitro DPPH (1, 1-diphenyl-2-picryl hydrazyl) radical scavenging activity was determined as per the method described by Bors et al. (1990), with minor modifications. Equal volume of test extracts dissolved in methanol was added to a methanolic extract of DPPH (100µM). For control, the mixture of methanol and DPPH (1:1) was used. Ascorbic acid dissolved in methanol was taken as standard. Test, standard and blank were checked for antioxidant activity in concentrations ranging from 1-1,000 µg/ml. After keeping the Test-tubes in dark for 30 minutes at room temperature, the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm and the percentage inhibition calculated by using the formula.

$$\text{Inhibition (\%)} = \frac{\text{Control-Test}}{\text{Control}} \times 100$$

Results and Discussion: Comparative analysis of active principles in shade dried and sun dried methanolic extract of P. granatum peel in this study showed that constituents like alkaloids, glycosides, ellagatanins etc. were present in both types of extracts while saponin was found to be present in shade-dried extract but absent in sun -dried extract. Previous published reports (Satheesh et al. 2012; Amina & Fouzia, 2013) also reveal the presence of various secondary metabolites such as triterpenoids, steroids, glycosides, flavonoids, tannins, carbohydrate in the peel extract of P. granatum. In the present investigation, the scavenging activity of shade dried and sun dried peel extract of P. granatum and standard was recorded to be 9. 50 %, 8.64 % & 1.92 % respectively at a concentration of 1µg/ml . (table-1) . The table also shows increse in % scavenging activity with the increase in concentration. Temperature sensitive active principles like tannins were probably decreased in sun dried peel extract of Punica granatum due to ultra violet light which in turn decreased its % scavenging activity. No reports

were available to compare the antioxidant activity in shade-dried and sun- dried peel of Punica granatum. However, the scavenging activity of shade-dried peel extract of P. granatum in the present study are in consensus with the findings of De Silva et al.(2014) who reported increase in free radical scavenging by P. granatum peel with the increase in concentration. Similarly, Ashoush et al.(2013) also recorded high radical scavenging activity (96.24%) of peel powder of P. granatum. Chemical constituents of shade dried & sun dried extract of Punica granatum peels have been presented in table-2.

Table-1: % radical scavenging activity of methanolic extract of P. granatum

Sample	% Free radical Scavenging			
	1 µg/ml	10 µg/ml	100 µg/ml	1000 µg/ml
Shade-dried P. granatum	9.50	56.86	97.83	99.26
Sun-dried P. granatum	8.64	54.9	95.74	97.85
Ascorbic acid	1.92	59.47	91.79	93.65

Table-2: Chemical constituents of shade dried & sun dried extract of Punica granatum peel

S. No.	Chemical constituent	Shade-dried extract	Sun-dried extract
1.	Alkaloids	-	-
2.	Flavonoidss	+	+
3.	Tanins	+	+
4.	Proteins	+	+
5.	Carbohydrates	+	+
6.	Saponin	+	_
7.	Glycosides	+	+

Conclusion: The shade-dried fruit peel extract of P. granatum has more free radical scavenging activity than sun dried extract of the same plant. This could be probably due to decrease or loss of some temperature sensitive constituents in the P. granatum extract. Furthermore, % radical scavenging activity increased in a concentration dependent manner.

Thus, the findings of the present investigation justify its extensive therapeutic use in traditional system of medicine.

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