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# Polyphenolic Compounds Possessing Radical Scavenging Activity (RSA) in Pomegranate Peel

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## Introduction

Pomegranate is important fruit of tropical and subtropical region of India. It is used in medicine for ancient time (Asish et al., 1999). The scientific name of pomegranate is *PunicagranatumL.*. In India, pomegranate is commonly known as aanar. Pomegranate fruit are good source of bioactive compounds, organic acid, vitamin, mineral, polyphenol (shown antioxidant effect) and sugar (Jaiswal et al. 2010). Pomegranate peel is a rich source of tannins, flavonoids and other phenolic compounds (Li et al. 2006). Its juice also contains various constituents such as polyphenols, tannins, anthocyanins, including vitamin C, vitamin E, and lipoic acid (Vroegrijk et al. 2011) and punicalagin bioactive constituent responsible for more than 50% of the antioxidant activity of pomegranate juice (Seeram et al. 2005). Pomegranate peel shows highest antioxidant activity it prevent atherosclerosis disease. The various product available in market such as juices, flavour and extracts, wine, powder form etc. The pomegranate juice show higher antioxidant properties as compared to other.Pomegranate peels are rich in tannins (Al-Zoreky, 2009; Machado et al., 2003; Voravuthikunchai et al., 2004). They have been used traditionally for their medicinal properties as anticancer, anti-inflammatory, antioxidant and antihelminthic (Hayouni et al., 2011; Mousavinejad et al., 2009) and for other purposes such as tanning, dyeing (Adeel et al., 2009; Lloyd, 1897) and heavy metal removal (Najim and Yassin, 2009).

## Material and Methods

### Material

Pomegranates were obtained from local market at Sirsa and the pomegranates were bought three days before the experiment is run and they were stored in sealed plastic bags in refrigerator. For this analysis, there is specific species of the pomegranate fruit is use which is the pomegranate that was cultivated in the great Himalayas.

### Preparation of sample as hot water extract of dried peel powder

Hot water extract of dried pomegranate peels were prepared by the procedure given by Xu et al. (2008). The sample peel powder (5g) were added to 100 ml of boiling distilled water and infused for 30 min. Then the extract was cooled to ambient temperature and centrifuged at 5000rpm for 20 min. The supernatant was collected and made a final volume of 100 ml by addition of distilled water and the liquors of each sample were analysed for radical scavenging activity (RSA%), total carotenoid content and p-carotene by spectrophotometric methods.

### Analytical methods

The fruit peels was analysed for the analysis of antioxidant like total carotenoids content, Beta-carotene, ascorbic acid content and radical scavenging activity (RSA%).

### Total carotenoid content

Total carotenoid content of samples were determined using method described by Arnon, (1949). The samples (100-500 mg) were homogenized with 10-15 ml of 80% acetone in pestle-mortar until tissue become colorless. A pinch of calcium carbonate was also added to avoid the destruction of chlorophyll and other pigments. The liquid was centrifuged at 2000 rpm for 10-15 min. and filtered it. The volume was made to 10 ml with acetone and the absorbance was recorded at 480 and 510 nm using spectrophotometer.

Calculations

$$\text{Total carotenoids} = \frac{7.6*A\,480 - 14.9*A\,510 * V}{a*1000*w}$$

Where

A= Absorbance (nm)

a= Light path (cm)

V= Volume (ml)

W=Weight of sample (mg)

**Beta-Carotene content (BCC)**

The beta-carotene content of pomegranate peel was evaluated using the method described by (Srivastava et al. 2001). The sample (5g) was crushed in 10-15 ml of acetone and followed by addition of anhydrous sodium sulphate, with the help of pestle and mortar. Decanted off the supernatant in a beaker. The process was repeated twice and the combined supernatant was transfer to a separatory funnel, 10-15 petroleum ether added and thoroughly mixed. Two layers will separated out on standing. The lower layer was decanted off and upper layer was collected in a 100ml volumetric flask then the volume was made to 100 ml with petroleum ether and optical density was recorded at 452 nm using petroleum ether as blank in spectrophotometer.

Calculation

$$\text{Beta-carotene } (\mu g/100g) = \frac{O.D \times 13.9 \times 10^4 \times 100}{\text{Wt. of sample} \times 560 \times 1000}$$

**Radical scavenging activity (RSA%)**

RSA was evaluated by method given by Brand-Williams et al. (1995), based on the measurement of the scavenging ability of antioxidant towards the stable radical DPPH. The methanolic solution (3.9 ml) of 0.063 mM DPPH was added to the test tube and 0.1 ml of each extract was added and shaken vigorously then the test tube was allowed to stand at 27˚C for 20 min. A control reaction was prepared as above without any extract and methanol was used for baseline correction and the absorbance was measured at 515 nm in spectrophotometer.

Calculations

Radical scavenging activity (RSA%) was expressed as the inhibition % and was calculated by using the following equation

% RSA = (control Abs – sample Abs/ control Abs) × 100

**Ascorbic acid content**

Standard Ascorbic acid (5ml) was added to 5ml of $HPO_3$ and the burette was filled with dye. Then the standard ascorbic acid solution was titrated with dye solution to pink color, which had persisted for 15 seconds.

Dry factor = 0.5/ titre

**Preparation of sample:**

Sample (10mg) was mixed with 3% $HPO_3$ and made the volume to 100 ml with $HPO_3$ and after, it was centrifuged at 2000 rpm for 20 min.

**Assay of Extract**

The Aliquot (5 ml) of $HPO_3$ extract was taken and titrated with the standard dye to pink end point which had persisted for 15 min.

Calculation

$$\text{Ascorbic acid (mg/100g)} = \frac{\text{Titre} \times \text{Dye factor} \times \text{vol. made up} \times 100}{\text{Aliquot of extract for estimation} \times \text{wt. of sample for estimation}}$$

**Result**

**Total carotenoid content (TCC),Beta-carotene content (BC), Radical scavenging activity (RSA %) and Ascorbic acid content (ACC)**

The pomegranate showed the total carotenoid content (TCC) was different in different samples. The TCC ranged from 1.460 to 1.462 mg/100g.

**Table 1: Total carotenoid content (TCC),Beta-carotene content (BC), Radical scavenging activity (RSA %) and Ascorbic acid content (ACC)in different samples of pomegranate**

| Samples (peel) | TCC (mg/100g) | BC (mg/100g) | RSA % | ACC (mg/100g) |
|---|---|---|---|---|
| **Samples 1** | 1.462 | 0.033 | 0.215 | 13.60 |
| **Samples 2** | 1.461 | 0.031 | 0.211 | 13.20 |
| **Samples 3** | 1.460 | 0.029 | 0.218 | 13.00 |

The beta-carotene of pomegranate peel was measured in different samples. The beta-carotene content ranged from 0.0.29 to 0.033 mg/100g. The radical scavenging activity was 0.211 to 0.218 %. Pomegranate peel showed the 13.60 mg/100g in sample 1, 13.20 in sample 2 and 13.00 in sample 3. The study reveals that the beta-carotene content, total carotenoid content and ascorbic acid content may also indicate the presence of natural antioxidant in the samples taken. As it is found that beta-carotene, total carotenoid content and ascorbic acid content were found. Thus, the incorporate the extracted antioxidant from pomegranate peels into different foods commodities to prevent oxidation/rancidity.

**Summary and conclusion**

The study was to determine the amount of antioxidants i.e total carotenoid content, beta-carotene, ascorbic acid and RSA (%) naturally present in the peel of the fruit. The research showed that the peels from pomegranate are a potential resource for phenolics, proanthocyanidins and flavonoids. The antioxidant activity of pomegrant peel was attributed to the total phenolics. The pomegranate peel extracted with methanol gave the highest total extract yield, followed by water, ethanol, acetone and ethyl acetate.

Fruit peels are good source of natural antioxidant. India may offer a novel source of antioxidant for the food industry for boosting both the shelf-life and nutritional content of food. As plants produce significant amount of antioxidants to prevent the oxidative stress caused by photon and oxygen, they represent a potential source of new compounds with antioxidant activity. Therefore, if a systems practiced in India can offer promising leads for the discovery of potent antioxidant that can have therapeutic and dietary use globally.

The consumption of pomegranate has grown tremendously due to its reported health benefits. Pomegranate and derivates, such as juice, peel and seeds, are rich sources of several high-value compounds with potential beneficial physiological activities. The rich bioactive profile of pomegranate makes it a highly nutritious and desirable fruit crop. Accumulating research offers ample evidence that routine supplementation with pomegranate juice or extract may protect against and even improve several diseases, including diabetes and cardiovascular disease; it may even help to prevent and arrest the development of certain cancers, in addition to protecting the health of the mouth and skin. Side effects are very rare. Using concentrated, low-cost pomegranate juice or standardized pomegranate extract capsule offers consumers a way of reaping the broad spectrum of health benefits of this fruit.

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