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Antioxidant activity of pomegranate peel and seed powder extracts

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Abstract
The present study was devised to explore the antioxidant activity of pomegranate peel powder (PPP) and pomegranate seed powder (PSP). Total phenolic content was determined by Folin Ciocalteu reagent method and antioxidant activity by DPPH and ABTS radical scavenging activity methods. The study revealed that the total phenolic content (mg GAE/ g) of PPP was significantly higher ($P<0.05$) than that of PSP. The values were observed to be numerically higher ($P>0.05$) in the methanolic extracts compared to the water extracts. The percent DPPH-RSA of PPP was significantly higher ($P<0.05$) than the PSP (91.81 ± 0.62). Similarly, M*e* showed significantly higher ($P<0.05$) values compared to W*e*. The percent ABTS-RSA of PPP was significantly higher ($P<0.05$) than the PSP. However, the M*e* and W*e* exhibited non-significant differences ($P>0.05$), the values being numerically slightly higher in the former. Thus it was concluded that pomegranate peel and seed powder could find several applications as functional food ingredients due to the antioxidant properties.

Keywords: Antioxidant, functional, ingredients, pomegranate, peel, powder, seed

1. Introduction
Pomegranate fruit is berry like with a leathery rind (husk or peel) enclosing many seeds surrounded by juicy arils. The husk is composed of two parts: pericarp and mesocarp (albedo). The edible part of the pomegranate fruit (50%) consists of 40% arils and 10% seeds. Arils contain 85% water, 10% total sugars and bioactive compounds such as phenolics and flavonoids, principally anthocyanins (Rafraf *et al.,* 2017) [1]. Pomegranate peel comprises about 50% of the total fruit weight and is an important source of minerals especially potassium, calcium, phosphorus, magnesium, and sodium; complex polysaccharides and high levels of diverse range of bioactive compounds such as phenolics, flavonoids, proanthocyanidin compounds and ellagitannin (ETs), such as punicalagins and its isomers, as well as lesser amounts of punicalin, gallagic acid, ellagic acid, and ellagic acid glycosides. Pomegranate seed oil (PSO) contains an exceptional conjugated fatty acid called punicic acid (trienoic acid) that makes up approximately 65% to 80% of the oil from pomegranate seeds. Punicic acid is also referred as a super conjugated linolenic acid whose effect is even more potent than that of an ordinary conjugated linolenic acid. Seeds also contain protein, crude fibers, vitamins, minerals, pectin, sugars, polyphenols, isoflavones, the phytoestrogens, coumestrol and the sex steroid, estrone (Aruna *et al.,* 2016) [2]. Recently, it has been revealed that PP powder contains much higher content of lysine, leucine, aromatic fatty acids (phenylalanine and tyrosine), threonine and valine, while having less concentration of sulphur containing amino acids (methionine and cysteine), than the reference protein pattern of FAO/WHO (FAO/WHO, 1973). Also, pomegranate seed powder contains sulfur containing amino acids (methionine and cysteine), aromatic fatty acids (phenylalanine and tyrosine), leucine and isoleucine were much higher than the corresponding mentioned in reference protein pattern of FAO/ WHO (Syed *et al.,* 2007) [3]. Several scientific studies have confirmed pomegranate biological activities and medicinal effects of the edible part of the fruit, but very few data exist about the bioactivity of pomegranate peel, seed, powder and extracts. Therefore, more research has to be done in that field.

The industrial transformation of vegetables and fruits generates large quantities of co-products rich in bioactive compounds that may well be suitable for other purposes. The importance of natural food additives are increasing due to a more extensive use of natural compounds in food, cosmetics and pharmaceuticals industries rather than synthetic compounds. The vast majority of by-product streams generated throughout industrial processes give rise to immense environmental, societal and economic related issues. The development of strategies for the valorization of these industrial residues will not only address these problems but also promote

bioeconomy, satisfying sustainable development principles. Pomegranate peel (PP) and pomegranate seed (PS) are valuable sources of bioactive phytochemicals, the vast majority of which hold a great potential through appropriate processes to be converted into value added products. So, pomegranate by-products could be used as substrate for the production of nutritionally valuable and biologically active components that could find several applications as functional food ingredients, food additives, nutraceuticals and supplements and in phenolic-rich diets. To date, there has been limited assessment about the potential of converting non-edible pomegranate production process residues, through the development of novel efficient systems, to value added products such as antioxidants, dietary fibers, industrial enzymes and single cell protein. Up-grading of this by-product to value-added products is therefore, of interest to the pomegranate juice industry. The aim of the present study was to determine the antioxidant activity of pomegranate peel and seed powder as a potential source for food enrichment.

2. Materials and methods

Fresh ripened pomegranate fruits shall be procured from the local market and used as per experimental requirements.

2.1. Preparation of pomegranate peel powder (PPP)

Pomegranate fruits shall be washed with distilled water and cut manually to separate the arils 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 ± 5ºC 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 20 mesh sieve, packed in high density polyethylene bags and stored at room temperature (25 ± 5ºC) until use

2.2. Preparation of pomegranate seed powder (PSP)

Pomegranate fruits shall be washed with distilled water and cut manually to separate the arils 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 ± 5ºC 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 ± 5ºC) until use.

2.3 Antioxidant activity

2.3.1 Preparation of pomegranate peel and seed powder water and methanolic extracts (PPP $_{We/Me}$ / PSP $_{We/Me}$): The two extracts were prepared according to Shiban *et al.* (2012) [4], wherein pomegranate peel and seed powder (5 g) was blended separately for 2 min with 300 ml of distilled water or 80% methanol. Mixture was then left, in the dark; at room temperature for 1 hr prior to filtration (Whatman No. 1) and centrifuged at 3500rpm for 10 min. Extracts were kept at -20°C prior to analysis.

2.3.2 Total Phenolic Content

Total phenolic content of pomegranate peel powder and pomegranate seed powder was analyzed by Folin Ciocalteu method (Zheng and Wang *et al.*, 2001) [5]. Twenty ul of sample was mixed with 1.58ml of distilled water followed by 100 ul of Folin-Ciocalteu reagent. The mixture was vortexed vigorously, left to stand at room temperature for 15 min and 300 ul of sodium carbonate solution (20%) was added. The mixture was left to stand at room temperature for 2 hrs then the absorbance was measured with a spectrum UV-Vis spectrophotometer (HITACHI, UV-Spectrophotometer U-1800, Japan) at 765 nm. Each assay was carried out in triplicate.

Standard curve preparation: 20 ul of 10-100 mg/L concentration of gallic acid solution was taken in place of sample for standard curve preparation.

Calculations: Based on absorbance the total phenolic content of sample was calculated using equation of standard curve (Fig. 1):

$$y = 0.02x - 0.008$$

where
y is absorbance at A765nm (sample)
x is mg/L concentration of gallic acid

The results were expressed in terms of mg gallic acid equivalent (GAE)/g for PPP/PSP

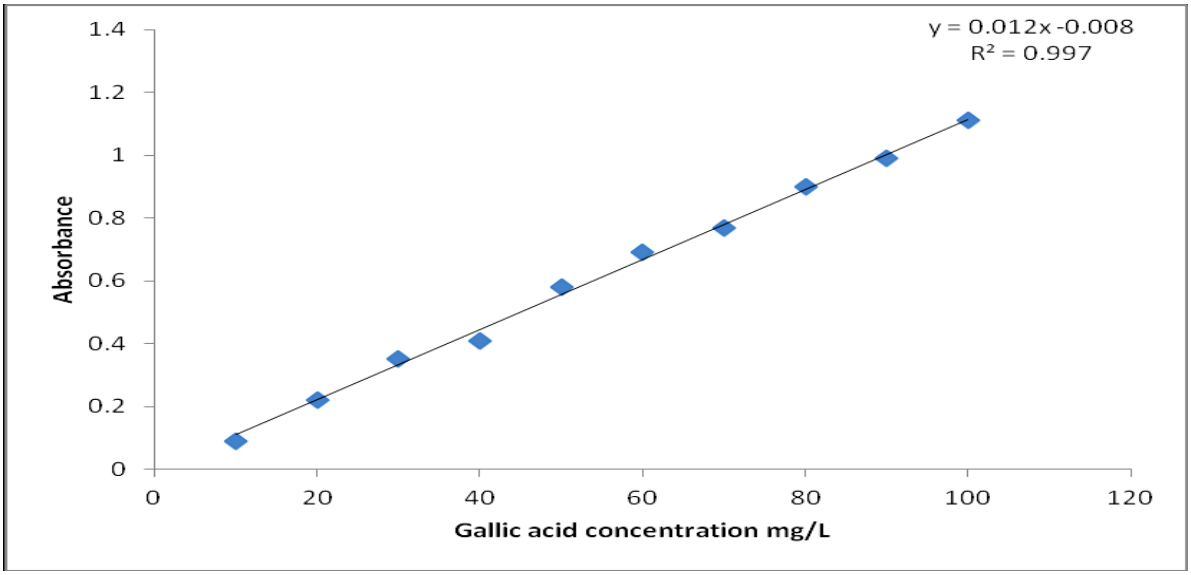

Fig 1: Standard curve for estimation of total phenolic content

2.3.3 Measurement of antioxidant activity by ABTS method

The 2, 2'- Azino-bis (3-ethyl-benzothiazoline-6-sulfonic acid) (ABTS) radical scavenging assay of pomegranate peel powder and seed powder was done, following the method given by Re *et al.* (1999) [6]. Aliquot (100 µl) of each sample extract was mixed with 3ml of prepared ABTS working solution and the change in absorbance was observed at 734nm using UV-VIS spectrophotometer (HITACHI, UV-Spectrophotometer U-1800, Japan).The ABTS radical scavenging capacity of the sample was calculated by the following formula:

ABTS Radical scavenging activity (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$

2.3.4 Assay for DPPH radical scavenging activity

Antioxidant activity based on DPPH (2,2 diphenyl-1-picryl hydrazyl) radical for extracts of pomegranate peel and seed powder were analyzed following the method given by Brand Williams *et al* (1995) [7]. An aliquot of 100µl of the sample extract was mixed with 2.9ml of freshly prepared DPPH working solution in 10ml test tube. The contents were mixed and reaction mixture was left in dark for 30 min after covering the test tube with aluminium foil. The absorbance of the solution was measured at 517 nm against methanol using UV-VIS spectrophotometer. Antioxidant activity was expressed as percentage inhibition of the DPPH radical and was determined by the following equation:

Radical scavenging (%) = $[(A_{blank} - A_{sample}) / A_{blank}] \times 100$

2.4 Statistical analysis

The statistically analyzed results has been tabulated and interpreted. The results were tabulated and analyzed statistically using one way and two way ANOVA using the software of Statistical Package for Social Sciences (SPSS-Base 20). The results were expressed as Mean ± S.E at 5% level of significance.

3. Results and discussion

3.1 Total Phenolic Content (TPC) and antioxidant activity

The phenolic content can be used as an important indicator of antioxidant capacity of any product when intended for use as a natural source of antioxidants in functional foods. The results have indicated that the PPP has a significantly higher ($P<0.05$) TPC value than PSP (Fig.2). Our results are in agreement with the findings of Li *et al.* (2005) [8], Pande and Akoh (2009) [9], Elfalleh *et al.* (2012) [10] and Altunkaya (2014) [11] who also reported that the phenolic content was higher in pomegranate peel extracts than seeds. The results also indicated that there was no significant difference ($P>0.05$) between water (W*e*) and methanolic extracts (M*e*) of either PPP or PSP, however M*e* had numerically a little higher values for both PPP and PSP. Our findings are concomitant with those of Cam and Iyer (2015) [12] who reported no statistically significant difference ($P>0.05$) between water extracts and methanolic extracts for extraction of pomegranate peel phenolics. However, Nuamsetti *et al.* (2012)[13] reported that the total phenolic content of hot-water extracts of pomegranate fruit peels was the highest (166.83 mg GAE/100g dry weight), followed by the ethanol (152.65 mg GAE/100g dry weight) and acetone extracts (85.48 mg GAE/100g dry weight), respectively. The differences found can be attributed to the fact that phenols are a heterogeneous group of complete mixture of organic substances the quality and quantity of which vary with growth stages, ecological conditions, extraction conditions, extraction solutions used and other factors based on which the phenolics are extracted (Celiktas *et al.*, 2007) [14].

Fig 2: Total Phenolic content of pomegranate peel powder (PPP-W*e*/M*e*) and pomegranate seed powder (PSP-W*e*/M*e*)

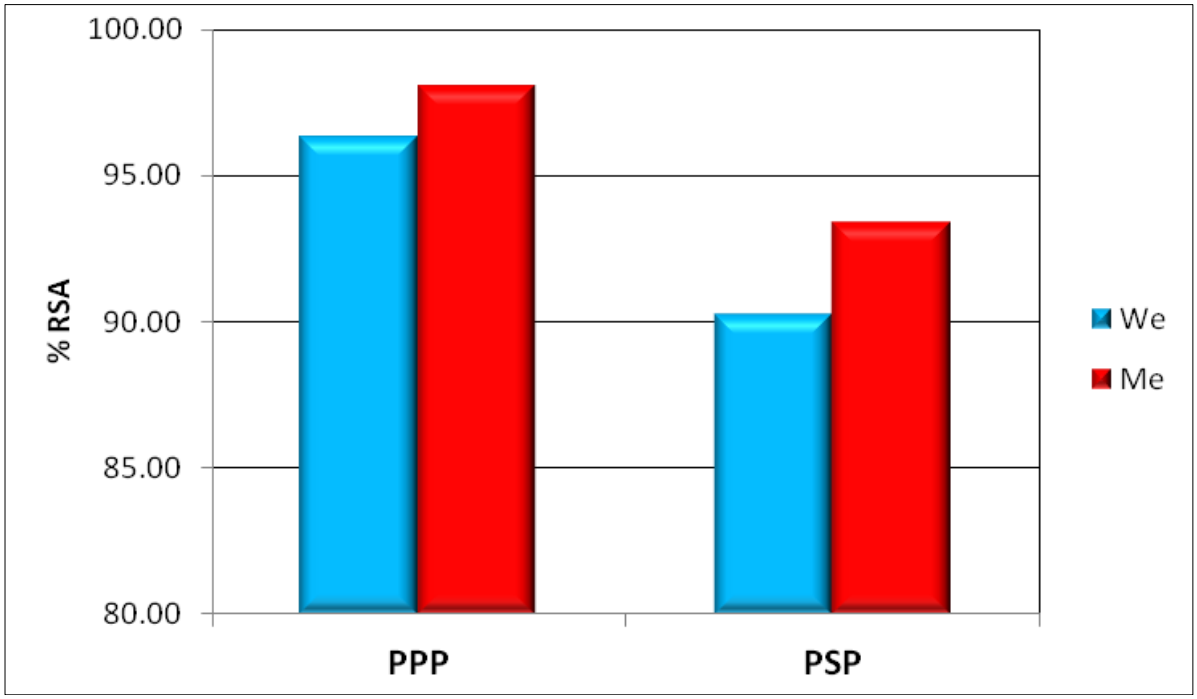

Fig 3: DPPH-RSA activity of pomegranate peel powder (PPP-W*e*/M*e*) and pomegranate seed powder (PSP-W*e*/M*e*)

The stable DPPH radical model is a widely used, relatively quick and precise method for the evaluation of free radical scavenging activity. DPPH is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule. Antioxidants on interaction with DPPH, transfer electron or hydrogen atom to DPPH and thus neutralizing its free radical character and convert it to 1-1, diphenyl-2-picryl hydrazine and the degree of discoloration indicates the scavenging activity of the sample. It is visually noticeable as a change in color from purple to yellow. The reduction capacity of DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants.

Results in present investigation have indicated that the percent DPPH-RSA of pomegranate peel powder (PPP) samples were significantly higher ($P<0.05$) than the pomegranate seed powder (PSP) samples (Fig.3). Li *et al.* (2006) [15] reported that pomegranate peel had the highest antioxidant activity among the peel, pulp and seed fractions. Our results are in agreement with the findings of Elfalleh *et al.* (2012) [10], Sood and Gupta (2015) [16], Malviya *et al.* (2013) [17], Manasathien *et al.* (2012) [18] and Patel *et al.* (2017) [19]. Similarly, the methanolic extracts (M*e*) showed significantly higher ($P<0.05$) values compared to water extracts (W*e*). Somewhat similar findings have been reported by Singh *et al.* (2002) [20] who noticed that a methanolic extract of pomegranate peel had much higher antioxidant capacity than that of seeds. Our results are also in agreement with the findings of Basiri (2015) [21] who reported that the methanolic extract proved to have higher antioxidant efficiency compared to aqueous extracts. The results also corroborate the finding of Negi and Jayaprakasha (2003) [22], Zahin *et al.* (2010) [23] and Shiban *et al.* (2012) [4].

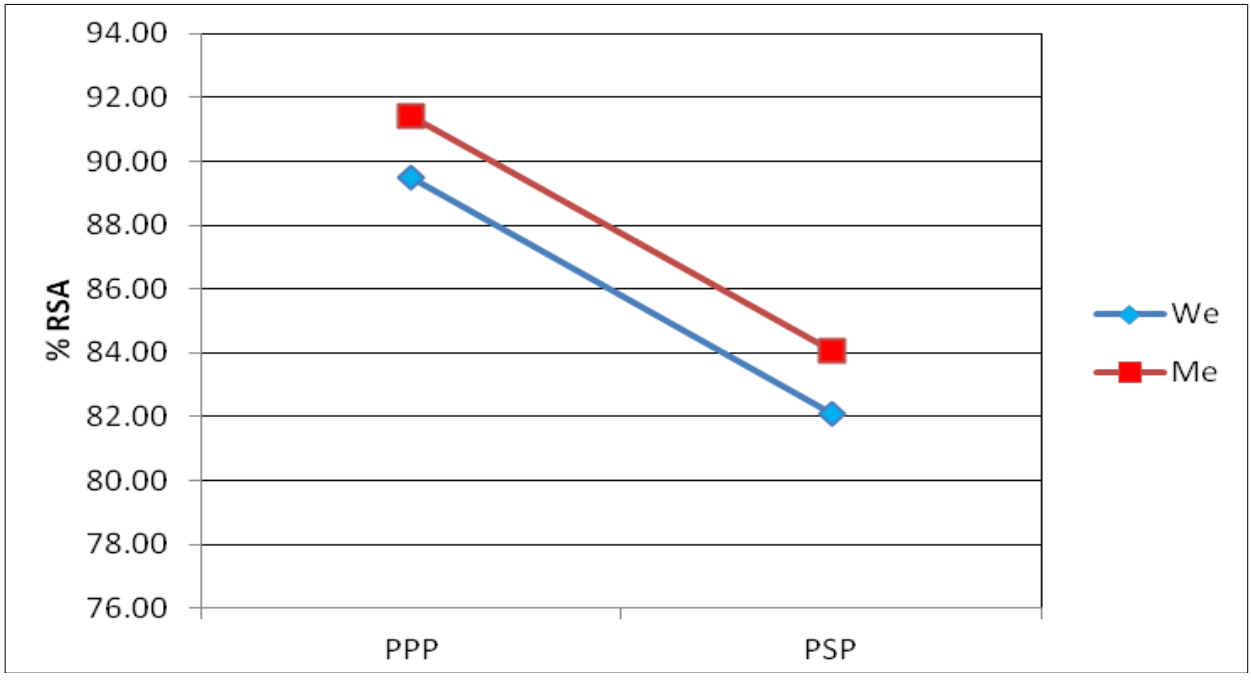

Fig 4: ABTS-RSA activity of pomegranate peel powder (PPP-W*e*/M*e*) and pomegranate seed powder (PSP-W*e*/M*e*)

In ABTS radical scavenging assay, ABTS is converted to its radical cation by addition of potassium persulfate. This radical cation is blue in color and absorbs light at 734 nm. The ABTS radical cation is reactive towards most antioxidants including phenolics, thiols and ascorbic acid. During this reaction, the blue ABTS radical cation is converted back to its colorless neutral form. The results have indicated that the percent ABTS-RSA of pomegranate peel powder samples were significantly higher ($P<0.05$) than the seed powder samples (Fig.4). These results correlate well with the findings of Elfalleh *et al.* (2012) [10] who reported significantly higher ABTS activities of pomegranate peel and flower than seed and leaf. These differences are due to the content and quality of the phenols in the different fruit part extract. Also, the methanolic and water extracts exhibited non-significant differences ($P>0.05$), the values being relatively higher in the former. Our results are in agreement with the findings of Marchi *et al.* (2015) [24], Malviya *et al.* (2013) [17] and Rajan *et al.* (2011) [25] who reported that the methanolic extracts of the different part of pomegranate fruit showed stronger antioxidant activities than the water extracts.
The slight difference in the values between the two methods (DPPH and ABTS-RSA) are most likely because of differences in reaction ability of the free radicals in the assays and reaction rates of the antioxidants under actual analytical conditions (Brand Williams *et al.*, 1995) [7]. The antioxidants in fruit preparations apparently vary sufficiently in chemical structure to give different levels of antioxidant capacity when two methods are compared. The differences in antioxidant capacity could be explained by different mechanism of analytical methods.

4. Conclusion

The present study provides enough evidence that both extracts of *Punica granatum* fruit (PPP/PSP; W*e*/M*e*) have higher total phenolic content and antioxidant activity thus suggesting a potential source of natural antioxidants. The activity of the extracts is attributed to their hydrogen donating ability. It is well-known that free radicals cause autoxidation of unsaturated lipids in food. On the other hand, antioxidants are believed to intercept the free radical chain of oxidation and donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of the lipids. The results obtained revealed that the PPP and PSP extracts are free radical inhibitors and primary antioxidants that react with free radicals. Thus, suggesting their potential application as natural additives and functional ingredient after its incorporation in a real food model.

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