

“Comparison of Antioxidant Properties of Pomegranate Peel Extract by Different Methods”

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Abstract: Cheaper and safer antioxidants of natural origin are the focus of research in recent times due to increased in safety concerns about synthetic antioxidants by the consumers. Pomegranate (*Punica granatum L.*) fruit both peel and pulp are abundant in antioxidants, the antioxidant activity in the former is greater than the latter. The effects of three extracting solvents methanol, acetone, ethyl acetate was studied using hot percolation (Soxhlet apparatus) extraction technique. The responses were extract yield, phenolics compounds and antioxidant activity of pomegranate peel extract. DPPH assay, FRAP assay and TEAC assay were used as the standards in determination of the antioxidant capacity of the pomegranate peel extract. Experiments revealed that all the peel extracts exhibited marked antioxidant capacity, with the methanol extract demonstrating the highest antioxidant capacity with significant difference with the other extracts ($p < 0.05$) and the ethyl acetate extract the lowest. The methanol extract of peels showed 91% free radical scavenging activity at 100ppm using DPPH model systems. Similarly, the TEAC value and the FRAP value of the methanol extract were reported as 5.26 ± 0.001 mM/mg extract as trolox equivalents and 756.44 ± 78.4 mg Fe^{II} /g as ferrous sulphate equivalents respectively. HPLC analysis showed that presence of catechin followed by chlorogenic acid in the acetone extract of pomegranate peel while the presence of chlorogenic acid followed by caffeic acid in the methanol extract of pomegranate peel. The overall results showed that the pomegranate peel extracts have antioxidant properties and may be exploited as biopreservatives in food applications and nutraceuticals.

Keywords: Pomegranate, Antioxidant activity, peel extracts, nutraceuticals

1. Introduction

Dietary habits are vital in human health. In such a fast moving world, where the industrialization and globalization are taking place at such a pace consumer needs are also altering day by day. Not only the scenario is prevalent in India but it can also be seen in abroad as well. This has led to the varying eating habits of the consumers in every part of the world. Due to the same, erratic growth and development is seen among individuals of different age groups. Epidemiological studies on the inter-relationship between dietary habits and disease risk have shown that the food has a direct impact on the health of a person. Besides studies have shown that consumption of fruits and green vegetables with high phenolic and flavonoid compounds are correlated with reduced cardiovascular [1, 2] inflammation [3, 4] cancer mortality [5,6] and disease rates [7, 8]. Antioxidant is defined as any substance that when present at low concentrations compared with those of an oxidizable substrate, significantly delays or prevents oxidation of that substrate [9]. The search for economical and abundant sources of natural antioxidants is attracting worldwide interest. Thus the need to study the antioxidant property is slowly and steadily gaining importance as the free radicals find an ease in attacking the body of an organism due to the absurd eating habits of the individual. In this context, people are exposed to a large number of both chemical and physiological changes occurring in nature. Free radicals generated in the body can be eradicated by body's own natural antioxidant defenses; for instance, glutathione, catalase, etc. However, endogenous antioxidant defenses are not completely sufficient. For this purpose, the dietary antioxidants is a boon to modern lifestyle and are required to lessen the overall effect of antioxidative stress caused by free radicals in the human body.

Pomegranate fruit (*Punica granatum L.*) is one of the most popular fruits native to Iran. There is growing interest in this fruit as it is considered to be a functional product of great benefit in the human diet as it contains several groups of substances that are useful in disease risk reduction [10]. Pomegranate extract is standardized and contains the important polyphenols found in pomegranates: ellagic acid, gallic acid, and punicalagin. These three compounds are potent antioxidant which promotes health by destroying cell damaging free radicals. The

different antioxidant compounds present in fruits are responsible for the high antioxidant capacity. Many experiments have reported the antioxidant activity of fruit juice and fruit pulp from edible fruits [11, 12]. However, there is a little information on the antioxidant activity in fruit peels. Peels are often the waste part of various fruits and are generally discarded off rather than being used up for nutritional purposes. These wastes have not generally received much attention with a view to being used or recycled rather than discharged. Perhaps, this might be due to their lack of commercial application [13]. Interestingly, the peel and seed fractions of some fruits have higher antioxidant activity than their pulp fractions. For instance, pomegranate peel has a higher antioxidant activity than its pulp [14]. The aim of the present study was to study the effect of solvents on extraction of pomegranate peel extracts and characterization of the pomegranate peel extract using allied assays viz antioxidant properties and through HPLC (High Performance Liquid Chromatography).

2. Materials and Methods

2.1 Preparation of pomegranate peel extracts

Ripened pomegranates (Ganesha variety) were obtained from Azadpur market, New Delhi. The peels were manually removed, air dried and powdered. Powder was extracted with a Soxhlet extractor using ethyl acetate, acetone and methanol for 6 h each. The extract was filtered through Whatman No. 41 filter paper for removal of peel particles and concentrated under vacuum at 40°C using a rotary evaporator [15]. The extract so obtained was finally vacuum dried and stored in desiccators for further evaluation purposes. The moisture content of peel powder was found to be 17.65%.

2.2 Determination of total phenolics

The concentration of phenolics in the pomegranate peel extract was determined with Folin–Ciocalteu reagent as per the method of Singh *et al.* [15]. Results were expressed as (+) tannic acid equivalents (TAE) and were specified by the tannic acid calibration curve.

2.3 Evaluation of antioxidant capacity

% radical scavenging activity as DPPH of pomegranate peel extracts was determined. FRAP (Ferric Reducing Antioxidant Power) assay done as described by Benzie and Strain [16]. The final result was expressed as the concentration of antioxidants having a ferric reducing ability equivalent to that of 1 mM FeSO₄. TEAC (Trolox Equivalent Antioxidant Capacity) assay was conducted according to the modified method of Re *et al.* [17]. The results were expressed in mM trolox/mg extract. The total antioxidant capacity of pomegranate peel extracts was evaluated by phosphomolybdenum method as per of Prieto *et al.* [18]. Water-soluble antioxidant capacity was expressed as equivalents of ascorbic acid (μmol/g of extract). All determinations were performed in triplicate.

Three replicates of each sample were used for statistical analysis and the values were reported as mean ± standard deviation (SD). All the extracts were found to be significant as they exhibited differences at $p < 0.05$.

3. Results and Discussion

The pomegranate peel contains many diverse antioxidants, including those possibly not so far well characterized. Pomegranate peel also had been shown to be loaded with polyphenols [19]. It is time-consuming to purify all antioxidants, one by one, from pomegranate peel. From the technical point of view, a suitable extracting procedure must be developed to recover as many antioxidants as possible before an extract rich in natural antioxidants could be further explored for possible application in health-promoting supplements for the food industry. The yield (w/w, db) of MeOH, acetone and EtOAc extracts were found to be 29.55 %, 6.38% and 2.62% respectively. The phenolic content (tannic acid equivalents) was highest in the MeOH extract, as 2.4 ± 0.013 mg/g followed by acetone (2.19 ± 0.089 mg/g) and EtOAc (0.7 ± 0.039 mg/g) extracts. The total phenolics content calculated in the results are different from the result reported elsewhere in literature. This deviation particularly in the values is likely to be due to the difference in extraction and phenolic content determination procedures [20]. Singh *et al.* [15] reported a phenolic content of 0.9 mg/g, 2.2 mg/g and 0.15 mg/g for ethyl acetate, methanol and water extracts respectively when expressed as tannic acid equivalents.

The FRAP assay treats the antioxidants which are present in the samples as reductants in a redox-linked colorimetric reaction and the value signifies the reducing power of the antioxidants. The reducing power of the

antioxidants has been reported to be highest in methanol extract as 756.44 ± 78.4 mg Fe^{II}/g extract followed by acetone (659.61 ± 45.6 mg Fe^{II}/g extract) and ethyl acetate (495.37 ± 53.8 mg Fe^{II}/g extract). Variations in antioxidant capacity of methanol may be attributed to differences in their phenolic contents [21]. Among nine varieties of pomegranate peel, their FRAP value was ranged between 516.66 ± 118.71 to 699.97 ± 96.65 mg Fe^{II}/g extract [22].

Antioxidant reacts with DPPH, which is a stable free radical, and converts it to R, R-diphenyl- α -picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. At 50 ppm, ethyl acetate, methanol, and acetone extracts of pomegranate peel exhibit 41%, 78%, and 53% free radical scavenging activity, respectively. Whereas at 100 ppm they exhibited 50%, 91% and 68% free radical scavenging activity, respectively. The results of the present work are almost consistent with the outcome from the study of the antioxidant activity of pomegranate peel extracts using in vitro models. The results were that at 50 ppm, ethyl acetate, methanol, and acetone extracts of pomegranate peel exhibited 48%, 81% and 56% radical scavenging activity, respectively. Whereas at 100ppm the extracts exhibited 54%, 88% and 63% radical scavenging activity, respectively [15].

TEAC value of the pomegranate peel extract has also been reported. The total antioxidant capacity of pomegranate fruit extracts of peel as TEAC value for methanol was found to be 5.26 ± 0.001 mg/g. Moreover, the total antioxidant capacity of pomegranate fruit extracts of peel as TEAC value for acetone and ethyl acetate extract has been reported as 4.93 ± 0.0038 mg/g and 2.83 ± 0.016 mg/g respectively. The main reason that can be cited to support this is that methanol is a polar protic solvent on account of its ability to donate proton and its dielectric constant (33) is also greater than that of acetone (21) and ethyl acetate (6).

The dried pomegranate peel extracts were used to determine their antioxidant capacities by the formation of phosphomolybdenum complexes. This assay was based on the reduction of Mo (VI) to Mo (V) in the presence of antioxidant compounds and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH, which was measured at 695 nm. Antioxidant capacity was determined by phosphomolybdenum method in terms of ascorbic acid equivalent. The antioxidant capacity as determined by the phosphomolybdenum method of methanol, acetone, ethyl acetate extracts were found to be ranging from 126.98 ± 49.1 to 438.362 ± 39 , 112.012 ± 65.1 to 367.21 ± 89.2 and 88.58 ± 43.4 to 424.449 ± 53.6 mg/g of extract as ascorbic acid equivalent (AAE) respectively as shown in Table 1.

TABLE I: Antioxidant capacity (as ascorbic acid equivalent (AAE)/g extract) of pomegranate peel extract at different concentration

Solvents	Concentration of Extracts			
	25 μ g/mol	50 μ g/mol	75 μ g/mol	100 μ g/mol
Methanol	126.98 ± 49.1^b	231.13 ± 31.5^c	298.87 ± 73.9^c	438.36 ± 39^d
Acetone	112.01 ± 65.1^b	247.87 ± 34.4^c	314.37 ± 45.2^c	367.21 ± 89.2^c
Ethyl acetate	88.58 ± 43.4^a	236.58 ± 27.1^c	349.24 ± 21.5^c	424.44 ± 53.6^d
Difference letter in the same column indicates significant difference ($p < 0.05$).				

It is quite difficult to assign an order of antioxidant capacity to the extracts because of the differential responses at four concentrations. At 25 μ g/mol and 100 μ g/mol concentrations, the methanol (MeOH) extract showed strong antioxidant capacities, whereas acetone and ethyl acetate (EtOAc) extracts showed strong antioxidant capacities at 50 μ g/mol and 75 μ g/mol concentrations, respectively. The results followed the similar trend line as reported by Negi et al. [20] in which the methanol extract exhibited the strongest antioxidant activity at 25 and 100 μ g/mol concentrations whereas acetone and ethyl acetate (EtOAc) extracts showed strong antioxidant capacities at 50 μ g/mol and 75 μ g/mol concentrations, respectively. Also, methanol being a polar protic solvent possesses dissociable H⁺ ions and thus has higher antioxidant activity. All the extracts showed an increase in antioxidant capacity with increase in dose of ascorbic acid concentration.

HPLC analysis of the pomegranate peel extract treated with acetone and methanol was carried out. The amount of five antioxidants present in the extract was determined through chromatographic technique. The

compounds were chiefly as: gallic acid, caffeic acid, p-coumaric acid, epicatechin and chlorogenic acid. The HPLC patterns of these antioxidants in peel extracts (acetone and methanol) using the HPLC grades of catechin, gallic acid, caffeic acid, chlorogenic acid, p-coumaric acid, catechin and gallic acid are also studied. The presence of catechin followed by chlorogenic acid in the acetone extract of pomegranate peel is shown to be the major component. While the presence of chlorogenic acid followed by caffeic acid in the methanol extract of pomegranate peel is shown to be the major component (Table 2). Similar reports are found by Usman and Kumar [23] using methanol for ultrasound assisted extraction of polyphenols from pomegranate peel.

TABLE II: Amount of the compounds (on dry basis) obtained through HPLC on dry basis

Extracts/compounds	Acetone extract (mg/kg)	Methanol extract (mg/kg)
Caffeic acid	ND*	480.152
Chlorogenic acid	2094.152	4114.967
p-coumaric acid	216.766	183.951
Catechin	7693.029	ND*
Gallic acid	40.258	234.878
ND*: Not detected		

4. Conclusions

All the extracts obtained from the peel studied here showed antioxidant capacity, although with different efficiencies. Due to the low cost and easy availability of fruit peel, which otherwise would be discharged as waste in the environment, they should be regarded as potential nutraceutical resources. In a nutshell, the methanol extract of the pomegranate peel exhibited the strongest antioxidant activity with an ample quantity of the total phenolics. The extracts from fruit residue hold promise in food industry as sources of bioactive compounds. In addition, an established use of the fruit residue will also help alleviate pollution problems caused because of the poor disposal of such residues. It is however necessary to consider both environmental (waste management and protection against pollution) aspects and economical aspects (extraction profitability) before the extracts from fruit residue could be commercially exploited. The overall results showed that the pomegranate peel extracts have antioxidant properties and more research is needed to establish bioavailability and real benefits of these extracts obtained from fruit residues in vivo.

5. References

- [1] P. Knekt, J. Kumpulainen, R. Jarvinen, H. Rissanen, M. Heliovaara, A. Reunanen, T. Hakulinen and A. Aromaa, "Flavonoid intake and risk of chronic diseases," *American Journal of Clinical Nutrition*, vol.76, pp.560-568, 2002.
- [2] I.C. Arts and P.C. Hollman, "Polyphenols and disease risk in epidemiologic studies," *American Journal of Clinical Nutrition*, vol. 81, pp. 317-325, 2005.
- [3] I. Rahman, S.K. Biswas and P.A. Kirkham, "Regulation of inflammation and redox signaling by dietary polyphenols," *Journal of Biochemistry and Pharmacology*, vol. 72, pp. 1439- 1452, 2006.
<http://dx.doi.org/10.1016/j.bcp.2006.07.004>
- [4] L.M. Szewczyk and T.M. Penning, "Mechanism based inactivation of COX-1 by red wine m-hydroquinones: A structure-activity relationship study," *Journal of Natural Product*, vol. 67, pp. 1777-1782, 2004.
<http://dx.doi.org/10.1021/np0498410>
- [5] J.A. Menendez, A.V. Martin, R. G. Villalba, A. C. Poncorbo, C. O. Ferraros, A.F. Gutierrez, and A.S. Carretero, "Oncogene effects of phenolic compounds directly isolated from commercial extra-virgin olive oil (EVOO)," *International Journal of Oncology*, vol. 18, pp. 377-400, 2008.
- [6] M.A. Gates, A.F. Vitonis, S.S. Tworoger, B. Rosner, L.T. Ernstruff, S.E. Hankinson, and D.W. Cramer, "Flavonoid intake and ovarian cancer risk in a population based case control study," *International Journal of Cancer*, vol. 124, pp. 1918-1925, 2009.
<http://dx.doi.org/10.1002/ijc.23924>

- [7] K. Ono, M. Hirohata, and M. Yamada, "Alpha-synuclein assembly as a therapeutic target of Parkinson's disease and related disorders," *Current Pharmaceutical Design*, vol. 14, pp. 3247-3266, 2008.
<http://dx.doi.org/10.2174/138161208786404191>
- [8] V. Vingtdeux, U.D. Werringloer, H. Zhao, P. Davies, and P. Marambaud, "Therapeutic potential of resveratrol in Alzheimer's disease," *Journal of BMC Neuroscience*, vol. 9, pp. S6, 2008.
<http://dx.doi.org/10.1186/1471-2202-9-S2-S6>
- [9] B. Halliwell, "Antioxidant characterization: methodology and mechanism," *Journal of Biochemical Pharmacology*, vol. 49, pp. 1341-1348, 1995.
[http://dx.doi.org/10.1016/0006-2952\(95\)00088-H](http://dx.doi.org/10.1016/0006-2952(95)00088-H)
- [10] J.J. Martinez, P. Melgarejo, F. Hernandez, D.M. Salazar and R. Martinez, "Seed characterization of five new pomegranate (*Punica granatum L.*) varieties," *Journal of Scientia Horticulture*, vol. 110, pp. 241-246, 2006.
<http://dx.doi.org/10.1016/j.scienta.2006.07.018>
- [11] M.S. Mokbel and F. Hashinaga, "Evaluation of the antioxidant activity of extracts from buntan (*Citrus grandis* Osbeck) fruit tissues," *Food Chemistry*, vol. 94, pp. 529-534, 2006.
<http://dx.doi.org/10.1016/j.foodchem.2004.11.042>
- [12] K. S. Valcheva, P. Borisova, B. Galunska, I. Krasnaliev and A. Belcheva, "Hepatoprotective effect of the natural fruit juice from *Aronia melanocarpa* on carbon tetrachloride-induced acute liver damage in rats," *Journal of Experimental and Toxicologic Pathology*, vol. 56, pp. 195-201, 2004.
<http://dx.doi.org/10.1016/j.etp.2004.04.012>
- [13] Y.Y. Soong and P.J. Barlow, "Antioxidant activity and phenolic content of selected fruit seeds," *Food Chemistry*, vol. 88, pp. 411-417, 2004.
<http://dx.doi.org/10.1016/j.foodchem.2004.02.003>
- [14] Y. Li, C. Guo, J. Yang, J. Wei, J. Xu and S. Cheng, "Evaluation of antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract," *Food Chemistry*, vol. 96, pp. 254-260, 2006.
<http://dx.doi.org/10.1016/j.foodchem.2005.02.033>
- [15] R.P. Singh, K.N. Chidambara Murthy and G.K. Jayaprakasha, "Studies on the antioxidant activity of pomegranate (*Punica granatum*) peel and seed extract using in vitro models," *Journal of Agricultural and Food Chemistry*, vol. 50(1), pp. 81-86, 2002.
<http://dx.doi.org/10.1021/jf010865b>
- [16] I.F.F. Benzie and J.J. Strain, "The ferric reducing ability of plasma (FRAP) as a measure of antioxidant power: The FRAP assay," *Journal of Analytical Biochemistry*, vol. 239, pp. 70-76, 1996.
<http://dx.doi.org/10.1006/abio.1996.0292>
- [17] R. Re, N. Pellegrinni, A. Proteggente, A. Pannala, M. Yang and C. R. Evans, "Antioxidant activity applying an improved ABTS radical cation decolorization assay," *Journal of Free Radicals in Biology and Medicine*, vol. 26, pp. 1231-1237, 1999.
[http://dx.doi.org/10.1016/S0891-5849\(98\)00315-3](http://dx.doi.org/10.1016/S0891-5849(98)00315-3)
- [18] P. Prieto, M. Pineda and M. Aguilar, "Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E," *Journal of Analytical Biochemistry*, vol. 269, pp. 337-341, 1999.
<http://dx.doi.org/10.1006/abio.1999.4019>
- [19] C.B. Nasr, N. Ayed and M. Metche, "Quantitative determination of the polyphenolic content of pomegranate peel," *Zeitschrift für Lebensmittel Untersuchung Und Forschung*, vol. 203, pp. 374-378, 1996.
<http://dx.doi.org/10.1007/BF01231077>
- [20] P.S. Negi, G.K. Jayaprakasha and B.S. Jena, "Antioxidant and antimutagenic activities of pomegranate peel extracts," *Food Chemistry*, vol. 80, pp. 393-397, 2003.
[http://dx.doi.org/10.1016/S0308-8146\(02\)00279-0](http://dx.doi.org/10.1016/S0308-8146(02)00279-0)
- [21] M.I. Gil, F.A.T. Barberan, B.H. Pierce, D.M. Holcroft and A.A. Kader, "Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing," *Journal of Agricultural and Food Chemistry*, vol. 48, pp.4581-4589, 2000.

<http://dx.doi.org/10.1021/jf000404a>

- [22] M.S.A. Reza, M. Hajimahmoodia, M.O. Reza, N. Sadeghib, B. Jannatc, A.M. Ranjbara, N. Gholamb and T. Moridib, "Comparative antioxidant activity and total flavonoid content of Persian pomegranate (*Punica granatum L.*) cultivars," *Iranian Journal of Pharmaceutical Research*, vol. 10(3), pp. 519-524, 2011.
- [23] U. Ali and P. Kumar, "Effect of soxhlet and ultrasound assisted extraction on antioxidant activity of pomegranate peel extract", *International Journal of Food and Nutritional Sciences*, vol. 3(6): pp. 265-270, 2014.