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Research Article

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Valorization of pomegranate (*Punica granatum l.*) By-products

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Abstract Pomegranate peels are the major by-products of pomegranate juice industry; they are rich sources of natural bioactive compounds which play an important role in the prevention of diseases. There are many antioxidant compounds in pomegranate peel and seeds, and they could be used as a natural and very inexpensive alternative to synthetic food additives. The present study evaluates the effect of solvent (methanol, ethanol and water) on the efficiency of the extraction of antioxidants from pomegranate peel and seeds. Water extraction, which has the economic and safety merits, can be used as an environmentally friendly method for producing antioxidants from pomegranate peels. This work proves that conditions, such as extraction solvent, have a crucial impact on obtaining extracts rich in antioxidants from pomegranate biowastes.

Keywords total phenol, antioxidant, pomegranate, residues

1. Introduction

The valorization of agricultural co-products is receiving more attention with many researchers evaluating the conversion of co-products into food ingredients and other value-added materials [1].

Pomegranate (*Punica granatum* L.) is an important fruit of tropical and subtropical regions, which originated in the Middle East and India and has been used for centuries in ancient cultures for its medicinal purposes. It is widely reported that pomegranate exhibits antivirus, antioxidant, anticancer, and antiproliferative activities [2-4]. Pomegranate can be consumed fresh or in processed form as juice, wines, flavors, and extracts. Compared to other fruit juices, red wine, and green tea, commercial pomegranate juice has the highest antioxidant activities and currently is a high value product in the agricultural market [5].

In commercial juices extracted from whole pomegranates the pomegranate antioxidant activity is typically higher than in experimental juices obtained from the arils only [6]. This could be attributed to its high content of polyphenols in peel, such as condensed tannins and anthocyanins. The processing of pomegranate juice involves squeezing juice from the fruit with the seeds and the peels together [7]. The resulting marc on a weight basis consists of approximately 73 % peels and 27% seeds and has a high potential for value addition as a source of phenolics, proanthocyanidins and flavonoids which are herein also referred to as antioxidants [8-11].

Recently, natural antioxidants have become very popular for medical and food applications and are preferred by consumers than synthesized antioxidants, such as BHA and BHT [12-13]. For example, enrichment of ice creams with pomegranate by-products might provide consumers health benefits with striking functional properties of punicalagins in pomegranate peel, and punicic acid in pomegranate seed oil [14]. Also, addition of pomegranate to popular chicken meat products enhanced its shelf life by 2–3 weeks during chilled storage [15].

2. Materials and Methodology

2.1. Plant Material

Pomegranate fruits were collected from a local supermarket. The selected fruits were at mature stage; they were cut into and juice was squeezed till all juice recovered. The juice was filtered using a cheese cloth, the pulp part that remained on the cheese cloth was collected and dried. Mango peels were washed with tap water to remove any dirt particles. The peels were spread thin in trays and dried at 50 °C for 18 h to moisture content around 10 % (dry basis). The moisture content was determined by using oven drying at 105 °C until constant weight was achieved. The dried peels were powdered using a hammer mill and sieved.

2.2. Solvents

In the present work, three solvents with different polarities were used to identify the most suitable one for the recovery of antioxidant components from pomegranate peel and seeds. The polarity of a solvent besides the dipole moment, polarizability and hydrogen bonding determines what type of compounds it is able to dissolve. Three types of solvents were used in this experiment: deionized (DI) water (polar solvent with a dielectric constant of 80); ethanol (polar with a dielectric constant of 24); methanol (polar with a dielectric constant of 33). All chemicals used were of analytical grade. For each solvent, dried and ground peel and seeds was extracted in a thermostatic water bath shaker at 40 °C for 4 h in a conical flask. The liquid extract was separated from solids by vacuum enhanced filtration through Whatman No. 1 filter paper. The filtrates were air dried in hood at room temperature and residual moisture removed in a vacuum oven at 50±2 °C. The dried extracts were weighted to analyze the total extract yield, the contents and yield of antioxidant compounds including total phenolics. The reported results, as illustrated in equations 1-3, include the total extract yield (%) and the yield of total antioxidant (total phenolics) from the pomegranate peels (%).

$$Total\ extract\ yield\ (\%) = \frac{g\ dried\ \ extract}{100\ g\ pomegranate\ \ \ peels\ (or\ \ seeds\ )} x100 \quad (1)$$

$$Yield\ of\ antioxidant\ (\%) = \frac{g\ total\ \ of\ \ antioxidant}{100\ g\ pomegranate\ \ \ peels\ (or\ seeds\ )} x100 \quad (2)$$

$$Content\ of\ antioxidant\ (\%) = \frac{g\ total\ \ of\ \ antioxidant}{100\ g\ dried\ \ extract} x\ 100 \quad (3)$$

All reported weights and percentages are dry basis unless specified otherwise.

2.3. Methods

2.3.1. Total phenolic content

Total phenolic contents were assayed using the Folin-Ciocalteau reagent, following Singleton's method [12]. An aliquot (0.125 mL) of a suitable diluted methanolic, ethanolic and water seed and peel extract (0.25 $mg{\cdot}mL^{-1}$) was added to 0.5 mL of deionized water and 0.125 mL of the Folin-Ciocalteau reagent. The mixture was shaken and allowed to stand fot 6 min, before adding 1.25 mL of 7% Na_2CO_3 solution. The solution was then adjusted with deionized water to a final volume of 3 mL and mixed thoroughly. After incubation for 90 min at 23^0C, the absorbance versus prepared blank was read at 760 nm. The phenolic content of seeds and peels (three replicates per treatment) was expressed as mg gallic acid equivalents (GAE) per gram of dry weight through the calibration curve with gallic acid.

2.3.2. DPPH assay

The electron donation ability of the obtained methanol, ethanol and aqueous acetone extracts was measured by bleaching of the purple-coloured solution of 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) [13]. 2 mL of methanolic grape seed and peel extracts were added to a 0.5 mL of a 0.2 $mmol{\cdot}L^{-1}$ DPPH methanolic solution. The mixture was shaken vigorously and left standing at room temperature for 30 min. The absorbance of the resulting solution was then measured at 517 nm after 30 min. The ability to scavenge the DPPH radical was calculated using the following equation:

$$DPPH\ scavenging\ effect(\%) = \left[\frac{(A_0 - A_1)}{A_0}\right] \times 100$$

where A_0 is the absorbance of the control at 30 min, and A_1 is the absorbance of the sample at 30 min. Samples were analyzed in triplicate.

2.3.3. Statistics

Samples were assayed in triplicate and results are given as averages ± SD. Student's t test was used for the statistical evaluation and $p < 0.05$ was considered statistically significant.

3. Result and Discussion

Results for the total extract yields reported as percentage of g of extract per 100g pomegranate peel on dry basis indicated that the pomegranate peel extracted with methanol gave the highest total extract yield (41.01 ± 1.32), followed by water (39.32 ± 1.44), ethanol (15.33 ± 1.98). Results for the total extract yields reported as percentage of g of extract per 100g pomegranate seeds on dry basis indicated that the pomegranate seeds extracted with methanol also gave the highest total extract yield (8.12 ± 0.22), followed by water (8.03 ± 0.98), ethanol (2.32 ± 1.23).

The effect of different solvents on the yield of total phenolics from the pomegranate peels and seeds are shown in Fig. (1). Methanol and water gave the top two yields for pomegranate peels and also for the seeds.

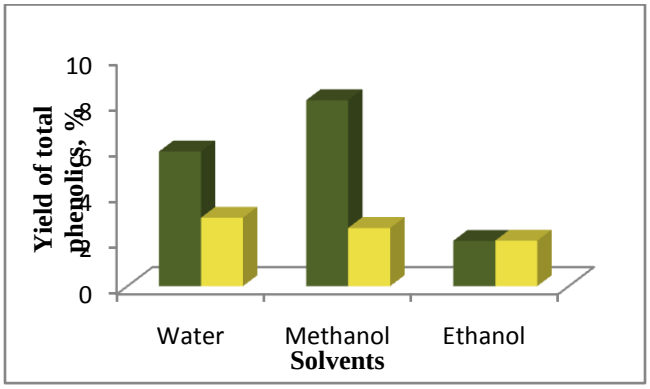

Figure 1: The effect of solvents on the yield of total phenolics from the pomegranate peels (■) and seeds (■)

The value for the total phenolic yield obtained using MeOH is comparable to that reported by other researchers [16]. This deviation particularly in the values is likely to be due to the difference in extraction and phenolic content determination procedures [17].

The concentration of total phenolics in the extracts content wise were the highest in the usage of methanol among the three different extraction solvents (Fig. 2). The total phenolics content was higher in methanol extract (13.21 %) than in water extract (11.21 %) and comparatively lower in ethanol extract (7.22 %). Our results show that the total phenolic content in the water extract and the MeOH extract was nearly the same: 11.21 % and 13.21 % respectively.

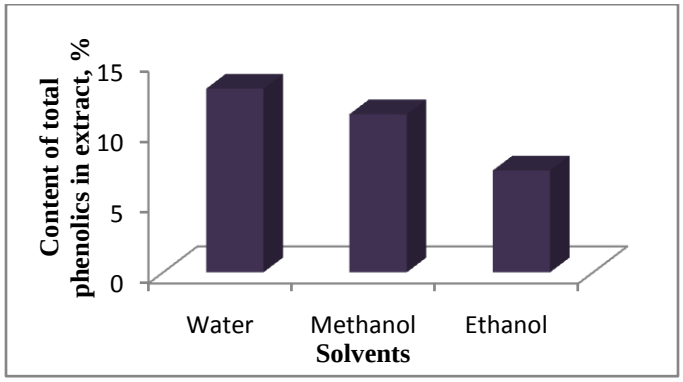

Figure 2: The effect of different solvents on the content of total phenolics from the pomegranate peels

Free radical scavenging potentials of pomegranate peel and seed extracts were tested by the DPPH method, and the results are shown in Figure 3. DPPH is a stable free radical which dissolves in methanol or ethanol, and its purple color shows a characteristic apsorption at 517 nm. When an antioxidant scavenges the free radical by hydrogen decoloration, the color from the DPPH assay solution becomes light yellow. Antioxidant reacts with DPPH and convert it to α,α-diphenyl-β-picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. For pomegranate peel, methanol, ethanol and water extracts of pomegranate peel exhibit 91.68, 70.36, and 88.3 % free radical scavenging activity, respectively, according to this method. The activity of the extracts is attributed to their hydrogen donating ability [16]. On the other hand, antioxidants are believed to intercept the free radical chain of oxidation and to donate hydrogen from the phenolic hydroxyl groups, thereby forming a stable end product, which does not initiate or propagate further oxidation of the lipid [18]. The data obtained reveal that the extracts are free radical inhibitors and primary antioxidants that react with free radicals.

Thus, the results of the present work indicate that the selective extraction of antioxidant from natural sources by appropriate solvent is very important in obtaining fractions with high antioxidant activity.

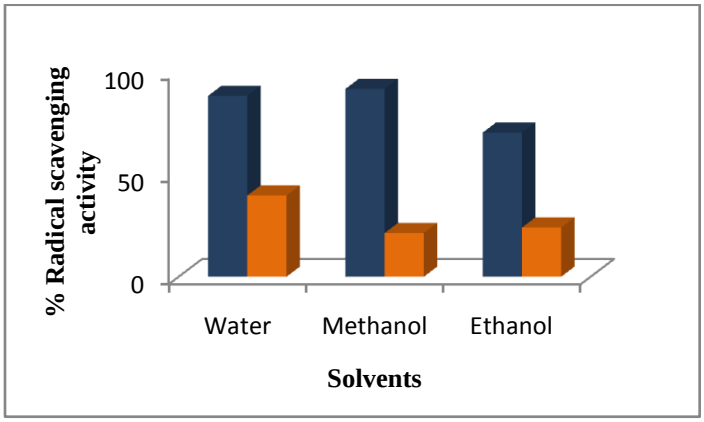

Figure 3: Radical scavenging activity of pomegranate peel (■) and seeds extracts (■) by DPPH method

4. Conclusion

The results of the present work indicate the presence of compounds possessing antioxidant activity from peel and seeds of pomegranate with peel as an enriched source of the antioxidants exhibiting higher activity as compared to seeds. The difference in the antioxidant activity of the peel and seed may be ascribed to their different phenolic compositions. Further studies are needed on the isolation and characterization of individual phenolic compounds to elucidate their different antioxidant mechanisms and the existence of possible synergism, if any, among the compounds.

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