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Total phenol, Total Flavonoids and Antioxidant Activity of Pomegranate Peel

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Abstract: Pomegranate (*Punica granatum* L.) is a nutrient dense food rich in beneficial phytochemicals. In this study, three types of solvent extracts of Pomegranate peel were used to examine the effects of extraction solvent on total phenolics content (TPC), total flavonoids content (TFC) and antioxidant activity by 1,1-diphenyl-2-picryl hydrazyl radical scavenging (DPPH) and ferric reducing antioxidant power (FRAP) were determined. Results showed that extraction solvent had significant effects on TPC, TFC, and antioxidant activity of acetone extract. The highest content of TPC, TFC and antioxidant activity (FRAP and DPPH) were found in 50% acetone extracts. The TPC for pomegranate peel from84.15 to 168.26 mg gallic acid/100 g dry weight, and TFC were between 42.40 to 87.21 mg QE/100g dry weight and antioxidant activity (FRAP from 86.21 to 142.21 mg Trolox equivalents/100 g dry weight), DPPH were between 45%to88.46%). The largest amount of TPC and TFC which leads to more effective radical scavenging effect was shown by 50% acetone extract. Moreover, amount of phenolic compounds and antioxidant activities increased in acetone extract. Acetone 50% solvent showed the greatest capability in extracting antioxidants and inhibiting the free radicals produced. It was concluded that extraction solvent play important roles on the phenolics compounds and their antioxidant activity of pomegranate peel extract.
Keyword: *Punica granatum*, Solvent, Antioxidant activity, Phenolic compounds.

Introduction

Pomegranate (*Punica granatum*L.) have been variously placed in the *Lythraceae*or *Punicacea* family, depending on the taxonomist and whether they are considering morphological or molecular data[1]. Pomegranate grows as a shrubor small tree reaching 4-10 m.The fruit size can vary from 6-12 cm in diameter and has a tough, leathery skin[2]. Pomegranate and its derivates such as juice, peel and seeds are rich source of several high-value compounds with beneficial physiological activities. Its high AA has led to applications in functional food formulation, mainly for heart and prostate health. The health benefits attributed to the consumption of fruits, vegetables and cereals are related, at least in part, to their antioxidant activity. Many constituents of these dietary components may contribute to their protective properties, including: vitamins C and E, Selenium and other trace minerals and micronutrients, carotenoids, phytoestrogens, allium compounds, glucosinolates and indoles, dithiolthiones, isothiocyanates, protease inhibitors, fiber and folic acid. These compounds may act independently or in combination as anti-cancer or cardioprotective agents by a variety of mechanisms. One such protective mechanism is radical-scavenging activity[3].Oxidation can be delayed by antioxidants of a few substrates in a chain reaction. Thus, antioxidants has a crucial role in diseases prevention, and can be used in increasing doses as technological and economic advancement are obtained[4]. The decline in morbidity and mortality due to heart disease and cancer are often associated with the consumption of fruits and vegetables[5]. Pomegranate the main source of many vitamins, such as vitamin C containing also vitamin E, pectin, and carotenoids. Among the different factors (sample pre-treatment, solvent/sample ratio, solvent type, extraction time, and extraction temperature) affecting extraction efficiency, solvent type has been the most analyzed [6] .

Frequently used solvents for antioxidant extraction include methanol, ethanol, and acetone either alone or in combination with an aqueous solution[6]. Therefore, this study aimed to determine the effect of solvent for extracting antioxidant compounds from pomegranate peel

Materials and Methods

Sample collection and preparation of pomegranate peels extract

The peels of pomegranate (*Punica granatum*L.) were obtained from the market in Thi-Qar city, Iraq. The peels of pomegranate were cleaned and cut into small pieces, and then oven dried at 50^{o}C for 48 h. The dried sample was then pulverized using a mechanical grinder and passed through a 250 μm mesh and then stored at 4^{o}C until use. The different types of solvent used were absolute methanol, ethanol, acetone, water and their aqueous solutions at 50%and 80% concentrations. All tests were performed at room temperature.

Total phenolic content (TPC)

The amount of total phenolics content (TPC) in pomegranate was determined with the Folin-Ciocalteu reagent base on[7]. About 0.5 mL of Folin-Ciocalteau (10%, v/v) was added to 0.1 mL of pomegranate extract sample. The mixture was swirled and allowed to stand for 6 min followed by the addition of 1 mL 7.5% (w/v) of sodium carbonate (Na_2CO_3) and samples were mixed. Solutions were allowed to stand for 2 h at room temperature and the absorbance were read at 765 nm wavelength using spectrophotometer. The results were express as milligrams of gallic acid equivalents per 100 g of sample (mg GAE/100 g of DW).

Total flavonoid content (TFC)

The TF content was determined by the colorimetric method as described by[8]. A total 0.5 ml of the extract was mixed with 2.25 ml of distilled water in a test tube, followed by the addition of 0.15 mL of 5% (w/v) $NaNO_2$ solution. After 6 min, 0.3 ml of a 10% $AlCl_3 \cdot {}_6H_2O$ solution was added, and the reaction was allowed to stand for another 5 min before 1.0 ml of 1M NaOH was added. The mixture was mixed well by vortexing, and the absorbance was measured immediately at 510 nm using a spectrophotometer. The results were expressed as milligrams of quercetin equivalents (QE) per 100 g of fresh sample (mg QE/100 g of FW).

Radical-scavenging activity (DPPH)

The DPPH free radical scavenging assay will be measured using the method of[9] technique. The 2,2-diphenyl-1-picrylhydrazyl was dissolved in of methanol to prepare the DPPH solution. The DPPH solution was dilute several 42 times with methanol to obtain 0.9 absorbance at 516 nm, using spectrophotometer. 1 ml of DPPH solution was added to 100 μl of pomegranate extract solution. The mixture was shaken in a vortex and kept for 2 h in dark place. After 2 h, the mixture was transferred to micro plate plastic and absorption of DPPH solution after the addition of the sample was measured at 516 nm using the spectrophotometer. The changing in absorption of each sample computed as difference between the plank and sample readings. The following equation (3.1) calculates the percentage of DPPH scavenging activity:

The percentage of DPPH scavenging activity was calculated using the following equation:

Radical scavenging (%) = $[(A_0 - A_1 / A_0) \times 100]$

Where A_0 is the absorbance of the control and A_1 is the absorbance of the sample extracts.

Ferric reducing antioxidant power (FRAP)

[10]method used to determine the antioxidant capacity of each sample. FRAP reagent was prepared by using 300 mM acetate buffer, (pH 3.6; 3.1 g of sodium acetate trihydrate, plus 16-mL glacial acetic acid and the distilled water made up to total volume of 1L) 10 mM TPTZ (2,4,6-tri (2-pyridyl)-striazine), in 40 mM HCl and 20 mM $FeCl_3 {}_6H_2O$ in the ratio of 10:1:1. Freshly prepared FRAP reagent (1000 μL), warmed at 37 °C, was mixed with 100 μl sample, standards. Samples were kept for 30 min and after that the mixture was transferred to micro plate plastic. The absorbance was measured at 595 nm wavelength using spectrophotometer. The result was express as milligrams of Trolox equivalents per 100 g of sample (mg TE/g of DW).

Statistical analysis

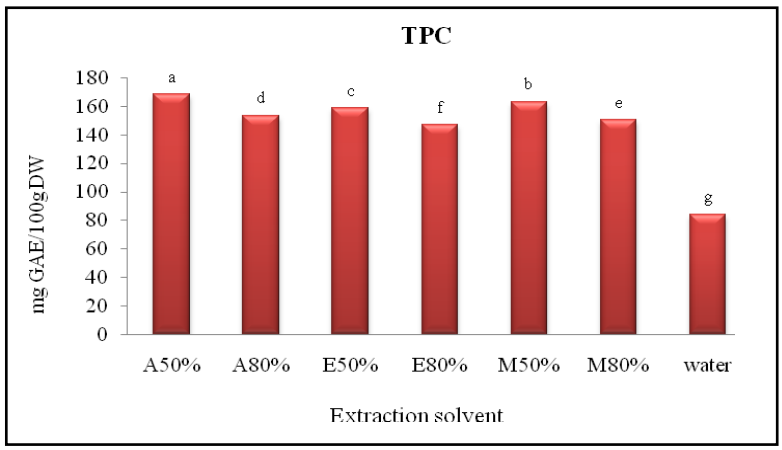
The experiment was carried out in triplicate. Statistical analysis of the data was performed by one-way ANOVA using (SPSS 19 software). Significant differences (P<0.05) among the four types of solvent were analyzed by Duncan triplicates range test[11]

Results and Discussion

Effect of solvent system

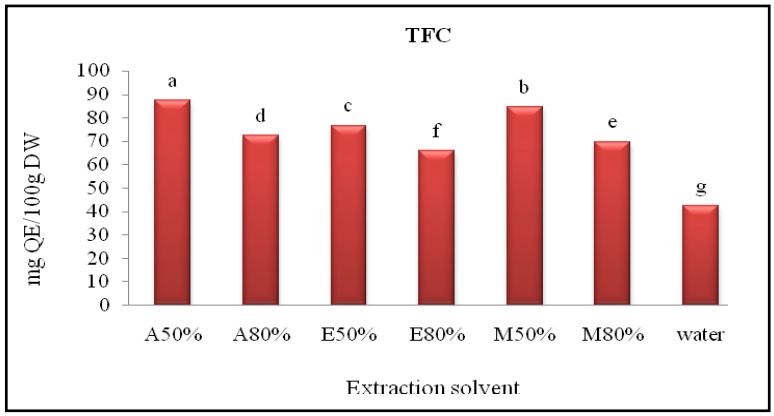
Frequently used solvents for antioxidant compound extraction (from fresh fruits/vegetables at different concentrations) include acetone, ethanol, methanol, propanol, and ethyl acetate[12,13]. The solubility of antioxidant compounds in solvent was found to have a significant effect on the recovery of compounds at the time of extraction. Thus, the polarity of solvents has an indirect function in the extraction process because it can raise the solubility of antioxidant compounds [13]. It was impossible to develop a standard solvent that was suitable for the all kinds of antioxidant compounds extraction from plants. Thus, the screening process is important to identify the best solvent for a specific extraction procedure and thus complete the optimal antioxidant task for a certain sample.

The results show that the recovery of antioxidant activity is based on the type and polarity of solvent. For TPC, TFC, FRAP and DPPH assays, 50% acetone solvent showed the best extraction power among the other solvent systems (Figures1,2,3 and 4). However, for phenolics content (TPC and TFC) and antioxidants activity (FRAP and DPPH) assays, 50% acetone showed the best extraction power for pomegranate peel. According to some studies, add water to the solvent improved the extracting power and antioxidant properties [14]. This finding concurs with that of[15,16] who found that 50% water is the best solvent for extraction in TPC, TFC and DPPH assays in terms of antioxidant activity. [17] found that methanol is the most powerful solvent for the extraction of antioxidant compounds from ginger fruit. However, no solvent screening was performed by the two latter groups of authors. Thus, the extraction efficiency of acetone was compared with that of ethanol. The difference in extraction capability of solvents at different types and concentrations might be attributed to changes in relative polarity [15]. Most pure solvents (acetone, ethanol, and water) exhibit weak extraction powers. The better extraction power of aqueous solvent indicates that the mixing of a non-polar solvent with water may increase the polarity index of solvents, thereby consequently enhancing the extraction power of a certain solvent. Our findings are consistent with those of [15], who found that the increase in polarity of a solvent (up to 50% water) enhances the solubility of antioxidant compounds. Therefore, in banana, the extraction power of a distilled solvent is the weakest among other solvents.



[a-g]Mean with different letters are significantly different (P< 0.05)

Figure 1: Effect of extraction solvents on the total phenolic content of pomegranate peel



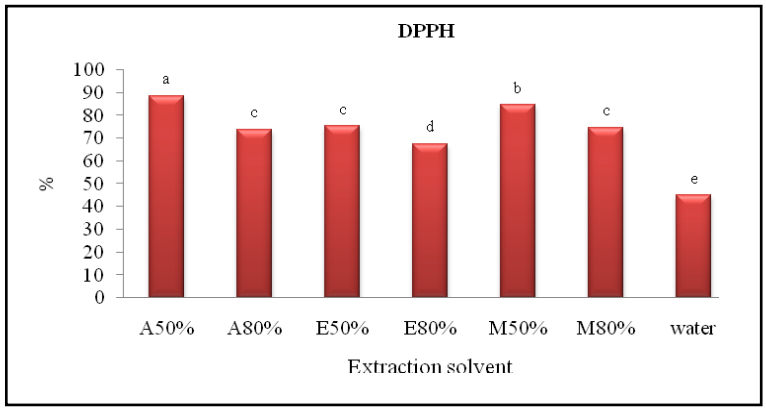
[a-g]Mean with different letters are significantly different (P< 0.05)

Figure 2: Effect of extraction solvents on the total flavonoids content of pomegranate peel

Antioxidant properties of pomegranate peel

Figure 1 and 2 showed significant difference (P<0.05) in the total phenolic and total flavonoids of pomegranate peel. Acetone 50% gave the highest phenolic content when compared with other solvents. The content of phenolic compounds in different solvent extracts (acetone, ethanol and methanol) of pomegranate peels Figure 1. With increase in solvent polarity, TP and TF content increased in extract. High content of TP (168.26 mg EGA/ 100g DW) and TF (87.21mg/g DW) were obtained from acetone extract. After acetone 50%, methanol 50% had high content of phenolic compounds in extract. As found in this study, in a mixture with no aqueous content, the extraction efficiency was low and negative. It is clear that the addition of some amount of water enhance the extraction efficiency. One possible reason for the increased efficiency with the presence of some water might be due to the increase in bulge of plant material by water, which increased the contact surface area between the plant matrix and the solvent [18]. Our result is similar to that reported by[17]where aqueous solvent was most effective in extracting phenolic components from ginger fruit. Research conducted by [15] confirmed the ineffectiveness of acetone, methanol and water for the extraction of total phenols of grapes seeds (*Vitisvinifera*).

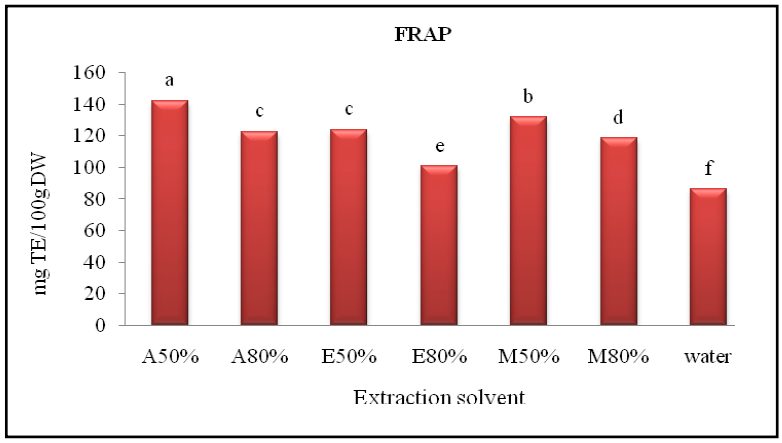
Antioxidant has two main categories: primary and secondary. DPPH assays are often used to measure the capability of primary antioxidants in plants as these antioxidants react to scavenge free radicals from the DPPH solution, thus suppressing the formation of the free radical initiation chain and disrupting the propagation chain by donating hydrogen atoms or electrons. This process converts free radicals into a more stable product [19,20] This condition leads to the discoloration of the DPPH solution from purple to yellow. As shown in Figure 2, the DPPH scavenging percentages for pomegranate peel were from 45- 88.46 %, depending on the type of solvent used. Generally, acetone had the best capability in a DPPH scavenging system, followed by methanol. To approximate the efficiency of antioxidant capacity, FRAP assays are frequently conducted in plants to contend with the FRAP reagent and reduce the ferric into ferrous.



[a-e]Mean with different letters are significantly different (P < 0.05)

Figure 3: Effect of extraction solvents on DPPH of pomegranate peel

Working antioxidant compounds are categorized as secondary antioxidants. In this case, these antioxidants suppress radical formation and prevent oxidative damage. Moreover, secondary antioxidants are also active in metal chelating and oxygen scavenging. The reduction of ferric in a FRAP reagent will contribute to the formation of a blue-colored product called TPTZ complex. In FRAP assays, Acetone50% had the highest TE content at 142.21 122.74 mg TE/100 g DW, followed by methanol 50%at 122.74 mg TE/100 g DW. This difference is again attributed to the type of solvent used (Figure4).The FRAP contents for ethanol 50%was 100.92 mg TE/100 g DW to 98 mg TE/100 g. TPC assays are dependent on oxidation, whereas FRAP assays are dependent on reduction reaction. In plants, these values can be correlated with the redox properties of antioxidant compounds.[21] discovered that acetone has the highest antioxidant activity. The difference in findings might possibly be attributed to the different extraction methods and solvents used [22].



[a-f]Mean with different letters are significantly different (P< 0.05)

Figure 4: Effect of extraction solvents on FRAP of pomegranate peel

Correlation of TPC, TFC, DPPH, and FRAP assays

A correlation analysis among phenolic compounds (TPC and TFC) assays, and antioxidant activity (FRAP, DPP) was performed regardless of the extraction solvent used. A high correlation (Table 1) was found between TPC, TFC and antioxidant activity (FPAP and DPPH). Thus, we can reasonably conclude that in the extract, antioxidant activity is related to the active component. Findings of researches of correlation analyses among TPC, TFC, and antioxidant activities (FRAP and DPPH) are high[12]. There have been significant effects on the antioxidant activities of pomegranate peel based on the solvent.

Table 1. Correlation coefficients of antioxidants activities of pomegranate peel.

Correlation coefficient (R^2)	FRAP	DPPH
TPC	0.92	0.86
TFC	0.91	0.88

Conclusion

The results of this study showed that the type of solvent used had a significant effect (P<0.05) on the extraction of antioxidant compounds from pomegranate (*Punica granatum*L.). TPC, TFC, FRAP and DPPH values of pomegranate peel extracts decreased with increase in the organic solvent concentration. In fact, it can be concluded that the extracts obtained using higher polar solvents were more effective than less ones. The addition of 50% water to methanol, acetone or ethanol can enhance the extracting power and antioxidant activity estimation especially methanol and acetone. The phenolic compounds (TPC and TFC) assays showed a good correlation with antioxidant activity FRAP and DPPH of pomegranate peel.

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References

1. Still D.W., Pomegranates: A Botanical Perspective. In N.P. Seeram, R.N. Schulman & D. Heber, Pomegranates : ancient roots to modern medicine., 2006, (pp. 199-209). New York: CRC Press.
2. Eccles J., An R and D strategy for the Australian pomegranate industry. Rural Industries Research and Development Corporation of Australian Government. Retrieved from www.rirdc.gov.au on., 2009, 19.04.2010.
3. Rice-Evans C.A, Miller N.J. andPaganga G., Antioxidant properties of phenolic compounds. Trends in Plant Science., 1997, 2, 152-159.
4. Halliwell B., Gutteridge J.M.C. and Cross C.E., Free radicals, antioxidants and human disease: where are we now? Journal of Laboratory and Clinical Medicine., 1992, 119: 598-620.
5. Aruoma O.I., Free radicals, oxidative stress, and antioxidants in human health and disease. Journal of the American Oil Chemists Society., 1998, 75, 199-212.
6. Spigno G, Tramelli L, Faveri D.M.D., Effects of extraction time, temperature and solvent on concentration and antioxidant activity of grape marc phenolics. Journal of Food Engineering., 2007,81, 200-208
7. Slinkard K. and singleton V. L., Total phenol analysis; Automation and comparison with manual methods. American journal of Enology and Viticulture.,1977,28:48-55.
8. Abu Bakar M., Mohamed M., Rahmat A, and Fry J., Phytochemicals and antioxidant activity of different parts of bambangan (*Mangifera pajang)* and tarap (*Artocarpus odoratissimus)*. Food Chemistry., 2009, 113 (2): 479-483.
9. Brand-Williams W., ME Cuvelier, and C. Berset., Use of a free radical method to evaluate antioxidant activity. LWT-Food Science and Technology Journal.,1995,28 (1):25-30.
10. Benzie I. F. and Strain J. J., The Ferric Reducing Ability of Plasma (FRAP) as a Measure of ‘‘Antioxidant Power: The FRAP Assay. Analytical Biochemistry Journal.,1996,239:70-76.
11. BrymanA.and D. Cramer. Quantitative data analysis with IBM SPSS 17, 18& 19; A guide for social scientists.,2012., Routledge.
12. Mahattanatawee K, Manthey JA, Luzio G, Talcott ST, Goodner K, Baldwin EA., Total antioxidant activity and fiber content of select Florida-grown tropical fruits. Journal of Agriculture and Food Chemistry.,2006, 54, 7355–7363.
13. Alothman M., Bhat R. and Karim A.A., Antioxidant capacity and phenolic content of selected tropical fruits from Malaysia, extracted with different solvents. Food Chemistry.,2009, 115: 785-788.
14. Zhou K, Yu L., Effects of extraction solvent on wheat bran antioxidant activity estimation. LWT – Food Sci. Technol., 2004, 37, 717–721.
15. Musa KH, Abdullah A, Jusoh K, Subramaniam ., Antioxidant activity of pink-flesh guava (*Psidium guajava* L.): effect of extraction techniques and solvents. Food Analytical Methods., 2011, 4 (1), 100-107.
16. Wissal K, Fatma A, Christophe B, Hamadi A, Souhail B., Effects of extraction solvents on phenolic contents and antioxidant activities of Tunisian date varieties (*Phoenix dactylifera* L.). Industrial crops and products.,2013, 45, 262-269.
17. Ali G, Hawa ZE, and Asmah R., Effects of solvent type on phenolics and flavonoids content and antioxidant activities in two varieties of young ginger (*Zingiber officinale* Roscoe) extracts. Journal of Medicinal Plants Research., 2011, 5(7), 1147-1154.
18. Rostagno M. A., Palma M, and Barroso C. G., Ultrasound-Assisted Extraction of Soy Isoflavones. Journal of Chromatography A., 2003, 1012(2), 119-128.
19. Nurliyana R., Syed Zahir I., Mustapha Suleiman K., Aisyah MR., and Kamarul Rahim K., Antioxidant study of pulps and peels of dragon fruits: a comparative study. International Food Research Journal., 2010, 17, 367-375.
20. Yan LY., Teng LY, and Jhi TJ., Antioxidant properties of guava fruit: comparison with some local fruits. Sunway Academic Journal., 2006, 3, 9-20.
21. Mazandarani M ., Zarghami MoghaddamP., Zolfaghari M. R., Ghaemi E. A. and Bayat H. Effects of solvent type on phenolics and flavonoids content and antioxidant activities in *Onosma dichroanthum* Boiss. Journal of Medicinal Plants Research., 2012, 6(28), 4481-4488.
22. Uma DB., Ho CW. And Wan AWM., Optimization of extraction parameters of total phenolic compounds for Henna (*Lawsonia inermis*) leaves. SainsMalaysiana., 2010, 39,119-128.

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