

Extract of Phenolics From Pomegranate Peels

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Abstract: The effects of different solvents, temperature conditions, solvent-solid ratios and particle sizes on solid-solvent extraction of the total phenolics, proanthocyanidins and flavonoids herein also referred to as antioxidant from pomegranate marc peel (PMP) was studied. Water, methanol, ethanol, acetone, and ethyl acetate extraction efficiencies at extraction times of 0.17 to 10 min, extraction temperatures of 25 to 95°C, ratios of solvent/solid of 5:1 to 50:1 and particle sizes of 10 to 40 mesh were evaluated. At 40 °C, solvent/solid ratio of 15 : 1, extraction time of 240 min and particle size of 40 mesh, methanol gave the highest extract yield of the total phenolics (8.26%), followed by water (5.90%), ethanol (1.55%), acetone (0.37%), and ethyl acetate (0.18%), respectively. However, at an extraction temperature of 95°C, the total phenolics extract yield with water was 11.15% for particle size of 40 mesh, solvent/solid ratio of 15:1, and extraction time of 2 min. Despite the lowest extract yield at extraction temperature of 40 °C, solvent/solid ratio of 15 : 1, extraction time of 240 min and particle size of 40 mesh, ethyl acetate extraction gave the highest content of the total phenolics (20.24%), proanthocyanidins (2.65%) and flavonoids (3.92%) in the extract. The DPPH antioxidant activity of extracts had a linear relationship with the total phenolics content in the extracts ($R^2=0.9779$). This study revealed that water extraction, which has the economic and safety merits, can be used as an environmentally friendly method for producing antioxidants from the PMP.

Keywords: Antioxidant, pomegranate, total phenolics, extract, temperature, time, ratio of solvent/solid, particle size, marc, residues.

1. INTRODUCTION

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 antiviral, antioxidant, anticancer, and antiproliferative activities [1-3]. Pomegranate is consumed fresh and in processed form as juice, wines, flavors, and extracts. Commercial pomegranate juice has the highest antioxidant activities compared to other fruit juices, red wine, and green tea and currently is a high value product in the agricultural market.

The pomegranate antioxidant activity is typically higher in commercial juices extracted from whole pomegranates than in experimental juices obtained from the arils only. This can 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. 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.

It has been reported that the peel in particular possesses relatively higher antioxidant activity than seed and pulp and

therefore might be a rich sources of natural antioxidants [4-7]. 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. For instance, the use of agricultural wastes such as wine-making wastes as alternative low-cost sources of phenolics compounds has been on the increase [8-10]. Extraction is the first step in the commercial isolation of these antioxidant compounds from pomegranate. However, efficient methods for extraction of antioxidants embedded in the pomegranate peels such as phenolics, proanthocyanidins and flavonoids and the determination of kinetic parameters which are important for designing efficient extraction process for their production from peels have not been studied. Accordingly, the objective of this research was to evaluate solid-solvent extraction of antioxidants from the pomegranate marc peel (PMP) and further elucidate how different solvents, temperature conditions and solvent-solid ratios affect the extraction of the antioxidant compounds.

2. MATERIALS AND METHODS

2.1. Materials

Pressed pomegranate marcs (from 'Wonderful' variety of pomegranate) were kindly provided by Stiebs Pomegranate Products (Madera, CA), a pomegranate juice processor. They were collected after juicing and kept at -18°C until used. Prior to experiments, the samples were thawed at 4°C followed by hot air oven drying at 40 °C to a moisture content of about 8% (dry basis). The moisture content was determined by using oven drying at 105 °C until constant weight

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was achieved. The peels and seeds were manually separated. The dried peel was ground in a mill (WBB-6, Gruendler Pulverizing Co., St. Louis, MO) equipped with a 5 mm opening sieve. The ground material was sieved through 10, 20, 30, 40 mesh screens. Five groups of different particle size samples were obtained: >10, 10~20, 20~30, 30~40, and <40 meshes.

2.2. Extraction Procedures and Effects of Different Parameters on Total Phenolics

2.2.1. Solvents

The extraction yield of antioxidant compounds from plant materials is influenced mainly by the conditions under which the process of liquid-solid extraction is carried out to separate a soluble fraction from a permeable solid [11]. In the present work, five solvents with different polarities were used to identify the most suitable one for the recovery of antioxidant components from pomegranate peel. The polarity of a solvent besides the dipole moment, polarizability and hydrogen bonding determines what type of compounds it is able to dissolve. Five 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); acetone (polar with a dielectric constant of 21) and ethyl acetate (non polar with a dielectric constant of 6). All chemicals used were of analytical grade. For each solvent, dried and ground peel was extracted in a thermostatic water bath shaker (R/76, New Brunseick Scientific Co., Inc., Edison, NY) with a 15:1 (w/w) ratio solvent/sample (dry weight) 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, proanthocyanidins and flavonoids. The reported results, as illustrated in equations 1-3, include the total extract yield (%), the yield of total antioxidant (either phenolics or proanthocyanidins or flavonoids) from the PMP (%), and the content of antioxidant (either phenolics or proanthocyanidins or flavonoids) (%) in extract respectively:

$$\text{Total extract yield (\%)} = \frac{\text{g dried extract}}{100\text{g PMP}} \times 100 \quad (1)$$

$$\text{Yield of antioxidant (\%)} = \frac{\text{g total of antioxidant}}{100\text{g PMP}} \times 100 \quad (2)$$

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

All reported weights and percentages are dry basis unless specified otherwise. All the extraction trials were carried out in triplicate.

2.2.2. Extraction Time and Temperature Effect

To study the effect of extraction time, samples of 3 g PMP powder (40 mesh) were mixed with 45 g DI water and extracted at 25, 60, and 95 °C for 0.167, 0.333, 0.5, 1, 1.5, 2, 4, 6, 8 and 10 min. The liquid extract was separated from solids by vacuum enhanced filtration through Whatman No.

1 filter paper. The filtrate was transferred to a 50 ml flask after filtration, and DI water was added to make the finally volume to 50 ml. After the filtrate volume adjustment, the total phenolics concentration was measured.

To determine the effect of extraction temperature on the recovery of phenolics, temperatures of 20, 40, 60, 80, 95°C were tested during a 2 min extraction. Samples (40 mesh) of 5, 3, 1.8 g were mixed with 45 ml DI water to achieve the following ratios: 9, 15, 25(w/w).

2.2.3. Solvent-Solid Effect

The effect of solvent-solid ratio on the total phenolics extraction was studied. Samples (40 mesh) were mixed with 45 g DI water at ratio of solvent-solid from 5 to 50, and extraction performed at temperature of 60 °C for 2 min. The phenolics yields were determined.

2.2.4. Particle Size Effect

The PMP samples of five particle sizes were investigated in this study: >10, 10-20, 20-30, 30-30 and <40 mesh. Extractions were conducted at 60°C for 2, 20 and 60 min. The ratio of solvent/solid used was 15 and the phenolics yields were measured.

2.3. Analysis Assay

2.3.1. Total Phenolics Content

The total phenolics content in the extract was determined by the Folin-Ciocalteu method [5]. The 0.05 g of dried extracts was dissolved in 5 ml methanol or the filtrate made up to 50 ml were used directly. Aliquots of 10 µl of samples were mixed with 2.5 ml of 10-fold-diluted Folin-Ciocalteu reagent and 2 ml of 7.5% sodium carbonate. The total volume of the mixture was adjusted to 25 ml using DI water and allowed to stand for 30 min at room temperature before the absorbance was measured at 760 nm using a spectrophotometer (Hewlett Packard 8452A, Diode Array, USA). The total phenolics content in the extract was calculated and expressed as tannic acid equivalents (TCE; g/100 g dry mass) using a tannic acid (0–0.004 mg/ml) standard curve.

2.3.2. Flavonoid Content

The flavonoids content was measured using a modified colorimetric [5]. A quantity of 0.05 g of dried extracts was dissolved in 5 ml methanol or the filtrates made up to 50 ml were used directly. A volume of 0.4 ml of the solution was transferred to a 25ml flask containing 5 ml of 30% ethanol and mixed with 0.75 ml of 5% sodium nitrite for 5 min. Then, 0.75 ml of 10% aluminum nitrate was added. After 6 min, the reaction was stopped by adding 5 ml of 1 M sodium hydroxide. The mixture was further diluted with 30% ethanol up to 25 ml. The absorbance of the mixture was immediately measured at 510 nm. The flavonoids content was calculated and expressed as rutin equivalents (RE, g/100 g dry mass) using a rutin (0–0.03 mg/ml) standard curve.

2.3.3. Proanthocyanidin Content

Determination of Proanthocyanidins was based on the procedure reported in literature [5]. A quantity of 0.05 g of dried extracts was dissolved in 5 ml methanol or the filtrates made up to 50 ml were used directly. A volume of 1 ml solution was mixed with 3 ml of 4% vanillin–methanol solution

and 1.5 ml hydrochloric acid and the mixture was allowed to stand for 15 min at room temperature. The absorbance at 500 nm was measured and the Proanthocyanidins was expressed as catechin equivalents (CE, g/100g dry mass) using a catechin (0~0.08 mg/ml) standard curve.

2.3.4. Antioxidant Activity

The antioxidant activity of PMP extracts was measured in terms of hydrogen donating or radical scavenging ability, using a modified DPPH method [11]. A volume of 10 μ l of 0.01 g/ml of dried extract in methanol solution was added to 1 ml (500 μ M) of DPPH solution and diluted to 25 ml with methanol. The solution was shaken vigorously with vortex and incubated at room temperature (25 \pm 2 $^{\circ}$ C) for 20 min. The decrease in absorbance at 517nm was determined at the end of incubation period with a Spectrophotometer. The control was prepared as above without any extract and methanol was used as blank. Radical scavenging activity was expressed as the inhibition percentage (I %) and was calculated using the following formula:

$$I\% = ((A_c - A_s) / A_c) \times 100 \quad (8)$$

where, A_c is the absorbance of the control reaction (containing all reagents except the test compound) and A_s is the absorbance of the test compound.

3. RESULTS AND DISCUSSION

3.1. Effect of Extraction Procedures and Different Parameters

3.1.1. Influence of Solvents

Results for the total extract yields reported as percentage of g of extract per 100g pomegranate peel on dry basis indi-

cated that the pomegranate peel extracted with methanol gave the highest total extract yield (46.51 \pm 0.86), followed by water (43.19 \pm 2.24), ethanol (17.71 \pm 0.23), acetone (3.81 \pm 0.08) and ethyl acetate (0.88 \pm 0.08) when the extractions were done with the ratio of solvent/sample of 15:1 (w/w) at 40 $^{\circ}$ C for 4 h. It should be noted that, because of polarity differences between solvents, the solubility of the solute into the solvent is expected to be different. Water, methanol and ethanol are polar protic solvents of dielectric constants of 80, 33 and 24 respectively, while acetone and ethyl acetate are polar aprotic and non-polar solvents of dielectric constants of 21 and 6 respectively. It has been reported that pomegranate peel extract yield (% w/w) were 9.38, 7.53 and 1.04 for methanol, water and ethyl acetate respectively under the following experimental conditions: peel powder (25 g) extraction by mixing using a magnetic stirrer with 100 mL of the corresponding solvents at 30 $^{\circ}$ C for 1 h, filtration through Whatman No. 41, residue re-extraction with the same solvent, extract pooling and concentration under vacuum at 40 $^{\circ}$ C [11]. Our findings agree in terms of solubility trend but differ in the extracted yield.

The effect of different solvents on the yield of total phenolics, proanthocyanidins and flavonoids from the pomegranate peels are shown in Fig. (1). Methanol and water gave the top two yields of all three antioxidant components, which indicate that they are more effective than ethanol, acetone, and ethyl acetate for the antioxidants' extraction from the pomegranate peel. Particularly for the phenolic content, our results are different from the result reported elsewhere in literature [12] that the phenolic content from water extraction was the lowest among ethyl acetate (EtOAc), acetone, methanol and water. In the preceding results, the phenolic contents of EtOAc, acetone, MeOH and water extracts were

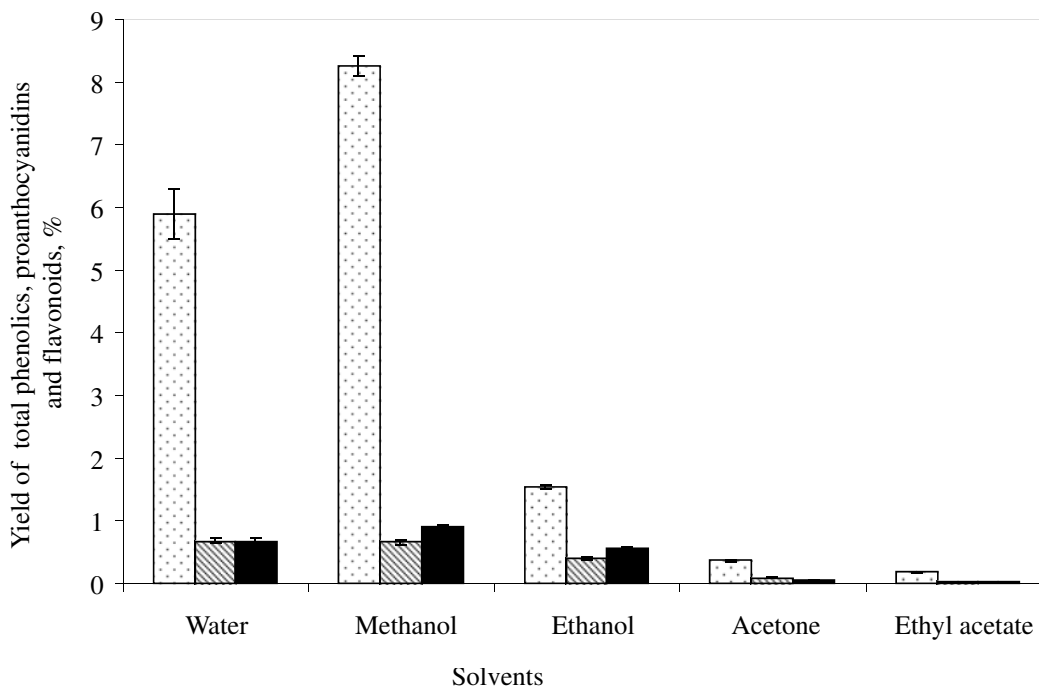


Fig. (1). The effect of different solvents on the yield of total phenolics, proanthocyanidins and flavonoids from the marc of pomegranate peels. Extraction was conducted at a temperature of 40 $^{\circ}$ C, a solvent/solid ratio of 15:1, a particle size of 40 mesh and an extraction time of 240 min: \square , Total phenolics; \square (hatched), Proanthocyanidins; \blacksquare , Flavonoids.

found to be 16.5, 52, 46.2 and 4.8%, respectively. That withstanding, the value for the total phenolic yield obtained using MeOH is comparable to that reported by other researchers [11]. This deviation particularly in the values is likely to be due to the difference in extraction and phenolic content determination procedures [12]. For instance, the powder from pomegranate peel was extracted with a Soxhlet extractor for 4 h [12], filtered, concentrated under vacuum at 40 °C [11] and then dissolved in methanol:water (6:4 v/v) (1 mg/ml) for evaluation of antioxidant capacity; the concentration of phenolics in the extracts was determined [11] and results were expressed as (+) catechin equivalents. In our determination, the results were expressed as tannic acid equivalents (TCE; g/100 g dry mass).

Despite the low yield of total phenolics, proanthocyanidins and flavonoids from the pomegranate peel (%: g total calculated weight of antioxidants/100 g PMP), the concentration of these compounds in the extracts content wise were the highest in the usage of ethyl acetate among the five different extraction solvents (Fig. 2). The total phenolics content was higher in methanol extract (18%) than in water extract (14%) and comparatively lower in ethanol extract (9%). It is reported [11] that pomegranate phenolics content was 44% with methanol, 3.0% with water, and 18% with ethyl acetate. Our results, however, show that the total phenolic content in the water extract and the MeOH extract was nearly the same: 14% and 18% respectively. Factors that have been attributed to bringing variation include the method of extraction [12], mixture of different solvents [5] and use of different materials [13] among others. A possible factor for higher content of phenolics, proanthocyanidins and flavonoids (%) in extract (g phenols/100 g dried extract) is due

to the higher purity of extract associated with using ethyl acetate. The use of methanol, ethanol, acetone, and water, generally yields a significant co-extraction of concomitant substances and decreases the yield of target antioxidants [14]. So whereas ethyl acetate may exhibit significant selectivity in respect of natural products, methanol and water allow for higher total extract yield (g dried extract/100 g PMP). The proanthocyanidins content of ethanol and acetone extracts were almost the same (3%), although higher than the contents of water extract (1%) and methanol extract (1%). The content of flavonoids in ethanol extract (4%) is much higher than water extract (1%), methanol extract (2%), and acetone extract (1%) (Fig. 2). Water would be a better extracting agent than methanol when the toxicity and cost aspects are considered. Hence, in furthering studies on pomegranate total phenolics extraction, water was chosen as the best solvent.

3.1.2. Influence of Extraction Time and Temperature

Fig. (3) shows the kinetics curves of total phenolics yield. The parabolic shaped curve had three distinct phases. The initial phase was characteristically almost linear (up to 2 min at 25 °C, 0.5 min at 60 °C, 0.33 min at 95 °C) with higher percentage of phenolics yield increments per unit time. The second phase displayed a lower percentage of phenolics yield increments per unit time before the final asymptotic ending of the third phase. The results indicate that extraction process is sensitive to the extraction time and temperature in the early stage [14]. A similar trend has been observed using different materials [14] in a study of the kinetics of extraction of proanthocyanidins from dry grape seeds using ethyl acetate with different contents of water (10, 15 and 20%). In

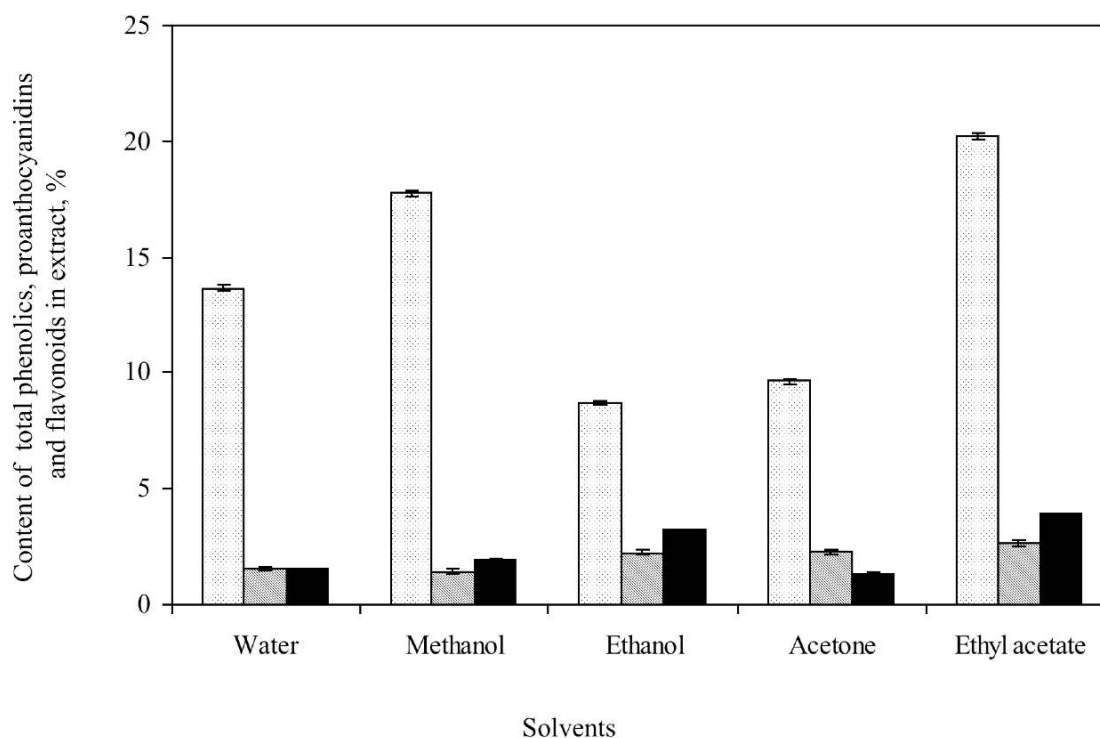


Fig. (2). The effect of different solvents on the content of total phenolics, proanthocyanidins and flavonoids from the marc of pomegranate peels (PMP) in the extract (g dried extract/100 g PMP). Extraction was conducted at a temperature of 40 °C, a solvent/solid ratio of 15:1, a particle size of 40 mesh and an extraction time of 240 min. □, Total phenolics; ▨, Proanthocyanidins; ■, Flavonoids.

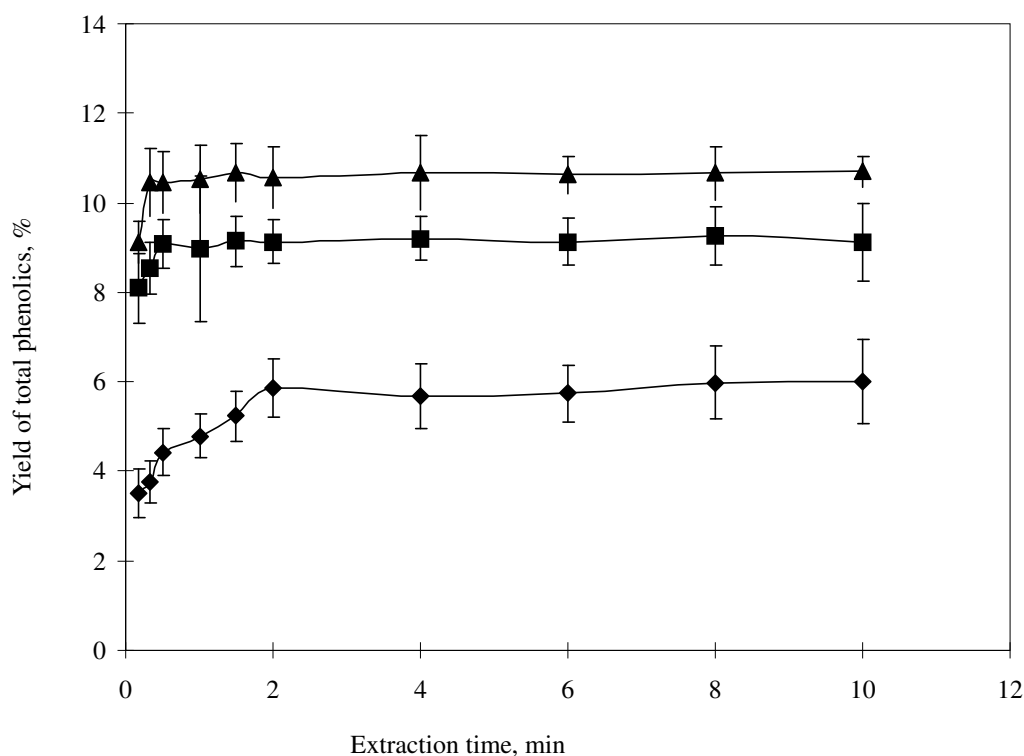


Fig. (3). The kinetics curves of water extracted total phenolics yield. The water extraction was done using a ratio of solvent to solid of 15: 1 and particle size of 40 mesh. \blacklozenge , 25°C; \blacksquare , 60°C; \blacktriangle , 95°C.

the study it was noted that the kinetic curves obtained were of parabolic shape, with the initial part being linear, thus reflecting a strong increase in the yield of proanthocyanidins, whereas their second parts showed a slower increase and an asymptotic ending [14]. Further, it has been identified that in water-extracts of grape, the yield of polyphenols gently increased with the time [8]. Shorter times (preferably <8 h) were reported for grape marc phenolics extraction at 60 °C [15]. The antioxidant phenolics extraction time is 90 min from pine sawdust (*Pinus pinaster*) at 50 °C with 5:1 of liquid–solid ratio [16].

The equilibrium times and concentrations were 2, 0.5, 0.33 min and 6.55, 9.14, 11.92 % at 25, 60, 95 °C respectively, as shown in Fig. (3). The diffusion of the dissolved solute within the solid into the solvent is the rate limiting step [17]. The short equilibrium time lies in three aspects: firstly, the fine particle sizes which enlarges the resolve surface area and shorten the mass transfer distance; secondly, the loose tissue of pomegranate peel with larger diffusion of the dissolved solute within the solid into the solvent and thirdly, the variety of phenolics.

The effect of extraction temperature on the extraction rate is shown in Fig. (4). An increase in temperature significantly increases diffusivity as established by the Einstein equation. The equilibrium concentration has a linear relationship with the extraction temperature. Temperature strongly influenced the total phenolics yields, but may enhance purity, probably because temperature increase favors extraction by increasing solubility and diffusion coefficient of any compounds, not only of antioxidants [10]. Extraction

temperature, however, is affected by the extract mass transfer velocity and the equilibrium concentration.

3.1.3. Influence of Ratio of Solvent/Solid

The solid-solvent ratio affects the concentration gradient within the particles of raw material (Fig. 5). The rates of extraction increased with a larger concentration gradient (Fig. 5) in the first stage, and then reached equilibrium when most of the phenolics had been extracted out. The equilibrium ratio of solvent/solid decreased at higher extraction temperature before reaching equilibrium: ratio of 25 at 95°C and ratio of 30 at 60°C.

3.1.4. Influence of Particle Size

Particle size is also a factor to be considered during extract processing. Smaller particle size reduces the diffusion distance of the solute within the solid and increases the concentration gradient, which ultimately increases the extraction rate. Since the path of solute to reach the surface is shorter, the extraction time is reduced. In Fig. (6), total phenolics extract yield of smaller particle size goes up when the extraction time is 2 min.

The foregoing results agree with earlier reports whereby higher yields in total phenolics and anthocyanins extraction resulted from a decrease in size of black currant juice press residues [9]. Fig. (6) also illustrates further the effect of extraction time on total phenolics extract.

3.2. Antioxidant Activity

The DPPH assay was employed. The DPPH assay has been widely used to determine the free radical-scavenging

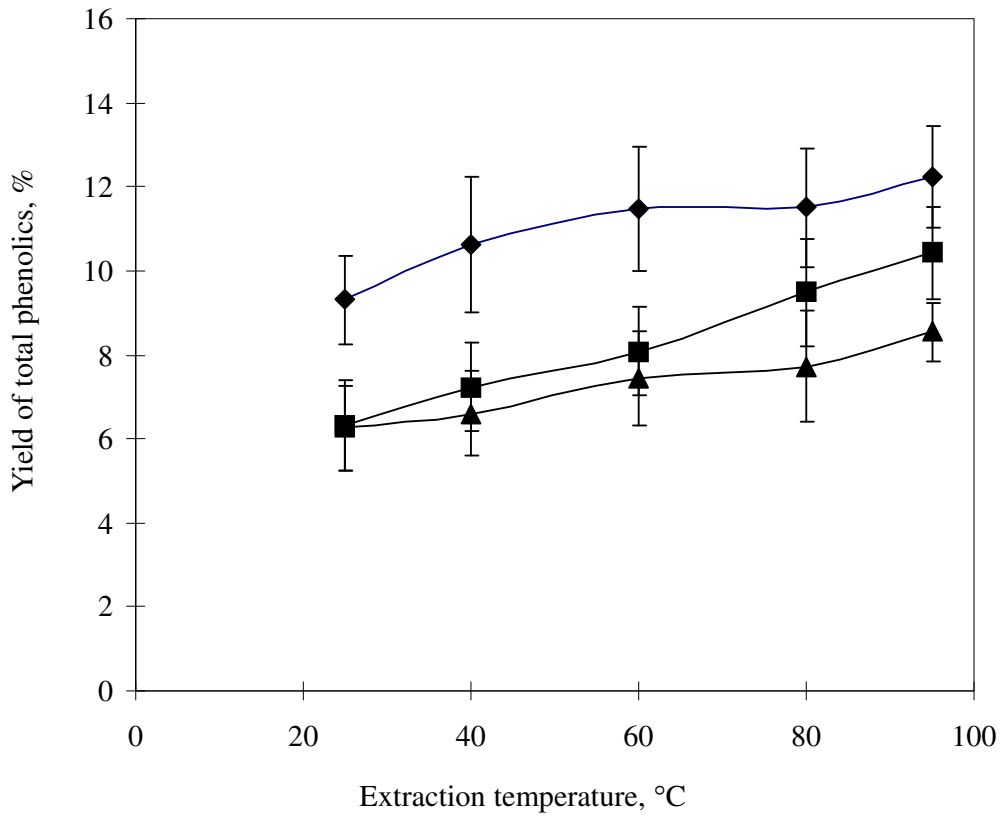


Fig. (4). The effect of extraction temperature on the yield of total phenolics for different solvent solid/ ratios. Extraction time was 2 min and the particle size of 40 mesh: \blacktriangle , 1:09; \blacksquare , 1:15; \blacklozenge , 1:25.

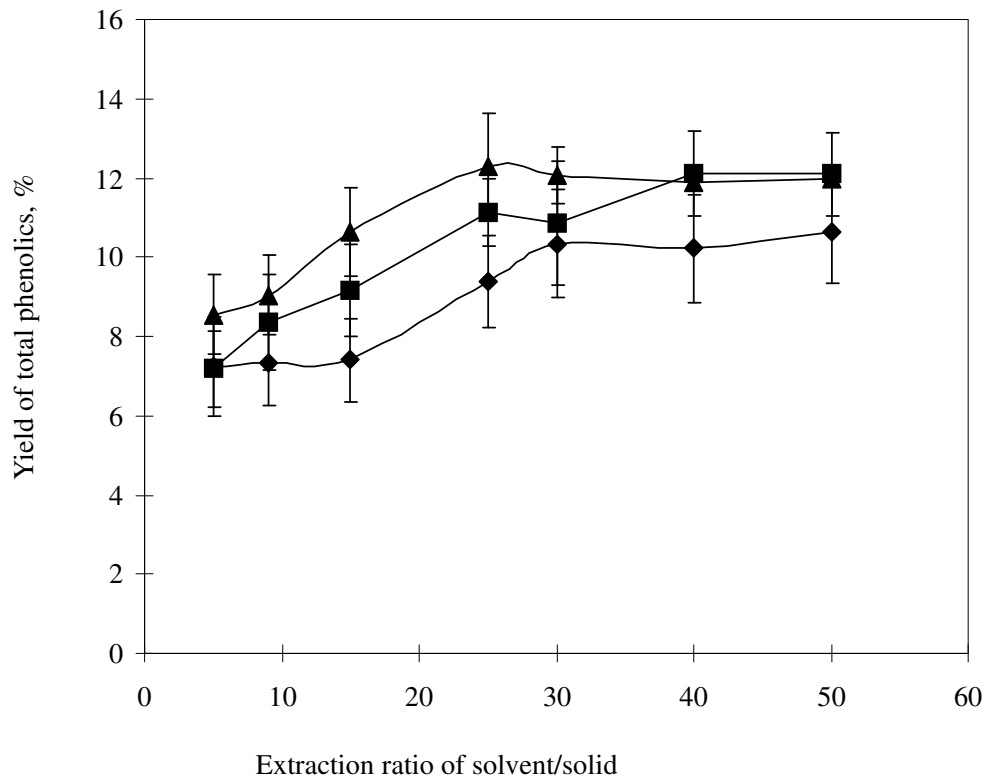


Fig. (5). The effect of the ratio of solvent /solid ($g\cdot g^{-1}$) used in the extraction on the yield of total phenolics. The extraction time was 2 min and the particle size of 40 mesh was used. \blacklozenge , 25°C; \blacksquare , 60°C; \blacktriangle , 95°C.

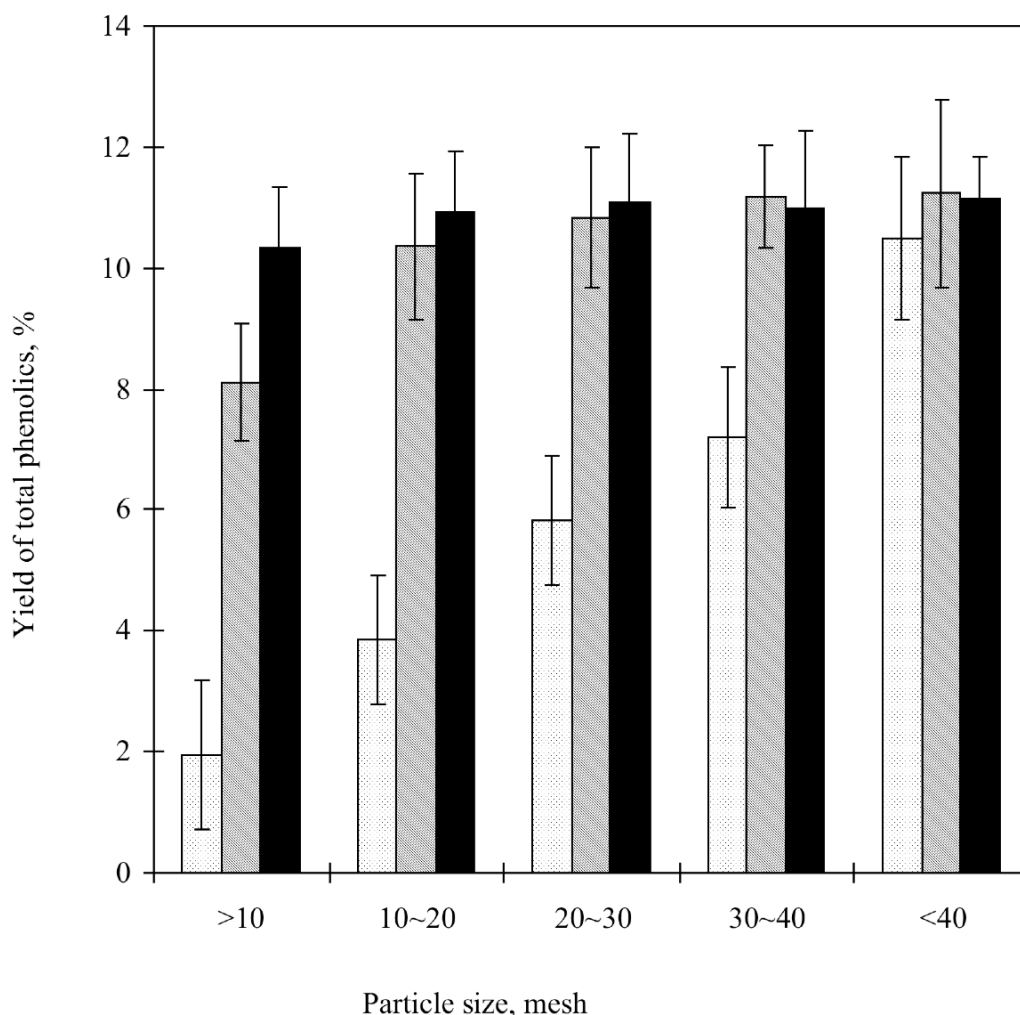


Fig. (6). The effect of particle size on the yield of total phenolics with different extraction times (2, 20 and 60 min). The extraction temperature was at 60°C. □, 2min; ▨, 20min; ■, 60min.

activity of various plants and pure compounds [18, 19]. DPPH is a stable free radical which dissolves in methanol or ethanol, and its purple color shows a characteristic absorption at 517 nm. When an antioxidant scavenges the free radical by hydrogen donation, the color from the DPPH assay solution becomes light yellow.

Our study of antioxidant activities of the extracts was carried out to investigate the correlations between the antioxidant activity and the content of phenolics, proanthocyanidins and flavonoids and results are shown in Fig. (7). The results indicate a strong correlation between DPPH and total phenolics ($R^2=0.98$), but no correlation existed with proanthocyanidins ($R^2=0.01$) and flavonoids ($R^2=0.05$). Other studies [11, 12] also reported that the antioxidant activity of pomegranate peel correlated to the total phenolics. Therefore, the total phenolics yield should be one of the most important indicators of effective extraction process for producing high quality product. Comparing methanol with water as the solvent in pomegranate antioxidant extraction, the total extract yield (dried extract/100 g PMP) were 43.18% and 46.51%, the yield of total phenolics (g total calculated weight of phenolic/100 g PMP) were 5.90% and 8.26%, the content of phenolics (g phenols/100 g dried extract) were

13.63% and 17.78%, and the DPPH antioxidant activities were 53.74% and 65.30%, respectively.

4. CONCLUSIONS

The research showed that the peels from pomegranate marc are a potential resource for phenolics, proanthocyanidins and flavonoids. The antioxidant activity of pomegranate 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 when the extractions were done with the ratio of solvent/sample of 15:1 (w/w) at 40 °C for 4 h. Water compared well to methanol as an extracting solvent and qualifies as a better agent than methanol when toxicity and cost aspects are considered. Comparing methanol with water as the solvent in pomegranate antioxidant extraction, the total extract yield were 43.18% and 46.51%, the yield of total phenolics were 5.90% and 8.26%, the content of phenolics were 13.63% and 17.78%, and the DPPH antioxidant activities were 53.74% and 65.30%, respectively. Shorter extraction time was needed with higher extraction temperature and smaller particle size. High yield was attainable with increased ratio of solvent/solid and was also affected by the extraction temperature. The total phenolics extract yield with water was

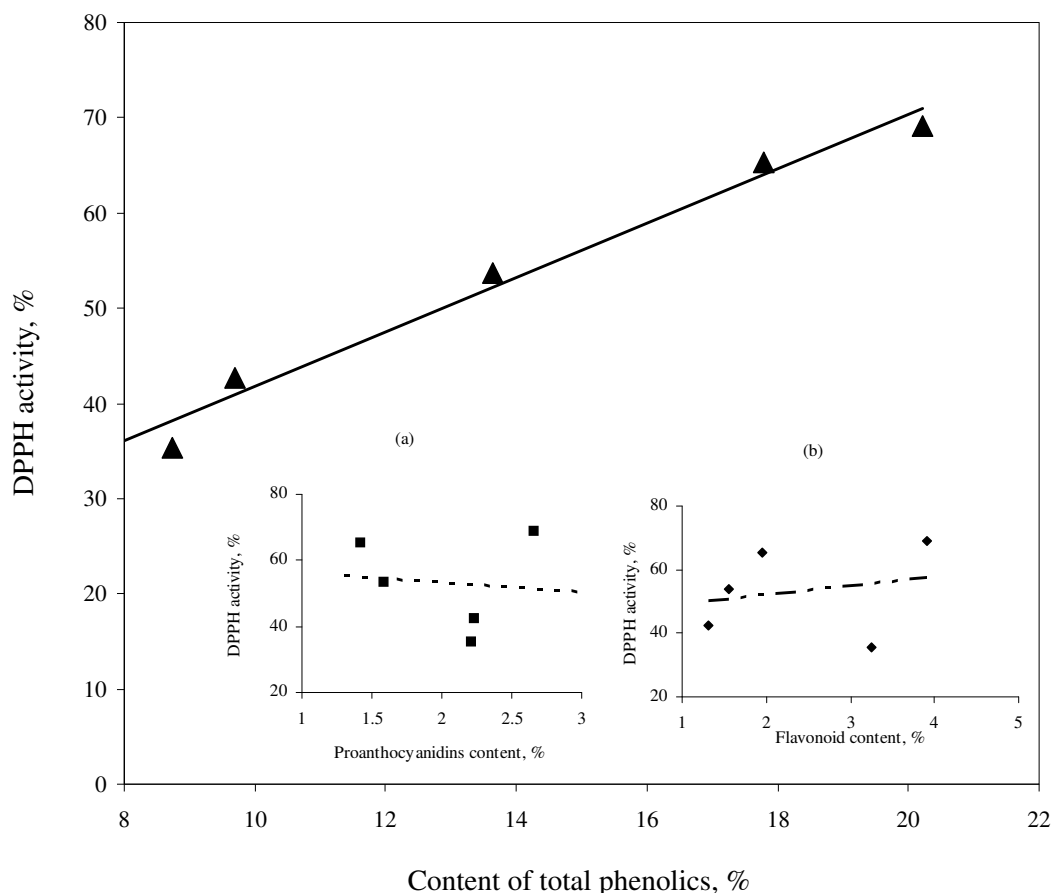


Fig. (7). The relationship between the content of total phenolics, proanthocyanidins and flavonoids of the pomegranate marc peel and the DPPH activity for the following extraction condition: extraction temperature of 40 °C, solvent/solid ratio of 15:1, extraction time of 240 min and particle size of 40 mesh. ▲, Total phenolics; —, Linear Total phenolics ($y = 2.8594x + 13.183$, $r^2=0.9779$); ■, Proanthocyanidins; - - - - -, Linear Proanthocyanidins ($y = -2.8245x + 58.951$, $r^2=0.0099$); ◆, Flavonoid; - - - - -, Linear Flavonoid ($y = 2.8881x + 46.336$, $r^2=0.0511$); DPPH activity (a) insert and proanthocyanidins content, DPPH activity (b) insert and flavonoid content.

11.15% at the suggested extraction temperature of 95°C, particle size of 40 mesh, ratio of solvent/solid of 15/1, and extract time of 2 min.

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