

Antioxidant Activity of Pericarp Extract from Different Varieties of Pomegranate Fruit

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ABSTRACT

The pomegranate *Punicagranatum* fruit pericarp, contain polyphenolic compounds including alpha and beta punicalagins and ellagic acid, which exhibit remarkable antioxidant activities. The aim of this study was to purify and quantify the phenolic components from different varieties of Pomegranate Pericarp Extracts (PPEs) and determine their antioxidant properties. Methanolic and aqueous extracts of four pomegranate cultivars (Shahvar, Siahsofreh, Torshsaz and Abdorahimkhany, from Shiraz, Iran) were prepared and total phenolic content of PPEs was determined. PPE components were further purified by XAD-16 column chromatography followed by LH-20 gel filtration. The eluted components were subjected to HPLC analysis to differentiate and quantify polyphenolic compounds. Antioxidant activity was measured using DPPH assay. The result revealed significant difference in total phenolic contents and phenolic components in four cultivars. Total phenolic content in methanolic PPE was significantly ($P < 0.05$) higher than aqueous extracts. Shahvar cultivar had the highest total phenols (11.72 ± 0.01 mg mL⁻¹ in water, 17.7 ± 0.12 mg mL⁻¹ in methanol) and exhibited the most antioxidant property among cultivars. Analysis of components of Shahvar PPE by HPLC showed that proportions of different phenolic components were alpha punicalagin ($28.34 \pm 2.12\%$), beta punicalagin ($39.75 \pm 2.14\%$) and ellagic acid ($3.49 \pm 0.93\%$). Antioxidant activity of alpha punicalagin, beta punicalagin, punicalagin (sum of alpha+beta) and ellagic acid was 50.8, 60.5, 56, and 27%, respectively. Taken together, these results indicate that PPE of Shahvar cultivar is an excellent source of natural antioxidants and can potentially replace the current synthetic antioxidants in food and pharmaceutical industries.

Keywords: DPPH assay, Polyphenolic compounds, Pomegranate cultivars, *Punicagranatum*.

INTRODUCTION

Pomegranate has been used as a folk medicine since ancient times and it is a symbol of life (Longtin, 2003), permanence, wellbeing, femaleness, fertility, knowledge, immortality, and holiness. Pomegranate is native to Iran and the surrounding countries (Levin, 1994; Seeram *et al.*, 2005b; Celik *et al.*, 2009; Viuda-Martos *et al.*, 2010). Many researchers have focused on the biological waste part of this wonder fruit, pomegranate, in order to discover the medical effects on human health. The potential therapeutic

properties of pomegranate peel are extensive, including treatment and prevention of cancer (Dikmen *et al.*, 2011; Hong *et al.*, 2008), cardiovascular diseases (Basu and Penugonda, 2009; Jurenka, 2008), diabetes (Banihani *et al.*, 2013; Middha *et al.*, 2012), dental conditions (Viuda-Martos *et al.*, 2010), erectile dysfunction (Kanatt *et al.*, 2010), protection from UltraViolet (UV) radiation (Kanatt *et al.*, 2010) and antimicrobial activity (Dahham *et al.*, 2010; Ferreira, 2007; Hayouni *et al.*, 2011). Other potential applications include prevention of infant brain ischemia, Alzheimer's disease (Kwak *et al.*, 2005; Middha *et al.*, 2012),

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male infertility, arthritis (Shukla *et al.*, 2008; Kanatt *et al.*, 2010), dermal wounds (Hayouni *et al.*, 2011), and obesity (Kanatt *et al.*, 2010). A survey revealed that the tannins from the pericarp of pomegranate exhibit antiviral activity against the genital herpes virus (Zhang *et al.*, 1995). In addition to pomegranate, the antioxidant activity has been extensively reported in many plant sources (Rafiee *et al.*, 2012, Rahmanian *et al.*, 2015; Taghvaei and Jafari, 2015; Taghvaei *et al.*, 2014). In pomegranate, pericarp constitutes almost 50% of the fruit weight and is a rich source of bioactive constituents, such as phenolics, flavonoids, ellagitannins, and proanthocyanidin compounds. Among these phenolic compounds, the most abundant is punicalagin, which is a large polyphenol hydrolysable tannin with the formula 2, 3-(S)-hexahydroxydiphenoyl-4, 6-(S, S)-gallagyl-D-glucose and a molecular weight of 1084. The chemical structure of punicalagin is shown in Figure 1 (Kraszni *et al.*, 2013). Punicalagin is one of the main compounds responsible for antioxidative

potential of pomegranate juice (Gil *et al.*, 2000; Singh *et al.*, 2002). Punicalagin is found in alpha and beta forms in pomegranates which can be interconverted (ratio constant, $K = [\beta]/[\alpha]$) (Lu *et al.*, 2008). Punicalagin concentration in husk depends upon the geographic area of fruit growth, processing and storage conditions (Lu *et al.*, 2008).

Ellagic acid, another antioxidant polyphenol from PPE, has potent anticancer (Mertens-Talcott and Percival, 2005) and anti-atherosclerosis properties (Rouanet *et al.*, 2010). Free ellagic acid in human plasma, is generated by the hydrolysis of the ellagitannins such as punicalagins (Seeram *et al.*, 2004). It has previously been reported that the antioxidant activity of pomegranate peel is related to phenolic content (Ricci *et al.*, 2006). Punicalagin and ellagic acid are inhibitor of the process of oxidation, even at relatively small concentration, and thus have diverse physiological role in the body. These antioxidants act as radical scavengers, and help in converting the radicals to less reactive species (Chidambara Murthy *et al.*,

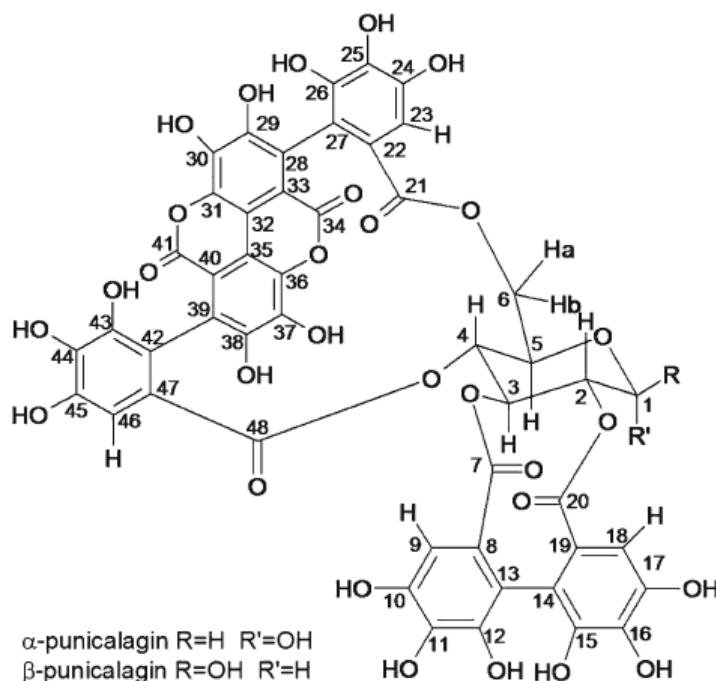


Figure 1. The chemical structure of punicalagin (Kraszni *et al.*, 2013).

2002). However, the antioxidant activity of purified phenolic compounds of PPE from different varieties and the relative contribution of each phenol component have not been compared yet. This communication reports a comparative study on the antioxidant activity of pericarp components from four cultivars of pomegranate.

MATERIALS AND METHODS

Punicalagin and ellagic acid (as a standard), formic and acetic acids, Folin-Ciocalteu reagent, DiPhenyl-2-PicrylHydrazyl (DPPH), Sephadex Lipophilic LH-20 and Amberlite XAD-16 resins, tryptic were purchased from Sigma Aldrich. All solvents were HPLC grade.

Preparation of Pericarp Extract

Fruits of four pomegranate cultivars (Shahvar, Abdorahimkhany, Siahsofreh, and Torshsabz), crop of 2014, were purchased from local markets in Shiraz, Iran. After removing the thin outer layer, their pericarps were manually separated from the aril and used for extraction. To prepare samples, 20 g fresh pericarps were soaked in 60 mL methanol or water and placed in shaker at 150 rpm for 4 hours. Sample were left in dark at room temperature for 12 hours, homogenized using a laboratory homogenizer and centrifuged at 9,000 rpm for 15 minutes. Supernatant were labeled as PPE and used for further studies (Basiri *et al.*, 2015).

Analytical Studies

Total phenol content of extracts was determined using Folin-Ciocalteu reagent and gallic acid as standard (Sadasivam and Manickam, 1996). Approximately 40 μ L of PPEs were mixed with 1.25 mL of 20% aqueous sodium carbonate solution. After 3 minutes, 250 μ L Folin-Ciocalteu's phenol

reagent was added to the mixture. After 30 minutes of incubation at room temperature, absorbance was measured at 750 nm against a blank. Total phenolic content was calculated on the basis of the calibration curve of gallic acid. A linear calibration curve was obtained in the range of 20-140 μ g mL⁻¹ gallic acid in methanol, with regression equation $y = 0.0712x + 0.0106$ and linear regression coefficient $r^2 = 0.999$. This assay was performed in triplicate and the results were reported as mean \pm standard deviation. Antioxidant activity of water and methanol PPEs was evaluated by 1, 1-DiPhenyl-2-PicrylHydrazyl (DPPH) assay (Kulkarni *et al.*, 2004). Different concentrations of each PPE (0-15 μ g mL⁻¹) were added to methanolic solution of DPPH. After 30 min at room temperature, the absorbance was recorded at 515 nm

The results were recorded as $A_{515\text{nm}}$ vs. phenolic content and percentage of Radical Scavenging Activity (RSA%) was calculated based on the following equation:

$$\begin{aligned} \text{DPPH Scavenging activity (\%)} \\ = \left[\frac{(A_{\text{blank}} - A_{\text{sample}})}{A_{\text{blank}}} \right] \times 100 \end{aligned}$$

Where, A_{blank} and A_{sample} are Absorbance of the control (containing all reagents except the extract) and extract, respectively. Tert-ButylHydroQuinone (TBHQ), a synthetic phenolic antioxidant, was used as positive control (0-15 μ g mL⁻¹).

Purification of Polyphenols

Purification of Total Pomegranate Tannin (TPT) from PPEs was performed by the method of Seeram *et al.* (2005b) with minor modifications. PPEs were dried using a drying oven at 50°C. The resultant semi-solid residues were re-dissolved in 20 mL water and loaded onto a 3x40 cm chromatography column packed with Amberlite XAD-16 resins (a hydrophobic polyaromatic resin normally used for removing hydrophobic compounds up to 40,000 MW). The column was washed with 2 L water. Ellagitannins were recovered



from the column using 400 mL methanol and were dried on rotary evaporator. This fraction was labeled TPT. One hundred mg TPT powder was dissolved in Sephadex lipophilic LH-20 gel filtration resin that was pre-equilibrated with H₂O and eluted with stepwise (0, 20, 40, 60, 80, 100%) methanol to give six fractions. Each fraction was analyzed by HPLC.

Before injection, samples were centrifuged (4 min at 5000 rpm) and the supernatant was filtered through a 0.4 µm filter. A Knauer reversed phase HPLC system (Germany) with a 4.6×250 mm C18 separation column at 20°C was used. The HPLC method was performed as described by Hayouni *et al.* (2011) with minor modifications, in which two solvents were used as mobile phase including solvent A consisting of 0.1% formic acid in water and solvent B consisting of 100% methanol (Hayouni *et al.*, 2011). The gradient was initially 1% B in A for 5 minutes followed by 1 to 20% B in A for 15 minutes, 20 to 40% B in A for 10 minutes, 40% for 5 minutes, 40 to 95% B in A for 5 minutes, and finally, 15% B in A for the next 15 minutes. Absorption was detected at 254 nm.

Ellagitannin (punicaladin and ellagic acid) rich fraction was evaporated and re-chromatographed, this time with stepwise increasing (0, 20, 40, 60, 80, 100%) concentration of ethanol (six fractions). The punicalagin-rich fraction was filtered through 0.45-µm filter and 100 µL was injected on the HPLC column for separation of punicalagin. Pure alpha and beta

punicalagins were collected manually. To detect alpha and beta punicalagin and ellagic acid, their standard samples were used as external standard and then area percentage was calculated.

Statistical Analysis

Triplicate experiments were performed and means were compared using SPSS statistical software (SPSS version 10.0.0; SPSS Inc., Chicago, IL). One-way Analysis Of Variance (ANOVA) and Tukey's post hoc tests were carried out to compare means. P-values less than 0.05 were considered to be significant.

RESULTS AND DISCUSSION

Table 1 presents total phenolic contents in pericarp of four pomegranate cultivars. Shahvar cultivar had the highest (0.74 ± 0.005 mg GAE mg⁻¹ pericarp) and Abdorahimkhany cultivar the lowest (0.23 ± 0.040 mg GAE mg⁻¹ pericarp) phenolic content in methanolic extracts. The aqueous extract of Shahvar contained the highest (0.49 ± 0.004 mg GAE mg⁻¹ pericarp) and Siahsofreh the lowest (0.30 ± 0.501 mg GAE mg⁻¹ pericarp) phenolic content. Interestingly, Table 1 shows that the water extract of Abdorahimkhany had a higher phenolic content than methanolic extract. The reason for this phenomenon is not known at present. It can be related to the

Table 1. Concentration of total phenols in four pomegranate cultivars.^a

Pomegranate cultivars	Methanolic extract (mg GAE mg ⁻¹ pericarp)	Aqueous extract (mg GAE mg ⁻¹ pericarp)
Shahvar	0.74 ± 0.005	0.49 ± 0.004
Siahsofreh	0.37 ± 0.016	0.25 ± 0.024
Torshsabz	0.48 ± 0.019	0.35 ± 0.021
Abdorahimkhany	0.23 ± 0.040	0.30 ± 0.501

^a Values are mean±SD.

difference in chemical and structural properties of each cultivar, which probably facilitated more extraction in water.

The results indicated that there were significant differences in total phenolic contents among the tested cultivars. It has been recognized that phenolic compounds show antioxidant activity and their effects on human nutrition and health are considerable (Kalaycıoğlu and Erim, 2017). Phenolic compounds are a class of antioxidant agents that act as free radical terminators (Shahidi *et al.*, 1992; Rahmanian *et al.*, 2015; Kalaycıoğlu and Erim, 2017). Free radicals are involved in many disorders like neurodegenerative diseases, cancer and AIDS (Kim *et al.*, 2015). Antioxidants through their scavenging power are useful for the management of those diseases (Taghvaei and Jafari, 2015). It has been

reported that the peel extract of pomegranate has the highest antioxidant activity amongst extracts from eight types of fruits (Okonogi *et al.*, 2007). Few studies have been conducted on pomegranate pericarp. A study illustrated that components of pomegranate pericarps have antioxidant activity (Zhou *et al.*, 2014). The antioxidant activity of PPEs from different cultivars is illustrated in Figure 2, which shows the decrease in absorbance of DPPH reagent due to the free radical scavenging ability of the PPEs. Four pomegranate pericarp cultivars (methanolic and aqueous extracts) showed different antioxidant activities. The result of the present study showed that the PPE of Shahvar cultivar, which contained the highest amount of phenolic compounds, exhibited the greatest antioxidant activity in aqueous and methanolic extracts,

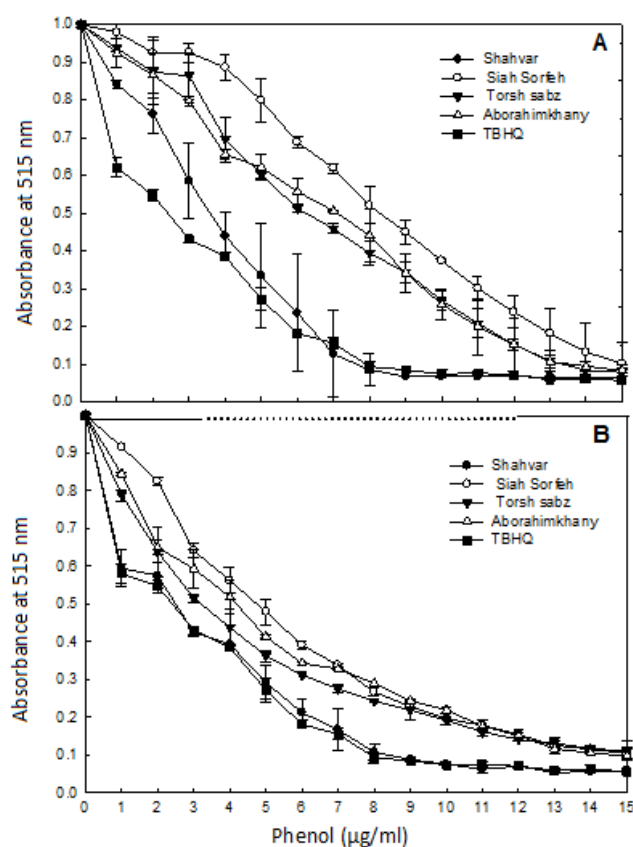


Figure 2. DPPH scavenging activity of methanolic (A) and aqueous (B) extracts of pericarp from four pomegranate cultivars.



approaching those of TBHQ. The PPE of Siahsofreh cultivar had the lowest antioxidant activity in both extracts (Figure 2). Previous studies also showed that the antioxidant activities of different pomegranate peels are different (Hajimahmood, *et al.*, 2008; Ozgen *et al.*, 2008). Ardekani *et al.* (2011) examined antioxidant activity of nine different cultivars of Iranian pomegranate peels and pulp. They found that the highest antioxidant activity and phenolic contents were in Sour summer cultivar for pulp and peel extracts, but the flavonoid content was not very high in pulp extract of this cultivar. Also, the antioxidant activity of pomegranate peel extract was 10 times higher than the pulp extract (Ardekani *et al.*, 2011). Hajimahmoodi *et al.* (2008) reported the antioxidant activity of ten Persian pomegranate cultivar hydro-extracts. Based on their findings, the highest FRAP value for pulp and peel was observed in Sour alac. Suitable properties for the selection of antioxidants include low concentration (0.010-0.01%), lack of undesirable effects on food properties, non-toxic, compatibility, and economical. This data could be useful to the pomegranate industry in identifying and developing cultivars having commercial value.

Pomegranate pericarp is an important source of phenolic active compounds that are valuable to human health. Among phenolic compounds, punicalagin (α and β) and ellagic acid can be useful as antioxidants in preventing many diseases (Zhou *et al.*, 2014). HPLC results showed significant differences in alpha and beta punicalagin isomers and ellagic acid in HPLC profile of four cultivars (both methanolic and aqueous extracts) (Figure 3). Alpha and beta punicalagin profiles and their elution order were similar in all four cultivars, but the peak areas varied significantly among them. As shown, the most polar phenolic compound in these cultivars was alpha punicalagin, which eluted first, followed by beta punicalagin and ellagic acid (Figure 3). The major phenolic components in PPEs were beta punicalagin,

alpha punicalagin and ellagic acid. Shahvar cultivar had the highest content of beta punicalagin both in methanol ($46.10 \pm 4.012\%$) and aqueous extracts ($39.57 \pm 2.143\%$) (Table 2). Siahsofreh cultivar in methanol extract ($34.22 \pm 2.122\%$) and Abdolrahimkhany in aqueous extract ($29.89 \pm 1.154\%$) had the highest alpha punicalagin, respectively. Punicalagin constant ratio ($K = [\beta]/[\alpha]$) was different in the four cultivars (Table 2). This ratio was the highest in Shahvar cultivar (1.85 in methanol extract and 1.39 in aqueous extract) and the lowest in Siahsofreh (1.06 in methanol extract and 0.94 in aqueous extract). Also, the highest content of punicalagins (sum of alpha and beta) in extracts was found in Shahvar cultivar (67.95% in aqueous extract), which is nearly 1.3 fold higher than in Siahsofreh (51.35% in aqueous extract). The results of this study indicate that the amount of punicalagins is significantly different among cultivars.

HPLC analysis showed that ellagic acid is the most non-polar component in the four cultivars of pomegranate pericarp. Amount of ellagic acid was also different among cultivars. Torshsabz cultivar had the highest ellagic acid in methanolic extract ($5.98 \pm 0.64\%$) and Shahvar cultivar had the highest ellagic acid in aqueous extract ($3.49 \pm 0.92\%$) (Table 2). These results predict that the punicalagin, especially β isoform, is responsible for the antioxidant activities of PPEs. To confirm this data, we purified components of PPE and determined their antioxidant activities. Further purification of the components was performed as described in the Materials and Methods section. Purification of pomegranate pericarp components was performed by, first, XAD-16 column chromatography using 100% methanol for separating TPT's. HPLC profile of TPT's showed four peaks corresponding to four ellagitannins (Figure 4-A). Each peak was identified based on the external standard. Peaks 1 and 2 were identified as alpha and beta punicalagin anomers and the 4th peak was ellagic acid. TPT's contain 50–71%

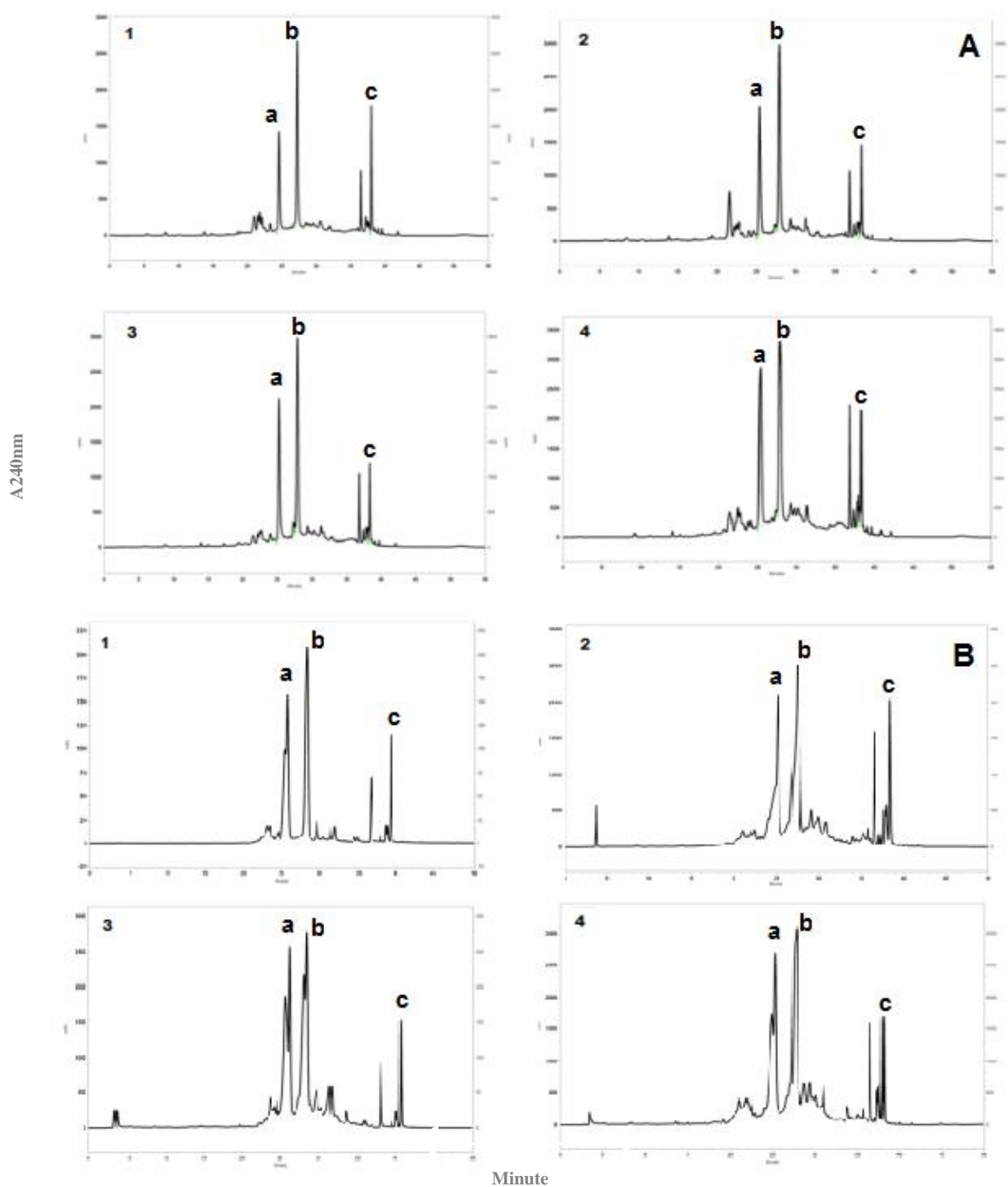


Figure 3. HPLC chromatogram of methanolic (A) and aqueous (B) extracts of pericarps from different pomegranate cultivars (1: Shahvar, 2: Siahsofreh, 3: Torshsabz and 4: Abdorahimkhany). Absorbencies are recorded at 254 nm (a: Alpha punicalagin, b: Beta punicalagin, c: Ellagic acid)

**Table 2.** HPLC-analysis of methanolic and aqueous extract of pericarp from pomegranate cultivars.

Punicalagins ellagic acid					
Cultivars	% α	% β	% ($\alpha+\beta$)	(β/α)	(%)
Methanolic extracts					
Shahvar	24.84 \pm 1.78	46.10 \pm 4.01	70.92 \pm 5.79	1.85	3.35 \pm 0.84
Siahsorfeh	34.22 \pm 1.34	36.46 \pm 1.34	70.68 \pm 3.46	1.06	4.65 \pm 0.39
Torshsabz	27.26 \pm 1.34	42.35 \pm 1.55	69.62 \pm 2.89	1.55	5.98 \pm 0.64
Abdollahimkhany	28.87 \pm 0.79	42.06 \pm 1.29	70.92 \pm 2.09	1.42	4.52 \pm 0.46
Aqueous Extract					
Shahvar	28.38 \pm 2.12	39.57 \pm 2.14	67.95 \pm 4.26	1.39	3.49 \pm 0.92
Siahsorfeh	26.52 \pm 4.92	24.83 \pm 2.05	51.35 \pm 6.97	0.94	2.70 \pm 0.53
Torshsabz	28.96 \pm 2.74	32.14 \pm 3.37	61.10 \pm 6.18	1.11	2.92 \pm 0.17
Abdollahimkhany	29.89 \pm 1.15	32.30 \pm 1.40	62.19 \pm 2.56	1.05	2.70 \pm 0.21

w/w of punicalagin anomers and 2-6% w/w ellagic acid. More purification was done by loading TPT's on LH-20 Sephadex column so that fraction of 65-75% of methanol had the highest amounts of punicalagin and ellagic acid (Figure 4-B). Punicalagin and ellagic acid were separated from each other by reloading this fraction on the LH-20 column and increasing the gradient of ethanol, as described in Materials and Methods section. Alpha and beta punicalagin isomers were isolated manually after loading on HPLC system (Figures 4-E and -D).

Antioxidant activity of purified pomegranate pericarp components was determined. Antioxidant activity of alpha punicalagin and beta punicalagin, their mixture and ellagic acid is shown in Table 3. As expected, beta punicalagin had the highest antioxidant activity (60.5 \pm 8.46%) while ellagic acid showed the least antioxidant activity (27.0 \pm 3.28%). The antioxidant activities of alpha and beta punicalagins were statistically different. This result confirms the HPLC result. The HPLC profile showed different composition of phenolic compounds in the four studied cultivars. The constant ratio ($K = [\beta]/[\alpha]$) of punicalagins were different in the four cultivars and it was higher in methanolic extract as compared with water extract. In Shahvar cultivar, constant ratio $K = [\beta]/[\alpha]$ is 1.4 in aqueous and 1.85 in methanolic extract. The equilibrium constant was reported to be equal to 1 in methanol and 4 in water (Doig *et al.*, 1999). The result of another study showed

a constant ratio of about 1.6 in different solvents (Jurenka *et al.*, 2008). Alpha and beta punicalagins interconvert.

Recently, there have also been numerous reports on the *in vitro* and *in vivo* antioxidant activities of punicalagin and ellagic acid, (Rosenblat *et al.*, 2013; Seeram *et al.*, 2005a; Xu *et al.*, 2015), but their antioxidant activity has not been compared. We purified alpha and beta punicalagins as well as ellagic acid in order to identify the main component of pomegranate pericarp with highest antioxidant activity. DPPH antioxidant assay showed that alpha and beta punicalagins have different antioxidant activities. This is the first report on the antioxidant activity of punicalagin anomers. Therefore, our finding further confirms that beta punicalagin is the major component of PPE that is responsible for antioxidant potential of pomegranate pericarp.

The result of antioxidant activity of beta punicalaginis is almost equivalent to antioxidant activity of TBHQ, a synthetic antioxidant that it used in foods (Table 1). Since there are considerable discrepancies regarding the safety of this and other synthetic antioxidants for human and animals, replacing them with natural antioxidants such as those reported in this study seems to be a desirable goal.

CONCLUSIONS

Our study showed that pomegranate pericarp extract from different cultivars have

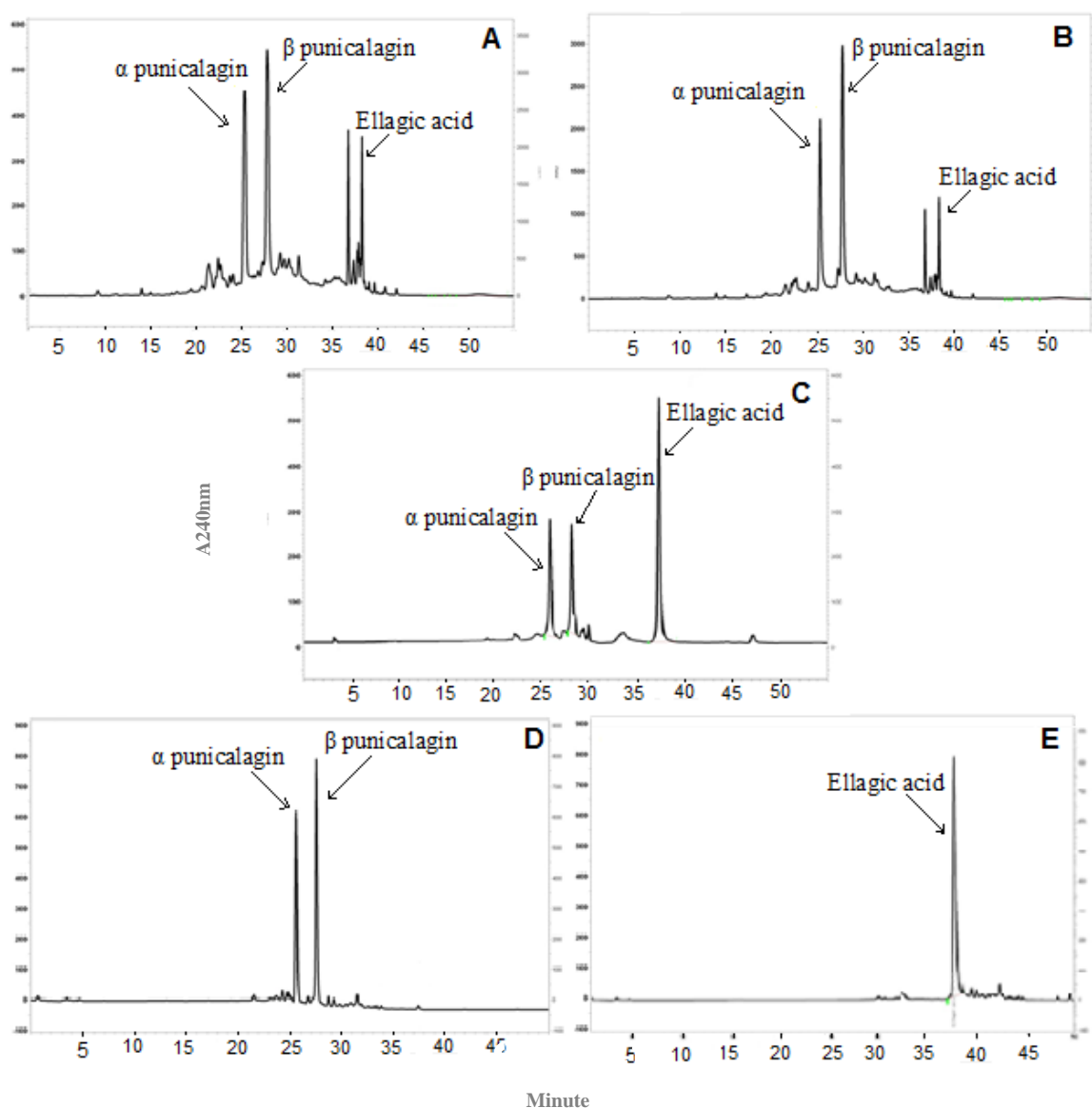


Figure 4. HPLC chromatogram of pomegranate pericarp extracts (A), TPT (B), alpha punicalagin, beta punicalagin and ellagic acid (C), alpha and beta punicalagin isomers (D) and ellagic acid (E).

Table 3. DPPH scavenging activity of alpha and beta punicalagin isomers, mixture of punicalagin (alpha+beta), ellagic acid, and TBHQ.

Phenol component	% Inhibition
α Punicalagin	50.8 \pm 3.8
β Punicalagin	60.5 \pm 8.5
α + β Punicalagins	56.0 \pm 3.2
Ellagic acid	27.0 \pm 3.3
TBHQ	66.9 \pm 6.4



different antioxidant activities. The difference between antioxidant activities of the four studied cultivars was related to difference in phenolic contents and composition. Among the cultivar studied, Shahvar was found to contain the highest phenolic constituents and highest antioxidant activity. Purified phenol compounds have different antioxidant activities. Our results confirmed that the punicalagin is the substance responsible for the antioxidant activity of the pomegranate pericarp. The therapeutic use of medicinal plants is becoming popular because of the absence of side effects and, in this regard, Shahvar cultivar can potentially replace the current synthetic antioxidants in food and pharmaceutical industries.

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REFERENCES

1. Aguilar, C. N., Aguilera-Carbo, A., Robledo, A., Ventura, J., Belmares, R., Martinez, D., Rodríguez-Herrera, R. and Contreras, J. 2008. Production of Antioxidant Nutraceuticals by Solid-State Cultures of Pomegranate (*Punicagranatum*) Peel and Creosote Bush (*Larreatri dentata*) Leaves. *Food Technol. Biotech.*, **46**: 218-222.
2. Ardekani, M. R. S., Hajimahmoodi, M., Oveisi, M. R., Sadeghi, N., Jannat, B., Ranjbar, A. M., Gholam, N. and Moridi, T. 2011. Comparative Antioxidant Activity and Total Flavonoid Content of Persian Pomegranate (*Punica granatum* L.) Cultivars. *Iranian J. Pharmacy Res.*, **10**: 519-524.
3. Banihani, S., Swedan, S. and Alguraan, Z. 2013. Pomegranate and Type 2 Diabetes. *Nutr. Res.* **33**: 341-348.
4. Basiri, S., Shekarforoush, S., Aminlari, M. and Akbari, S. 2015. The Effect of Pomegranate Peel Extract (PPE) on the PolyPhenol Oxidase (PPO) and Quality of Pacific White Shrimp (*Litopenaeus vannamei*) during Refrigerated Storage." *LWT-Food Sci. Technol.*, **60**: 1025-1033.
5. Basu, A. and Penugonda, K. 2009. Pomegranate Juice: A Heart-Healthy Fruit Juice. *Nutr. Rev.*, **67**: 49-56.
6. Celik, I., Temur, A. and Isik, I. 2009. Hepatoprotective Role and Antioxidant Capacity of Pomegranate (*Punicagranatum*) Flowers Infusion against Trichloroacetic Acid-Exposed in Rats. *Food Chem. Toxicol.*, **47**: 145-149.
7. Chidambara Murthy, K. N., Jayaprakasha, G. K. and Singh, R. P. 2002. Studies on Antioxidant Activity of Pomegranate (*Punicagranatum*) Peel Extract Using *in Vivo* Models. *J. Agric. Food Chem.*, **50**: 4791-4795.
8. Dahham, S. S., Ali, M. N., Tabassum, H. and Khan, M. 2010. Studies on Antibacterial and Antifungal Activity of Pomegranate (*Punicagranatum* L.). *Am. Eurasian J. Agric. Environ. Sci.*, **9**: 273-281.
9. Dikmen, M., Ozturk, N. and Ozturk, Y. 2011. The Antioxidant Potency of *Punicagranatum* L. Fruit Peel Reduces Cell Proliferation and Induces Apoptosis on Breast Cancer. *J. Medic. Food*, **14**: 1638-1646.
10. Doig, A. J., Williams, D. H., Oelrichs, P. B. and Baczynskyj, L. 1990. Isolation and Structure Elucidation of Punicalagin, a Toxic Hydrolysable Tannin, from *Terminalia oblongata*. *J. Chem. Soc. Perkin Trans.*, **1**: 2317-2321.
11. Ferreira, D. 2007. Antioxidant, Antimalarial and Antimicrobial Activities of Tannin-Rich Fractions, Ellagitannins and Phenolic Acids from *Punica granatum* L. *Planta Med.*, **73**: 461-467.
12. Gil, M. I., Tomás-Barberán, F. A., Hess-Pierce, B., Holcroft, D. M. and Kader, A. A. 2000. Antioxidant Activity of Pomegranate Juice and Its Relationship with Phenolic Composition and Processing. *J. Agric. Food Chem.*, **48**: 4581-4589.
13. Hajimahmoodi, M., Oveisi, M. R., Sadeghi, B., Jannat, M., Hadjibabae, M., Farahani, E., Akrami, M. R. and Namdar, R. 2008. Antioxidant Properties of Peel and Pulp Hydro Extract in Ten Persian Pomegranate Cultivars. *Pak. J. Biol. Sci.*, **11**: 1600-1604.
14. Hayouni, E. A., Miled, K., Boubaker, S., Bellasfar, Z., Abedrabba, M., Iwaski, H.,

- Oku, H., Matsui, T., Limam, F. and Hamdi, M. 2011. Hydroalcoholic Extract Based-Ointment from *Punica granatum* L. Peels with Enhanced *in Vivo* Healing Potential on Dermal Wounds. *Phytomedicine*, **18**: 976-984.
15. Hong, M. Y., Seeram, N. P. and Heber, D. 2008. Pomegranate Polyphenols Down-Regulate Expression of *Androgen-Synthesizing* Genes in Human Prostate Cancer Cells Overexpressing the Androgen Receptor. *J. Nutr. Biochem.*, **19**: 848-855.
 16. Jurenka, J. S. 2008. Therapeutic Applications of Pomegranate (*Punica granatum* L.): A Review. *Altern. Med. Rev.*, **13**: 128-44.
 17. Kalaycıoğlu, Z. and F. B. Erım. 2017. Total Phenolic Contents, Antioxidant Activities, and Bioactive Ingredients of Juices from Pomegranate Cultivars Worldwide. *Food Chem.*, **221**: 496-507.
 18. Kanatt, S. R., Chander, R. and Sharma, A. 2010. Antioxidant and Antimicrobial Activity of Pomegranate Peel Extract Improves the Shelf Life of Chicken Products. *Int. J. Food Sci. Technol.*, **45**: 216-222.
 19. Kaplan, M., Hayek, T., Raz, A., Coleman, R., Dornfeld, L., Vaya, J. and Aviram, M. 2001. Pomegranate Juice Supplementation to Atherosclerotic Mice Reduces Macrophage Lipid Peroxidation, Cellular Cholesterol Accumulation and Development of Atherosclerosis. *J. Nutr.*, **131**: 2082-2089.
 20. Kim, G. H., Kim, J. E., Rhie, S. J. and Yoon S. 2015. The Role of Oxidative Stress in Neurodegenerative Diseases. *Exp. Neurobiol.*, **24**: 325-340.
 21. Kraszni, M., Marosi, A. and Larive, C. K. 2013. NMR Assignments and the Acid-Base Characterization of the Pomegranate Ellagitanninpunicalagin in the Acidic pH-Range. *Analytical Bioanalytical Chem.*, **405**: 5807-5816.
 22. Kulkarni, A. P., Aradhya, S. M. and Divakar, S. 2004. Isolation and Identification of a Radical Scavenging Antioxidant-Punicalagin from Pith and Carpellary Membrane of Pomegranate Fruit. *Food Chem.*, **87**: 551-557.
 23. Kwak, H. M., Young Jeon, S., Sohng, B. H., Kim, J. G., Lee, J. M., Lee, K. B., Jeong, H. H., Hur, J. M., Kang, Y. H. and Song, K. S. 2005. β -Secretase (BACE1) Inhibitors from Pomegranate (*Punica granatum*) Husk. *Arch. Pharm. Res.*, **28**: 1328-1332.
 24. Levin, G. M. 1994. Pomegranate (*Punicagranatum*) Plant Genetic Resources in Turkmenistan. *Plant Genetic Resources Newsletter (IPGRI/FAO)*, **97**: 31-36.
 25. Longtin, R. 2003. The Pomegranate: Nature's Power Fruit? *J. Natl. Cancer I.*, **15**: 346-348.
 26. Lu, J., Ding, K. and Yuan, Q. 2008. Determination of Punicalagin Isomers in Pomegranate Husk. *Chromatographia*, **68**: 303-306.
 27. Mertens-Talcott, S. U. and Percival, S. S. 2005. Ellagic Acid and Quercetin Interact Synergistically with Resveratrol in the Induction of Apoptosis and Cause Transient Cell Cycle Arrest in Human Leukemia Cells. *Cancer Lett.*, **218**: 141-151.
 28. Middha, S. K., Usha, T. and RaviKiran, T. 2012. Influence of *Punica granatum* L. on Region Specific Responses in Rat Brain during Alloxan-Induced Diabetes. *Asian Pac. J. Trop. Biomed.*, **2**: 905-909.
 29. Okonogi, S., Duangrat, C., Anuchpreeda, S. and Tachakittirungrod. 2007. Comparison of Antioxidant Capacities and Cytotoxicities of Certain Fruit Peels. *Food Chem.*, **103**: 839-846.
 30. Ozgen, M., Durgaç, C., Serçe, S. and Kaya, C. 2008. Chemical and Antioxidant Properties of Pomegranate Cultivars Grown in the Mediterranean Region of Turkey. *Food Chem.*, **111**: 703-706.
 31. Rafiee, Z., Jafari, S. M., Alami, M. and Khomeiri, M. 2012. Antioxidant Effect of Microwave-Assisted Extracts of Olive Leaves on Sunflower Oil. *J. Agr. Sci. Tech.*, **14**: 1497-1509.
 32. Rahmanian, N., Jafari, S. M. and Wani, T. A. 2015. Bioactive Profile, Dehydration, Extraction and Application of the Bioactive Components of Olive Leaves. *Trends Food Sci. Technol.*, **42**: 150-172.
 33. Ricci, D., Giamperi, L., Bucchini, A. and Fraternali, D. 2006. Antioxidant Activity of *Punica granatum* fruits. *Fitoterapia*, **77**: 310-312.
 34. Rosenblat, M., Volkova, N. and Aviram, M. 2013. Pomegranate Phytosterol (β -Sitosterol) and Polyphenolic Antioxidant (Punicalagin) Addition to Statin, Significantly Protected against Macrophage foam Cells Formation. *Atherosclerosis*. **226**: 110-117.



35. Rouanet, J. M., Décordé, K., Del Rio, D., Auger, C., Borges, G., Cristol, J. P., Lean, M. E. J. and Crozier, A. 2010. Berry Juices, Teas, Antioxidants and the Prevention of Atherosclerosis in Hamsters. *Food Chem.*, **118**: 266-271.
36. Sadasivam, S. and Manickam, A. 1996. Phenolics. In: "*Biochemical Methods*". New Age International, New Delhi, PP. 195-198.
37. Seeram, N. P., Lee, R. and Heber, D. 2004. Bioavailability of Ellagic Acid in Human Plasma after Consumption of Ellagitannins from Pomegranate (*Punica granatum L.*) Juice. *Clin. Chim. Acta*, **348**: 63-68.
38. Seeram, N. P., Adams, L. S., Henning, S. M., Niu, Y., Zhang, Y., Nair, M. G. and Heber, D. 2005a. *In Vitro* Antiproliferative, Apoptotic and Antioxidant Activities of Punicalagin, Ellagic Acid and a Total Pomegranate Tannin Extract Are Enhanced in Combination with Other Polyphenols as Found in Pomegranate Juice. *J. Nutr. Biochem.*, **16**: 360-367.
39. Seeram, N. P., Lee, R., Hardy, M. and Heber, D. 2005b. Rapid Large Scale Purification of Ellagitannins from Pomegranate Husk, a By-Product of the Commercial Juice Industry. *Sep. Purif. Technol.*, **41**: 49-55.
40. Shahidi, F., Janitha, P. and Wanasundara, P. 1992. Phenolic Antioxidants. *Crit. Rev. Food Sci. Nutr.*, **32**: 67-103.
41. Shukla, M., Gupta, K., Rasheed, Z., Khan, K. A. and Haqqi, T. M. 2008. Consumption of Hydrolyzable Tannins-Rich Pomegranate Extract Suppresses Inflammation and Joint Damage in Rheumatoid Arthritis. *Nutr.*, **24**: 733-743.
42. Singh, R. P., Chidambara Murthy, K. N. and Jayaprakasha, G. K. 2002. Studies on Antioxidant Activity of Pomegranate (*Punica granatum*) Peel Extract Using *in Vivo* Models. *J. Agric. Food Chem.* **50**: 4791-4795.
43. Srivastava, A., Rao, L. J. M. and Shivanandappa, T. 2007. Isolation of Ellagic Acid from the Aqueous Extract of the Roots of *Decalepishamiltonii*: Antioxidant Activity and Cytoprotective Effect. *Food Chem.*, **103**: 224-233.
44. Taghvaei, M. and Jafari, S. M. 2015. Application and Stability of Natural Antioxidants in Edible Oils in Order to Substitute Synthetic Additives. *J. Food Sci. Technol.*, **52**: 1272-1282.
45. Taghvaei, M., Jafari, S. M., Sadeghi Mahoonak, A., Mehregan Nikoo A., Rahmanian, N., Hajitabar, J. and Meshginfar, N. 2014. The Effect of Natural Antioxidants Extracted from Plant and Animal Resources on the Oxidative Stability of Soybean Oil. *LWT-Food Sci. Technol.*, **56**: 124-130.
46. Viuda-Martos, M., Fernández-López, J. and Pérez-Álvarez, J. 2010. Pomegranate and Its Many Functional Components as Related to Human Health: A Review. *Comp. Rev. in Food Sci. Food Safety*, **9**: 635-654.
47. Vroegrijk, I. O., van Diepe, J. A., van den Berg, S., Westbroek, I., Keizer, H., Gambelli, L., Hontecillas, R., Bassaganya-Riera, J., Zondag, G. C., Romijn, J. A., Havekes, L. M. and Voshol, P. J. 2011. Pomegranate Seed Oil, a Rich Source of Punicic Acid, Prevents Diet-Induced Obesity and Insulin Resistance in Mice. *Food Chem. Toxicol.*, **49**: 1426-1430.
48. Xu, X., Li, H., Hou, X., Li, D., He, S., Wan, C., Yin, P., Liu, M., Liu, F. and Xu, J. 2015. Punicalagin Induces Nrf2/HO-1 Expression via Upregulation of PI3K/AKT Pathway and Inhibits LPS-Induced Oxidative Stress in RAW264 Macrophages. *Mediators Inflamm.*, **2015**: 380-388.
49. Zhang, J., Zhan, B., Yao, X., Gao, Y. and J. Shong. 1995. Antiviral Activity of Tannin from the Pericarp of *Punica granatum L.* against Genital Herpes Virus *in Vitro*. *China J. Chinese Materia Medica*. **20**: 556-558, 576.
50. Zhou, B. H., Tu, J., Zhou, J., Wu, Y. and Liu G. 2014. Purification of Pomegranate Pericarps (*Punica granatum L.*) Extracts with Macroporous Resins and Evaluation of Antioxidant Activities *in Vivo*. *Adv. Mat. Res.*, **1**: 156-162.

فعالیت آنتی اکسیدانی عصاره پریکارپ رقم های مختلف میوه انار

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چکیده

پریکارپ میوه انار حاوی ترکیبات پلی فنولی شامل آلفا و بتا پونیکالائین والائیک اسید است که فعالیت های آنتی اکسیدانی قابل توجهی را نشان می دهد. هدف از این مطالعه، بررسی و ارزیابی ترکیبات فنولی عصاره پریکارپ رقم های مختلف انار (PPE) و تعیین خواص آنتی اکسیدانی آن هاست. عصاره های متانولی و آبی از پریکارپ چهار رقم مختلف انار (شهور، سیاه سرفه، ترش سبز و عبدالرحیم خانی از شیراز، ایران) تهیه شد و میزان فنول تام PPE هاتعیین شد. سپس اجزای آن توسط ستون های کروماتوگرافی XAD-16 و LH-20 به روش کروماتوگرافی جذب سطحی و ژل فیلتراسیون خالص شدند. ترکیبات پلی فنولی خارج شده از ستون های کروماتوگرافی به وسیله HPLC مورد بررسی قرار گرفتند. فعالیت آنتی اکسیدانی با استفاده از آزمون DPPH اندازه گیری شد. نتایج نشان داد که اختلاف معنی داری در محتوای فنل تام و نوع ترکیبات پلی فنولی در عصاره پریکارپ چهار رقم مختلف انار وجود دارد. مقدار فنول تام در عصاره های متانولی به طور معنی داری بیشتر از عصاره های آبی بود ($P < 0.05$). رقم شهور با بیشترین فنول تام (11.72 ± 0.01 mg/mL) در عصاره آبی و 17.7 ± 0.12 mg/mL در عصاره متانولی) بیشترین خاصیت آنتی اکسیدانی را میان ارقام نشان داد. تجزیه و تحلیل با استفاده از HPLC نشان داد که عصاره پریکارپ رقم شهور دارای بیشترین میزان بتا پونیکالائین (2.14 ± 39.75 درصد) بود. سپس آلفا پونیکالائین با 2.12 ± 28.34 درصد والائیک اسید با 0.93 ± 3.49 درصد، بیشترین ترکیبات فنولی را به خود اختصاص داد. پس از خالص سازی و مقایسه ی خاصیت آنتی اکسیدانی پلی فنول های خالص پریکارپ انار مشخص شد که فعالیت آنتی اکسیدانی آلفا پونیکالائین خالص 50.8 درصد، بتا پونیکالائین 60.5 درصد، مجموع آلفا و بتا پونیکالائین 56 درصد والائیک اسید 27 درصد بود. نتایج این پژوهش نشان می دهد که در بین رقم های مختلف انار، شهور با دارا بودن بیشترین میزان بتا پونیکالائین منبع عالی آنتی اکسیدان های طبیعی است و می تواند به طور بالقوه جایگزین آنتی اکسیدان های مصنوعی در صنایع غذایی شود.