



Evaluation of phytochemical contents and antioxidant activity of pomegranate flower

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Abstract: Pomegranate is one of the most abundant fruits consumed in Turkey. This study aimed to determine the content of total phenolic, total flavonoid, and antioxidant activity of *Punica granatum* L. flower in different extracts. Antioxidant activities of different extracts were determined 2,2-diphenyl-1-picrylhydrazyl (DPPH•) radical scavenging, reducing power, and metal chelating methods. The results showed that the total phenolic content for the extracts ranged from 14.82 to 90.86 mg gallic acid equivalents (GAE) / g extract. The contents of flavonoids were found to range from 7.35 to 500.00 mg quercetin equivalents (QUE)/ g extract. All pomegranate flower extracts displayed remarkable antioxidant activity according to DPPH and reducing power assays. Especially the methanolic extract of pomegranate flower possesses significant scavenging activity against DPPH• (85.80 %), as well as the largest contents of flavonoids and phenolic compounds. The antioxidant capacity of the methanolic extract was also greater than those of BHT and α -tocopherol in DPPH and reducing power assays. The results demonstrated that the antioxidant activity of extracts of *Punica granatum* L. flower might, at least in part, have high content of flavonoids and other phenolics.

Keywords: Pomegranate flower, *Punica granatum* Linn., antioxidant activity, phenolic compounds.

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INTRODUCTION

Punica granatum Linn. known as pomegranate is a member of the Punicaceae family. Pomegranate is a famous ancient fruit originating from the Middle East. Main producers and exporters in the world are Turkey, India, Iran, China, United States, Spain, South Africa, Peru, Chile, and Argentina (1). In Turkey, the annual production of pomegranate is 537.847 tons and it is cultivated in Adiyaman, Mersin, Antalya, Adana, Gaziantep, and Şanlıurfa (2).

Pomegranate possesses a broad array of pharmacological properties such as antioxidant (3), anti-inflammatory (4), anti-cancer (5,6), anti-parasitic (7), analgesic (8), antimicrobial (9), neuroprotective (10), antifungal (11), antiulcer (12), and antidiarrheal (13). The flowers of pomegranate have been ethnomedically used for their anti-cholinesterase, anti-hyperglycemic (14), anti-diabetic, anti-obesity (15), anti-bacterial, and antioxidant (16) effects. The constituents including gallic acid (17), ellagic acid, ethyl brevifolin-carboxylate, maslinic acid, ursolic acid, oleanolic acid, asiatic acid, sterol, daucosterol, punicaflavone, pelargonidin 3,5-

diglucoside and pelargonidin 3-glucoside (18) are responsible for the pharmacological effects of pomegranate flower extract.

The content of phytochemicals that contributes to the antioxidant effect of the pomegranate flowers located in Adiyaman is unknown and during pomegranate juice production, antioxidant-rich flowers of pomegranates are discarded. Moreover pomegranate flowers constitutes an inexpensive source for the extraction of phytochemicals that might be utilized in the pharmaceutical, cosmetic and food industries. Thus, the objective of this research is to evaluate the nutritional quality of pomegranate flowers, which constitute the pomegranate fruit juice industry wastes. Another aim is to evaluate whether total phenolic and flavonoid contents of pomegranate flowers are correlated with antioxidant activity.

MATERIAL AND METHODS

Chemicals

Ferric chloride and 2,2-diphenyl-1-picrylhydrazyl (DPPH), AlCl_3 , NaNO_2 , ethanol, and standard compounds, quercetin and alpha-tocopherol were purchased from Sigma Chemical Co. (Sigma-Aldrich GmbH, Steinheim, Germany). Butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were obtained from Acros. Folin-Ciocalteu reagent, trichloroacetic acid, and methanol were purchased from Merck. All the other chemicals used were of analytical grade.

Plant material

Dried pomegranate flowers were bought from a local shop in Adiyaman, Turkey. The flowers were ground and then the powder was kept at 4 °C until the extraction process.

Preparation of Extract

The powdered flowers (10 g) were extracted with n-hexane (H), methanol (M), 1% acidified methanol (99% methanol, AME 1 and 70% methanol, AME 2) in a Soxhlet apparatus for 4 h. Extracts were concentrated using a rotary evaporator (40 °C) under vacuum and kept at 4 °C until analysis.

Determination of total phenolic content

The total contents of phenolic compounds were determined by the Folin-Ciocalteu method (19). The extracts were dissolved in methanol. 50 μL of samples were mixed with 450 μL deionized water, 250 μL of 1.0 N Folin reagent and 1250 μL of 7.5% Na_2CO_3 . The mixture was mixed in a vortex before allowed to rest for 120 minutes at room temperature and the absorbance was measured at 765 nm. The calibration curve was plotted by different concentration of gallic acid equivalents (in mg/g).

Determination of total flavonoid content

The overall flavonoid content of the *Punica granatum* L. flower extract was estimated using the aluminum chloride colorimetric method (20). 500 μL extract solution (1 mg/mL) was blended with 4500 μL of distilled water and 300 μL 5.0% NaNO_2 . Having waited for 5 min, 300 μL of AlCl_3 solution was added to the blend and allowed to stand for 6 min. Then, 2000 μL of 1.0 M NaOH was added and volume increased to 10 mL with distilled water. The absorbance of the mixture after it turned to pink was measured at 510 nm using UV/Vis spectrophotometer (Shimadzu UV-160). A calibration curve of standard reference was established using quercetin (range of concentration from 4 to 20 $\mu\text{g}/\text{mL}$) as standard reference plotted. Total flavonoid content was expressed as mg of quercetin equivalents g extract.

Determination of antioxidant activity by DPPH radical scavenging assay

The antioxidant activity of the *Punica granatum* L. flower extract was determined by the DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging assay (21). 1 mL of 0.10 mM DPPH solution was mixed with 3 mL of extract solution at different concentration. (12.5-125 $\mu\text{g}/\text{mL}$). Vigorously shaken mixture was incubated in dark at room temperature 30 min. The absorbance was measured at 517 nm by a UV/Vis spectrophotometer. Butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and alpha-tocopherol were utilized as standards. Using the equation below, radical scavenging percentage was calculated :

$$\text{Radical scavenging activity (\%)} = \left[\frac{(A_{\text{Control}} - A_{\text{Sample}})}{A_{\text{Control}}} \right] \times 100 \quad (\text{Eq. 1})$$

A_{control} is the absorbance at the addition of ethanol instead of the extract/standard; A_{sample} is the absorbance at the addition of extract/standard.

Reducing power assay

The reducing power of different *Punica granatum* L. flower extracts was evaluated by the method of Oyaizu (22). 1 mL of the extract at different concentrations was mixed with 2.50 mL of phosphate buffer (0.20 mol/L, pH 6.60) and 2.50 mL 1.0% potassium ferricyanide and vortexed. The mixture was incubated at 50 °C for 20 minutes in a water bath. 2.50 mL of 10% trichloroacetic acid was then added to the mixture, and centrifuged at 6000 rpm for 10 minutes. 1.25 mL of supernatant was mixed with 1.25 mL of distilled water and 0.50 mL of 0.10% ferric chloride and mixed well. The absorbance of final solution was measured at 700 nm using a UV/Vis spectrophotometer. Higher absorbance of the reaction mixture meant higher reducing power.

Metal chelating activity

The chelation of ferrous ions of the studied extracts was observed according to the method as described by Dinis et al. (23). 50 µL of 2.0 mM FeCl₂ was mixed with 3750 µL of the extracts having different concentrations (12.5-125 µg/mL) and vortexed. After 10 min of incubation, the reaction was started by adding 200 µL of 5.0 mM ferrozine and the solution was incubated at room temperature for 20 min and then the absorbance of the solution was measured at 562 nm using a spectrophotometer. EDTA was utilized as a positive control. The percentage inhibition of Fe²⁺ - ferrozine complex was calculated by the following equation:

$$\text{Inhibition (\%)} = \frac{(\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}})}{\text{Abs}_{\text{control}}} * 100 \quad (\text{Eq. 2})$$

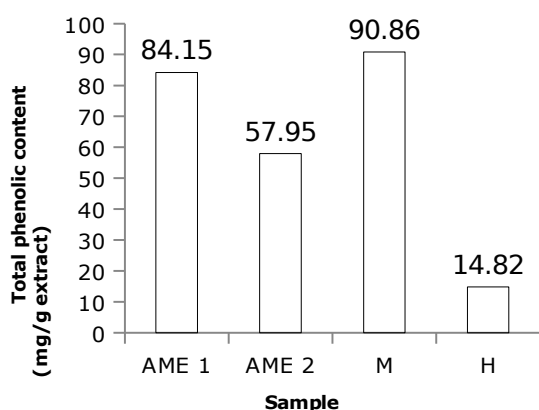


Figure 1. Total phenolic content as equivalent mg of gallic acid per g of AME 1, AME 2, M and H extracts of pomegranate flower.

A_{control} ; the absorbance at the addition of ethanol instead of the extract/standard, A_{sample} ; the absorbance at the addition of extract/standard.

RESULTS AND DISCUSSION

The yields of n-hexane, methanol, 1% acidified methanol (99% methanol and 70% methanol) extracts of the flower of *P. granatum* L. were 0.03%, 15.19%, 46.04% and 31.83%, respectively.

Total phenolic content

Total phenolic contents of the extracts of pomegranate flower determined by Folin-Ciocalteu method are presented as gallic acid equivalents (mg gallic acid/ g extract) in Figure 1. The amount of total phenolic contents in the determined extracts ranged from 14.82±0.01 to 90.86±0.01 mg GAE / g extract. The results showed that the highest total phenolic content was obtained in the methanolic extract, while the lowest was observed in the n-hexane extract. Eddebbagh et al.(24) found that the phenolic content of 80% methanolic extract of dried pomegranate flower is 90.73 mg GAE/ g of dry weight flowers. Abdolahi et al. (16) have found 28 mg GAE/ g dry weight in 70% ethanolic extract and Rashid and Shafi (25) have found 190.50 and 103.81 mg GAE/ g in methanolic and aqueous extracts in dried pomegranate flower. These differences in phenolic contents might be due to location, soil and climate.

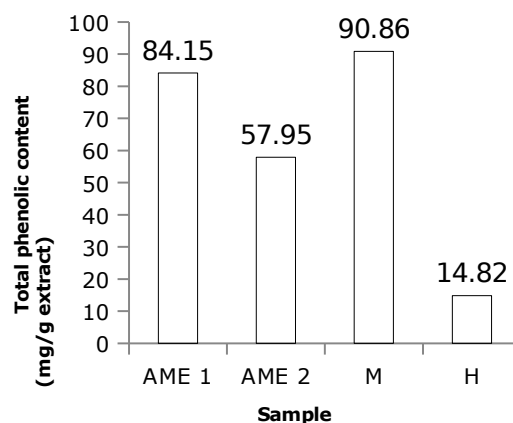


Figure 2. Total flavonoid content as equivalent mg of quercetin per g of AME 1, AME 2, M and H extracts of pomegranate flower.

Total flavonoid content

The total flavonoid contents in the determined extracts of pomegranate flowers are expressed in

terms of quercetin equivalent (mg quercetin/ g extract) in Figure 2. The flavonoid content was 350.74 ± 0.01 , 172.79 ± 0.02 , 500.00 ± 0.01 and 7.35 ± 0.01 mg QUE /g in AME 1, AME 2, M and H extracts, respectively. The results showed that the greatest total flavonoid content was obtained in the methanolic extract. Eddebbagh et

al. (24) have found 221.70 mg QUE/ g dry weight in 80% methanolic extract and Abdolahi et al. (16) have reported 64.38 ± 0.81 mg catechin equivalents per gram of dry weight in dried pomegranate flower. These differences might be related to solvent used and location.

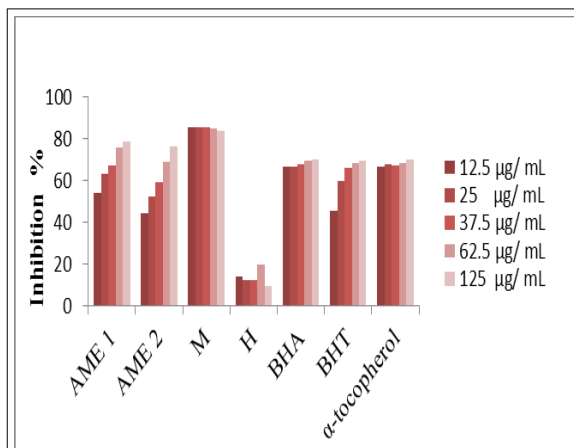


Figure 3. DPPH• radical scavenging activity(%) of AME 1, AME 2, M and H extracts of pomegranate flower.

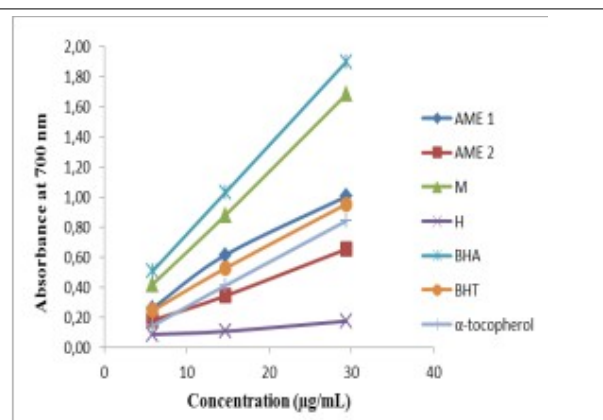


Figure 4. Reducing power activity(%) of AME 1, AME 2, M and H extracts of pomegranate flower.

DPPH radical scavenging activity

DPPH radical scavenging activity of the pomegranate flower extracts is summarized in Figure 3 and compared with the standard antioxidants such as BHA, BHT and α -tocopherol. The potential of different pomegranate flower extracts to scavenge free radical varied 54.12-78.54% in AME 1, 44.22-76.56% in AME 2, 83.82-85.80% in M and 9.57-19.80% in H. Among them, the methanolic extract exhibited the higher values of DPPH radical scavenging activity. Pomegranate flower extracts clearly showed that as the concentration got higher the antioxidant activity against DPPH radical in all extracts increased. Abdolahi et al. (16) have reported 91.04% inhibition at concentration of 100 $\mu\text{g/mL}$ pomegranate flower extract. On the other hand, Rashid and Shafi (25) have found 76.89% and 69.23% in methanolic and aqueous extract of flowers of pomegranate, respectively.

These differences can also be explained with soil and climate differences.

Reducing power activity

Reducing power activities of extracts are shown in Figure 4 with standard antioxidant compounds (BHA, BHT and α -tocopherol). The reducing power of the all extracts dose with the increase in concentration (Table 1). In addition, methanolic extract showed 2.93 times stronger antioxidant activity than that of α -tocopherol at an initial concentration of 5.88 $\mu\text{g/mL}$. In the literature, the reducing properties are usually related to the presence of reductones (reducing agents) which have been found to exert antioxidant ability to break the free radical chain by donating a hydrogen atom (26). So, the data gathered from the present study suggest that pomegranate flower might contain polyphenol which acts as reducing agents.

Table 1. Reducing power activity of AME 1, AME 2, M and H extracts of pomegranate flower.

Extracts/Standards	Reducing Power ($\mu\text{g/mL}$, 700 nm)		
	5.88	14.70	29.41
AME 1	0.260	0.613	1.004
AME 2	0.179	0.342	0.653
M	0.419	0.878	1.683
H	0.085	0.106	0.174
BHA	0.507	1.028	1.900
BHT	0.244	0.525	0.953
α -tocopherol	0.143	0.410	0.841

Metal chelating activity

Metal chelating activity of the pomegranate flower extracts and EDTA are presented in Table 2. n-Hexane extract possessed moderate metal

chelating activity whereas all the other extracts exhibited none. The highest metal chelating activity of 9.299% was determined in n-hexane extract.

Table 2. Metal chelating activity of AME 1, AME 2, M and H extracts of pomegranate flower.*ND; non-detected.

Extracts/ Standard	Inhibition, %				
	12.5 $\mu\text{g/mL}$	25 $\mu\text{g/mL}$	37.5 $\mu\text{g/mL}$	62.5 $\mu\text{g/mL}$	125 $\mu\text{g/mL}$
AME 1	*ND	ND	ND	ND	ND
AME 2	ND	ND	ND	ND	ND
M	ND	ND	ND	ND	ND
H	ND	0.104	1.149	1.985	9.299
EDTA	8.045	40.020	56.426	78.892	84.430

CONCLUSION

Our results show that the flowers of *P. granatum* may have health benefits when consumed. Our belief is that they display potential as a functional food or value-added ingredient for years to come. Also *P. granatum* flower might be considered a source of important antioxidant with bioactive properties to be explored for pharmaceutical applications. Further studies can be undertaken to investigate the effect of toxicity in these extracts.

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