General Medicine: Open Access

Anahita et al., General Med 2015, 3:1
DOI: 10.4172/2327-5146.1000164

Research Article Open Access

Evaluation of Total Phenolic Content, Total Antioxidant Activity, and Antioxidant Vitamin Composition of Pomegranate Seed and Juice

Anahita A[1*], Asmah R[1] and Fauziah O[2]

[1]*Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*

[2]*Department of Human Anatomy, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400, Serdang, Selangor, Malaysia*

[*]**Corresponding author:** Anahita A, Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia-43400, Serdang, Selangor, Malaysia, Tel: +60173972310; E-mail: asmah@upm.edu.my

Rec date: Nov 29, 2014 **Acc date:** Feb 16, 2015 **Pub date:** Feb 21, 2015

Abstract

This study aimed to determine total phenolic content (TPC), total antioxidant activity (TAA), antioxidant vitamin composition (A, C, and E) of pomegranate fruit. In addition, two edible parts of pomegranate juice, pomegranate seed, and combination of them were compared based on antioxidant properties. TPC was determined by using Folin-Ciocalteu (FC) method based on colorimetric reduction. Ferric reduction ability power (FRAP assay) was used to test the antioxidant activity. Vitamin assessments were conducted by using high performance liquid chromatography (HPLC). Results for antioxidant vitamin composition in pomegranate juice (PJ) showed that the concentration of vitamin A was 22.8 ± 0.69 µg/100 g, vitamin C was 57.8 ± 0.59 mg/100 g, and vitamin E was 0.07 ± 0.01 mg/100 g. Besides, TPC in PJ, pomegranate seed (PS), and pomegranate seed-juice (PSJ) was 2502 ± 54, 165 ± 49, and 2696 ± 49 mg GAE/L, and TAA was 32 ± 5.1, 20 ± 2.8, and 47 ± 5.5 mmol/L respectively. This study revealed that PSJ contained high level of phenolic compounds, antioxidant activity, and vitamin C. In addition, TPC was as main contributor to antioxidant activities, and positively correlated with TAA (r^2=0.91, $p<0.05$). Therefore, combination of pomegranate seed and juice may become an alternative and potential source of natural antioxidant.

Keywords Antioxidant activity; Phenolic content; Pomegranate; Vitamin composition

Introduction

Pomegranate (Punicagranatum) is one of the oldest known edible fruit that born on the small long-living tree, which is cultivated through the Mediterranean region, Himalayas, and Southeast Asian [1]. The pomegranate fruit can be divided into three anatomical origins: seeds, peel, and arils. Pomegranate fruit is considered as a nutritious fruit due to its biological actions; most of these effects were attributed to its high phenolic content and vitamin C [2]. Pomegranate juice is obtained from arils, which are rich sources of bioactive compound like phenolic and flavonoids [3]. Another part of pomegranate fruit is seeds, which are a rich origins of polyunsaturated (PUFA) mostly linolenic (n-3), and linoleic (n-2). Phenolic content is the main compound attribute for the most of the functional properties of many fruits such as pomegranate and grapes [4]. Reactive oxygen and nitrogen species are constantly produced in vivo for physiological purposes, and often over-produced in pathological situations cause oxidative damage [5]. All oxygen-consuming organisms are used antioxidant such as vitamin C, E, A, and phenolic to protect their possible damage to biological molecules [6]. In the recent years, more attention has been paid to the antioxidants contained in fruits; because epidemiological studies revealed that high fruit intake was associated with reduced mortality and morbidity of some chronic diseases such as cardiovascular diseases, cancers and neurological damage [2].

One of the possible mechanisms was attributed to the antioxidant activity presented infruits [7]. Some phenolic compounds are even more powerful as antioxidants than vitamin C, E *in vitro,* and significantly bioavailable, as demonstrated by animal and human studies [8]. Antioxidants have leading role in health maintenance based on their modulation of the oxidation processes in the body [9]. Consequently the search for inexpensive and abundant sources of natural antioxidants is attracting worldwide interest. Consumption of fresh fruits and vegetables to improve human health has been attributed primarily to their high contents of healthful phytochemicals and other micronutrients [10]. This study was conducted to determine antioxidant power of pomegranate fruit, and tocompare between two-edible parts of pomegranate (juice and seed) based on antioxidant properties.

Materials and Methods

Chemicals

L-ascorbic acid, α-tocopherol, trans-β-carotene, Ferric Chloride ($FeCl_3.6H_2O$), and TPTZ (2,4,6-tri[2-pyridyl]-s-triazine) were purchased from Sigma-Aldrich (St Louis, Mo., USA). Folin-Ciocalteu reagent was from Merck Chemical Supplies (MerckKGaA, Darmstadt, Germany). Gallic acid was obtained from NacaliaTesque, Kyoto, Japan.

Preparation of samples for analysis

Ripe sweet red pomegranate fruits were used in this study, were imported from Spain. The fruit was washed, peeled, and stored. The fruit arils were crushed and squeezed by squeezing machine (National Juicer/Blender). The pomegranate juice (PJ) was filtered to remove any water-insolublematerials. Liquid nitrogen and freeze-drying machine was used to make powder from pomegranate juice, and then stored at -18°C. The pomegranate seeds (PS) from the juice preparation were freeze dried at -20°C separately and groundinto powder. For

preparation of pomegranate seed-juice (PSJ), 0.8 g from PJ powder was added to 0.2 g of PS powder. About 100 g of the powder was mixed with 300 ml of 70% ethanol in distilled water and kept for 3 days at room temperature. The extract was then filtered. Solvent of ethanol was removed by using a rotary evaporator under vacuum at 50°C. The extract was obtained, and kept in the refrigerator.

Determination of total phenolic content (TPC)

Total phenolic content was determined according to the method of by using the Folin-Ciocalteu reagent based on colorimetric reduction [11]. The phenolic compounds are oxidized to phenolates by the reagent at alkaline pH in a saturated solution of sodium carbonate resulting in a blue complex. About 1.5 ml of Folin-Ciocalteau (10%, w/v,) is added to 300 µl sample, followed by the addition of 1.2 ml of aqueous Na_2CO_3 (7.5%, w/v). The mixture was allowed to stand in the dark for 90 minutes. The absorbance of the blue color solution was read at 760 nm on a UV visible spectrophotometer (Shimadzu, Kyoto, Japan), against blank (distilled water). Total phenolic concentration (mg/ml) of the samples were analyzed in triplicates, and extrapolated from a standard curve, constructed by using Gallic acid as a standard. Results were expressed as Gallic acid equivalents (GAE).

Determination of ferric reducing antioxidant power (FRAP) assay

The FRAP assay was presented as a method for assessing "antioxidant power" according to adapted procedure from [12]. Ferric to ferrous ion reduction at low pH causes a colored complex. The FRAP reagent was prepared with mixture 200 ml acetate buffer 300 mM (pH 3.6), 20 ml TPTZ (2,4,6-tri(22-pyridyI)-s-triazine), in 10 ml HCL, 40 mM; and 20 ml $Fecl_3.6H_2O$ in ratio 10:1:1 to give the working reagent. After preparing, FRAP regent kept in the water bath at 37°C. Then, 0.5 ml of pomegranate samples were mixed with 1 ml of FRAP regent and 30 ml of distilled water, and reading at 593 nm by using spectrophotometer (SECOMAN, France). Second reading was performed after 4 minutes at 593 nm. The results were calculated from the standard curve prepared by different concentration of $FeSO_4.7H_2O$, and expressed in mmol Fe^{+2}/L.

Estimation of β-carotene (vitamin A)

Estimation of β-carotene (vitamin A) was conducted by using high performance liquid chromatography (HPLC). Extraction of vitamin A was carried out according to the method described by [13]. The sample (10 g) was added with 10 ml of 100% (w/v) potassium hydroxide and 40ml of 99.8% ethanol and homogenized for 3 minutes. The mixture was saponified by a refluxing apparatus, and then was heated using an electric heating mantle for 30 minutes, and cooled to room temperature. The mixture was agitated frequently to avoid any aggregation. For the extraction step, the mixture was transferred into separatory funnel and then 50 ml n-hexane was added. The funnel was inverted, and shaken vigorously for a few seconds, and the layer was permitted to separate. The upper layer (hexane extract) was pipetted out and the aqueous layer was extracted twice, each time with (50 ml) of n-hexane. Then, the upper layer was washed and pooled with distilled water until free of alkali. Phenolphthalein solution (1%) was exploited to check for any alkali. The extract was then filtered to remove any water residue through anhydrous sodium sulphate. The hexane residues were removed using rotary evaporator under reduced pressure at 45°C. The resulting extract was diluted to (10 ml) with HPLC grade-hexane. Samples were conducted in triplicates, and separation condition in Table 1.The peak of β-carotene was established based on two techniques: comparing the retention time and spiking test with that of trans-β-carotene (Sigma, Co. Chemical, St. Louis, USA). 10 mg of trans-β-carotene was weighed and dissolved in 100 ml pure n-hexane to give a stock solution of 100 µg/ml. The solution was stored in a brown bottle and kept as stock in the fridge at 5°C. The standard solution of 1 µg/ml was prepared daily from the stock solution.

Conditions			
Parameter	**Vitamin A**	**Vitamin C**	**Vitamin E**
Moblie phase	(88:10:2)	(50:50)	(88:10:2)
Flow rate	1.0 ml/min	1.5 ml/min	1.0 ml/min
Detection	250 nm	254 nm	250 nm

Table 1: HPLC conditions for separation and identification of vitamin A, C, and E

Estimation of ascorbic acid (vitamin C)

Ascorbic acid (vitamin C) was conducted by using high performance liquid chromatography (HPLC). The sample was thoroughly cleaned using deionized water to remove adhering contaminants and estimation was done on the same day of purchase to counteract the instability of vitamin C in fruits. Extraction for ascorbic acid analysis was obtained by homogenizing 10 g of the sample in solution containing 20 ml meta-phosphoric acid (0.3 M), and acetic acid (1.4 M). The mixture was located in conical flask (wrapped with aluminum foil), and agitated at 100 rpm with an orbital shaker for 15 minutes at room temperature [14]. The mixture collected was filtered through Whatman (No. 4) filter paper (Milipore, USA), and 30 µl in triplicates was immediately used for HPLC analysis. The techniques were used to identify the peak of vitamin C on the chromatogram; comparing the retention time and spiking test with that of L-ascorbic acid (Sigma, Co. Chemical, St. Louis, USA). Ascorbic acid standard was prepared by dissolving 100 mg of L-ascorbic acid in a metaphosphoric acid (0.3 M) and acetic acid (1.4 M) solution at the final concentration of 1 mg/ml.

Estimation of α-tocopherol (vitamin E)

Estimation of α-tocopherol (vitamin E) was conducted by using high performance liquid chromatography (HPLC). Extraction for vitamin E was obtained according to the method described by Amin, Cheah and Abdulnabi [14,15]. 5 mg of sample was added with 20 ml methanol. Then, the mixture was mixed with 60 ml CCl_4 methanol (3:1) and agitated at 100 rpm with an orbital shaker for 20 minutes. The CCl_4 fraction was separated from the aqueous phase in a separatory funnel, and dried over Na_2SO_4. Rotary evaporator was exploited to evaporate the filtrate to dryness under pressure 45°C. The extract lipid fraction was saponified by refluxing in the presence of (0.5 g) ascorbic acid with 4 ml of 30% methanolic potassium hydroxide (KOH) for 30 minutes at the boiling point of methanol. After cooling, the flask at room temperature 15 ml of salted water added and the analogues of tocopherol were extracting twice with 40 ml petroleum ether in a separator funnel. The ether fractions were washed twice with distilled water, collected, and dried over anhydrous sodium sulphate (Na_2SO_4). Rotary evaporator was exploited to evaporate the solvent at 45°C. The residues were dissolved in 5 ml of

HPLC grade hexane. The vitamin E determined by a reverse-phase HPLC technique. Samples were conducted in triplicates, and separation conditions in Table 1. The peak for vitamin E also recognized based on comparing the retention time and spiking test what that of α-tocopherol (Sigma, Co. Chemical, St Louis, USA). 10 mg of α-tocopherol was weighed and dissolved in pure n-hexane to give stock solution 100 µg/ml. The solution was stored in a brown bottle and kept as stock in the fridge at 5°C.

Statistical analysis

All data were reported as mean ± S.E.M of triplicate determination. One-way analysis of variance (ANOVA) with significant differences between means determined at $p<0.05$, measured with post-hoc multiple comparisons and Tukey's test were performed with SPSS (version 21, IBM U.S.A). In addition, Pearson correlation was used to demonstrate the correlation between total antioxidant activity and total phenolic content.

Results and Discussion

Total phenolic content

The total phenolic content (TPC) of pomegranate was determined through analysis of Folin-Ciocalteau method that it is shown in Table 2. One-way ANOVA test was conducted to explore the level of phenolic content between PS, PJ, and PSJ, as measured by Gallic acid. Post-hoc comparison using the Tukey HSD test indicated that the mean score for PSJ was significantly ($p<0.05$) different from group PS and PJ. Moreover, The PSJ(2696 ± 49 mgGAE/L) exhibited high amount of TPC. This value for PJ was the same range for red wine (generally above 2000 mg/L), and twice that found in green tea (1029 ± 36) [16]. The total phenolic calculated for pomegranate juice reached 2100 mg/L, which was good in agreement with the Folin-Ciocalteu method. This finding showed that PSJ has high level of total phenolic content compared to other fruits juice such as orange and berries, even red wine. Besides, TPC in PSJ was higher than two other parts (PS and PJ) separately.

Epidemiology studies have demonstrated that consumption of fruits and vegetables with high phenolic content correlated with reduction of cardiovascular, cerebrovascular diseases, and cancer mortality [17]. Phenolic compounds in pomegranate may produce their beneficial effects by scavenging free radicals [2].

Parameter	TAA	TPC
Pomegranate juice (PJ)	32 ± 5.1^{a}	2502 ± 54^{a}
Pomegranate seed (PS)	20 ± 2.^{8b}	165 ± 49^{b}
Pomegranate seed + juice (PSJ)	47 ± 5.5^{c}	2696 ± 49^{c}

Table 2: Total antioxidant activity (TAA) of pomegranate by FRAP assay and Total phenolic content (TPC) by Folin-Ciocalteu method. Values were expressed as mean ± S.E.M (n=3), different lowercase letters indicate significant difference at $p<0.05$

Total antioxidant activity

The antioxidant activity of pomegranate was determined by using FRAP assay, which was ferric reducing antioxidant power (FRAP) that it is shown in Table 2. ANOVA test was conducted to explore the level of antioxidant activity between PS, PJ, and PSJ as measured by FRAP value. Post-hoc comparison using the Tukey HSD test indicated that the mean score TAA forPSJ (47 ± 5.5 mmol/L Fe^{+2}) was significantly ($p<0.05$) different from PS, and PJ. The antioxidant activity of Pomegranate components has been reported in the many studies [18-20]. The antioxidant capacity of pomegranate juice was shown to behigher than red wine, and green tea, based on the evaluation of the free radicals scavenging activity, and iron reducing capacity of the juice [16]. Furthermore, fruits have relationship between, antioxidant composition, and antioxidant activity, and those with high antioxidant activity generally contain more antioxidants [7]. Current study proved that pomegranatefruits were a rich possessed source of dietary antioxidants. Moreover, these nutrient characteristics are more significant in PSJ compared to other parts of pomegranate (seed or juice) separately.

Correlation between total antioxidant activity (TAA) and total phenolic content (TPC)

Data were obtained from this survey showed strong correlation between antioxidant activity and total phenolic content (Figure 1). Statistical analysis showed that there are strong positive correlation between TAA and TPC (Pearson correlation r2 = 0.91 at $p<0.05$). Phenolic contentmight act as important contributor of antioxidant activity effect in PJ.

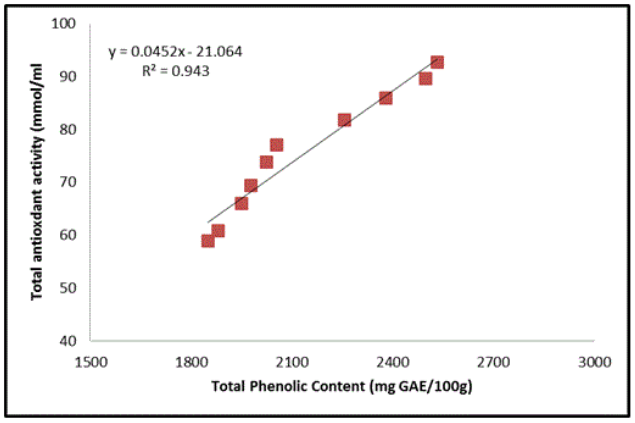

Figure 1: Linear correlation between the amount of total phenolic content and total antioxidant activity, coefficient of determination $R^2 = 0.943$, correlation is significant at 0.05

Antioxidant vitamins composition

Antioxidant vitamins composition in pomegranate was measured by HPLC and the results are summarized in Table 3. Results showed that pomegranate juice could be a complimentary source of vitamin C and A (58 ± 0.6 mg/100 g, 22.8 ± 0.7 µg/100 g). These concentrations are comparable to other fruits or vegetables such as apples, apricots, carrots, cherries, or peaches and better than plums or pears [21]. Vitamin C is the most important vitamin in fruits and more than 90% of vitamin C in human diets are supplied by fruits, and vegetables. These vitamins work both or synergistically to prevent or delay oxidative reaction that lead to degenerative disease [22]. However, this study revealed that pomegranate juice contained low level of vitamin E (0.07 ± 0.01 mg/100 g).

Parameter	Juice
Vitamin C (mg/100 g)	57.8 ± 0.59
Vitamin A (µg/100 g)	22.8 ± 0.69
Vitamin E (mg/100 g)	0.07 ± 0.01

Table 3: Vitamin composition of pomegranate juice. Value expressed as mean ± S.E.M; each value is a mean of triplicate reading

Conclusions

These findings suggested that pomegranate seed-juice has high level of phenolic content and antioxidant activity, which were positively correlated. Moreover, pomegranate juice could be acomplementary source of vitamin C and A. In addition, the result concluded that the combination of seed and juice have higher antioxidant activity than two other parts (seed and juice) separately. Therefore, pomegranate seed-juice has possessed a potential source of natural antioxidant; which can be used as treatment for chronic diseases relative to over-production of free radicals.

Acknowledgement

The authors wish to thank Department of Nutrition and Dietetics, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia for their support and facilities.

References

1. Langley P (2000) Why a pomegranate? BMJ 321: 1153-1154.
2. Lansky EP, Newman RA (2007) Punica granatum (pomegranate) and its potential for prevention and treatment of inflammation and cancer. J Ethnopharmacol 109: 177-206.
3. Li Y, Guo C, Yang J, Wei J, Xu J, et al. (2006) Evaluation of ?antioxidant properties of pomegranate peel extract in comparison with pomegranate pulp extract. Journal of Food Chemistry 96: 254-260.
4. Viuda-Martos M, Ruiz-Navajas Y, Fernández-López J, Pérez-Alvarez JA (2011) Spices as functional foods. Crit Rev Food Sci Nutr 51: 13-28.
5. Fang YZ, Yang S, Wu G (2002) Free radicals, antioxidants, and nutrition. Nutrition 18: 872-879.
6. Jacob RA (1995) The integrated antioxidant system. Journal of Nutrition Research 15: 755-766.
7. Guo CJ, Yang JJ (2001) Progress in the study of antioxidant capacity of fruits and vegetables. China Public Health Journal 17: 87- 88.
8. Cao G, Russell RM, Lischner N, Prior RL (1998) Serum antioxidant capacity is increased by consumption of strawberries, spinach, red wine or vitamin C in elderly women. J Nutr 128: 2383-2390.
9. Lee MK, Bok SH, Jeong TS, Moon SS, Lee SE, et al. (2002) Supplementation of naringenin and its synthetic derivative alters antioxidant enzyme activities of erythrocyte and liver in high cholesterol-fed rats. Bioorg Med Chem 10: 2239-2244.
10. Opara UL, Al-Ani MR (2010) Antioxidant contents of pre-packed fresh-cut versus whole fruit and vegetables. British Food Journal 112: 797-810.
11. Singleton VL, Rossi JL (1965) Calorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. The American Journal of Enology and Viticulture 16: 144-158.
12. Benzie IF, Strain JJ (1996) The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. Anal Biochem 239: 70-76.
13. Tee ES, Kuladevan R, Young SI, Khor SC, Zakiyah HO (1996) Nutrient analysis of foods. Report of the Institute Medical for Research. Kuala Lumpur: Institute Medical for Research.
14. Abdulnabi AA, Emhemed AH, Hussein GD, Biacs PA (1997) Determination of antioxidant vitamin in tomatoes. Journal of Food Chemistry 60: 207-212.
15. Ismail A, Cheah SF (2003) Determination of Vitamin C, b-carotene and Riboflavin Contents in Five Green Vegetables Organically and Conventionally Grown. Malays J Nutr 9: 31-39.
16. Gil MI, Tomás-Barberán FA, Hess-Pierce B, Holcroft DM, Kader AA (2000) Antioxidant activity of pomegranate juice and its relationship with phenolic composition and processing. J Agric Food Chem 48: 4581-4589.
17. Bravo L (1998) Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. Nutr Rev 56: 317-333.
18. Naveena BM, Sen AR, Kingsly RP, Singh DB, Kondaiah N (2008) Antioxidant activity of pomegranate rind powder extract in cooked chicken patties. International Journal of Food Science and Technology 43: 1807-1812.
19. Mousavinejad G, Emam DZ, Rezaei K, Khodaparast MH (2009) Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. Journal of Food Chemistry 115: 1274-1278.
20. Tezcan F, Gultekin OM, Diken T, Ozcelik B, Erim FB (2009) Antioxidant activity and total phenolic, organic acid and sugar content in commercial pomegranate juices. Journal of Food Chemistry 115: 873-877.
21. Davey MW, Van Montagu M, Inze D, Sanmartin M, Kanellis A, et al. (2000) Plant L-ascorbic acid: chemistry, function, metabolism, bioavailability, and effects of processing. Journal of Science Food and Agriculture 80: 825-860.
22. Elliot JG (1999) Application of antioxidant vitamins in foods and beverages. Journal of Food Technology 53: 46-48.