

Evaluation of biochemical and bioactive properties of native and imported pomegranate (*Punica granatum* L.) cultivars found in Bangladesh

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Abstract

Pomegranate (*Punica granatum* L.) is one of the oldest known and nutrient rich edible fruits. Peel and Arils of three available cultivars of this fruit in Bangladesh namely, Bangladesh pomegranate, Indian-Mridula and Egypt pomegranate were analyzed to evaluate their biochemical and bioactive properties. The ash, crude fiber, lipid, moisture, pH, titratable acidity, total soluble solid, total sugar as well as bioactive properties included DPPH radical scavenging activity, total phenolic content (TPC), total flavonoid content (TFC), total tannin content (TTC) and vitamin C were assessed in the study. The results of the analysis showed that the ash, pH, titratable acidity, moisture, total sugar ranged from 0.26-0.93%, 3.54-4.01, 1.40-1.87%, 75.43-81.20%, 9.02-10.12 g DE/100g for aril and 1-2.03%, 3.53-4.12, 1.75-1.88%, 71.69-76.65%, 21.14-29.19 g DE/100g for peel respectively. However, the seed of all cultivars contained significantly ($p < 0.05$) higher amount of lipid and crude fiber than peel especially high lipid content in the seed of Mridula (23.30 g/100g) and peel of Egypt pomegranate (11.76 g/100g) and high crude fiber content in the seed of Bangladesh pomegranate (64.96 g/100g) and peel of Mridula (15.61 g/100g). The peel showed markedly higher DPPH radical scavenging activity, TPC, TFC, TTC and vitamin C than aril. The cultivar behaved as the most influencing factor for variation among the values of individual parameters observed. The findings suggested that pomegranate peel appeared to have more potential as health supplement and rich in natural antioxidants than aril.

Keywords

Pomegranate

Biochemical

Bioactive

Vitamin C

Total phenolic content

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Introduction

Pomegranate (*Punica granatum* L.) is a fruit bearing deciduous shrub or small tree belongs to the family Punicaceae (previous Lythraceae) and native to central Asia, but since the pomegranate tree is highly adaptive to a wide range of climates and soil conditions, it is grown in many different geographical regions including the Mediterranean basin, Asia and California. The name “Pomegranate” derived from Latin pomum “apple” and granatus “seeded”. In Bangladesh, it is locally called “Dalim”. It is one of the oldest known edible fruits that has been an enormous source of food and herbal medicinal value. The fruit was seen by ancient Egyptians as a symbol of prosperity and ambition (Hassan *et al.*, 2012).

In Bangladesh it is mainly grown at homestead as ornamental tree and generally considered as an important minor fruit species (Rahman and Rahman, 2014). Recent scientific findings corroborate traditional usage of the pomegranate as a medical

remedy and indicate that pomegranate tissues of the fruit, flowers, bark and leaves contain bioactive phytochemicals that are antimicrobial, reduce blood pressure and act against serious diseases such as diabetes and cancer (Holland *et al.*, 2006).

There has been recently an explosion of interest in pomegranate fruit worldwide. Though freshly consumed, they are also used for processing: fresh juice, canned beverages, alcoholic beverages, and jellies, flavored and colored drinks. The edible parts of pomegranate fruit are named aril. They contain large amounts of organic acids, sugars, minerals, vitamins and polyphenols, showing high antioxidant activity and several medical benefits (Legua *et al.*, 2012). The fruit contains anticarcinogenic (Bell and Hawthorne, 2008), antimicrobial (Reddy *et al.*, 2007), and antiviral compounds (Kotwal, 2007).

Recent biological studies have proven that certain compounds contained in pomegranate juice, which has been shown to reduce blood pressure, are antiatherosclerotic and significantly reduce low

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density lipoprotein (LDL) oxidation (Aviram *et al.*, 2004). Pomegranate peel is generally non-edible but rich in polyphenols which exhibit various biological activities, such as eliminating free radicals, inhibiting oxidation and microbial growth and decreasing the risk of cardio and cerebrovascular diseases and some cancers. Many researchers have shown that preparations containing the pomegranate peel extract can be used to prevent and/or cure atherosclerosis, diarrhea, gastric ulcer, venereal disease and estrogen-related diseases (Opara *et al.*, 2009).

The natural process of oxidation produces energy to operate biological cycles in living organism. This oxidation in living beings causes the formation of free radicals or reactive oxygen species (ROS). The uncontrolled and excessive production of free radicals such as hydroxyl radical, hydrogen peroxide etc. cause damage to the body as well as assistive oxidative stress (Shin *et al.*, 2015). The alliance between free radicals or ROS and diseases can be clarified by the concept of "Oxidative stress". Human body has multiple mechanisms especially enzymatic and non-enzymatic antioxidant systems to protect the cellular molecules from ROS-induced damage. However, the endogenous defense may not be enough to severe or continued oxidative stress. Hence, certain amounts of exogenous antioxidants are constantly required to maintain an adequate level of antioxidants in order to balance the ROS in human body. Thus various fruits such as apple, orange, pomegranate, grape, berry etc. have received attention due to their antioxidant activity (Kar *et al.*, 2011; Rahman *et al.*, 2015).

In this 21st century, most population suffer from various chronic diseases principally cancer, heart disease and stroke which was probably a relatively rare disease in the ancient world. There are many cause behind these chronic diseases namely industrialization, environmental pollution, adulteration in food, less physical labor, imbalance diet and alcohol etc. But among all these 'The World Cancer Research Fund' has confirmed the central importance of diet as a major determinant of many forms of cancer across the globe. There are three general factors that are potentially important in any human population for causing cancer which includes presence of carcinogenic compounds in food, inadequacy of nutrient intake and inadequate intake of bioactive food components. Studies reported that there is a negative correlation found between dietary intake of phenolics and coronary heart diseases, stroke and cancer (Kar *et al.*, 2011; Zzaman *et al.*, 2013).

Previous researchers have reported the physicochemical properties and nutritional quality

attributes of pomegranate varieties grown in different parts of world such as Morocco (Legua *et al.*, 2012), Oman (Opara *et al.*, 2009), Iran (Fadavi *et al.*, 2005), Turkey (Özkan, 2005), Spain (Martinez *et al.*, 2006) and India (Khodade *et al.*, 1990). While the literature has an abundance of research reports on the biochemical attributes and health benefits of pomegranate, there seems to be lack of scientific data on pomegranate grown under Bangladeshi climatic conditions and available imported cultivars. Therefore, the current research focused on characterizing one local and two major imported cultivars to determine the biochemical and bioactive properties of the fruits.

Materials and Methods

Pomegranate samples

Fresh Pomegranate fruit of two imported cultivars were purchased from the Imported Fruit Wholesale Market, Badamtoli, Old Dhaka in Bangladesh and one cultivar of locally grown fruit was purchased from a local nursery of Chowhattah, Sylhet in Bangladesh. Thus, a total of three pomegranate cultivars were analyzed: (a) Bangladesh pomegranate, (b) Egypt pomegranate and (c) Indian-Mridula.

Chemicals

Aluminum chloride from Qualikems (India); Anthrone reagent, Auramine O dye, Phenolphthalein, Sodium hydroxide, Sodium salt of EDTA (Ethylenediaminetetraacetic acid) and Tannic acid were obtained from Loba chemie (India). Chloroform, Dextrose, Diethyl ether, Ethanol, Folin-Ciocalteu reagent, Gallic acid, Hydrochloric acid, L-Ascorbic acid, Methanol, Oxalic acid, Potassium iodate, Potassium iodide, Quercetin, Sodium acetate, Sodium carbonate, Sodium nitrite, Sulfuric acid from Merck (Germany); Sodium chloride from Uni-chem (UK) and DPPH (2,2-diphenyl-1-picrylhydrazyl) were purchased from Sigma-Aldrich (USA) and all chemicals and reagents used were analytical grade.

Preparation of sample

The fruits were cut into halves with a kitchen knife and peels and arils were manually separated. The juice of arils was extracted using a kitchen juicer (Model- MJ-M176P Juicer/ Blender, Panasonic, Osaka, Japan) and stored in individual glass bottles at refrigerator during the analysis. Separated peels and arils were directly Freeze-dried until a constant weight was reached and powdered in a grinder (Model- MJ-M176P Juicer/ Blender, Panasonic, Osaka, Japan). Seeds of the fruits were separated from the juice and

washed carefully to remove sugars and other adhering materials. Separated seeds were dried at Freeze drier until a constant weight was reached and powdered in a grinder. All powdered samples were kept in plastic boxes at room temperature until further analysis.

Preparation of aril and peel extracts

The powdered aril (1 g) and peel (2.5 g) were used to prepare methanol extracts. The extracts were procured by dissolving with 10 mL methanol: water mixture (70:30 v/v) and then mixed well using vortex for 1 min. The mixtures were then centrifuged at 3,500 rpm for 15 min. The mixtures were filtered through filter paper with Whatman No. 1 using Buchner funnel and the filtrates were considered as aril and peel extracts. Each sample was extracted in triplicate and the extracts were stored in a refrigerator for further analysis.

Biochemical properties

Biochemical properties include ash, crude fiber, lipid, moisture, pH, titratable acidity and total soluble solid were determined in triplicate in accordance with standards procedures. Ash content was determined in muffle furnace for about 550°C for 3hrs, until constant weight was obtained (AOAC, 2004). Crude fiber content in freeze dried pomegranate peel and seed powder was determined by acid digestion (H_2SO_4 0.255N) and alkali digestion (NaOH 0.313N) using (AOAC, 2005) method. Lipid content in freeze dried peel and seed powder was estimated using Chloroform: methanol solution (2:1) according to the method described by Folch *et al.* (1957). Moisture in aril and peel of pomegranate was determined by oven dehydration method at 105°C up to the constant weight. The loss in weight was moisture content (AOAC, 2004). The pH of pomegranate aril and peel was measured according to the method described by Defreitas *et al.* (1997) by directly using a digital pH meter (Model- PH500). The concentration of titratable acidity as citric acid in pomegranate aril and peel was determined by titration (Ranganna, 1986). Total soluble solid (TSS) of pomegranate aril and peel juice was measured according to (AOAC, 2005) by using a digital Hand refractometer (model - REF103bp) at room temperature with values being expressed as °Brix.

Total sugar content

The spectrophotometric method using anthrone reagent as described by Nwosuagwu *et al.* (2009) was used to determine total sugar in aril and peel. One gram of the crushed sample was dispersed after thorough shaking in 50 ml distilled water in a

conical flask and filtered through Whatman No. 1 filter paper of which 1ml of the filtrate was measured into a separate tube. 6 ml of anthrone reagent was run into the tube with the aid of a pipette under ice blocks. The tubes were later transferred into boiling water in a water bath at 100°C and allowed to boil for 10 min ensuring that the tubes were covered with aluminum foil to prevent evaporation. Then it was cooled in running water for 5 min. The absorbance of the solution was read at 620 nm using T60 UV-Visible Spectrophotometer against a reagent blank. A set of standard solutions of dextrose was read against a blank. Total sugar content was determined as g of Dextrose equivalent per 100gram using the equation obtained from a standard Dextrose calibration curve.

DPPH radical scavenging activity

The radical scavenging activity of the aril and peel of pomegranate was estimated according to the method described by Abedin *et al.* (2014). One mL of methanol extract was placed into a test tube and mixed with 4 mL of DPPH solution (0.1 mM in methanol) then shaken vigorously. The mixture was left to stand for 30 min at room temperature in dark place. After incubation, the absorbance was read at 517 nm using T60 UV-Visible Spectrophotometer. Triplicate measurements were carried out for each sample. The percentage of DPPH radical scavenging was measured from the following equation where the absorbance of DPPH solution without extract was used as control:

$$\% \text{ scavenging of DPPH} = (1 - \text{Absorbance of sample} / \text{Absorbance of control}) \times 100$$

Total phenolic content

Total phenolic content was measured based on Folin and Ciocalteu assay as reported by Amorim *et al.* (2008). An aliquot of 0.2 mL methanol extract was taken into a test tube and added with 8.3 mL distilled water. Then 0.5 mL of Folin–Ciocalteu reagent was added in the same tube and kept at room temperature for 5 minutes. After that 1 mL of 35% saturated aqueous solution of sodium carbonate (Na_2CO_3) was transferred to the tube and shaken well. The solution was incubated at room temperature for 20 min. The absorbance was read at 765 nm using T60 UV-Visible Spectrophotometer against a reagent blank. Gallic acid was used as standard in calibration curve preparation (10 – 60 mg/100 mL). Quantification (mg/g of aril and peel powder) was obtained by reporting the absorbance in the calibration curve. The results of total phenolic were expressed in terms of Gallic acid equivalent in mg/g of dry powder.

Total flavonoid content

Total flavonoid content of the peel and aril was determined according to the method described by Zzaman and Yang (2014). The methanol extract (1 mL) was added with 4 mL of distilled water into 10 mL of graduated flask. After that 0.3 mL of sodium nitrite solution (5%) was transferred to the same flask. Then 0.3 mL of aluminum chloride solution (10%) and 2 mL of sodium hydroxide (1 M) were added after 5 and 6 min respectively. The volume of the flask was made up with distilled water and the mixture was mixed well. Following this, the absorbance of the mixture was measured against blank at 510 nm using T60 UV-Visible Spectrophotometer. The total flavonoid content in each extract was measured using standard curve made using Quercetin (20 – 100 mg/L) and the results were expressed as mg Quercetin equivalent (mg QE/g) of aril and peel.

Total tannin content

Total tannin content was measured based on Folin and Ciocalteu assay as reported by Amorim *et al.* (2008). An aliquot of 0.2 mL of methanol extract was taken into a test tube and then added with 8.3 mL distill water. Then 0.5 mL of Folin–Ciocalteu reagent was added in the same tube and kept at room temperature for 5 minutes. After that 1 mL of 35% saturated aqueous solution of sodium carbonate was transferred to the tube and shaken well. The solution was incubated at room temperature for 20 min. The absorbance was read at 725 nm using T60 UV-Visible Spectrophotometer against a blank reagent. Total tannin content was determined as mg of tannic acid equivalent per gram (mg TAE/g) using the equation obtained from a standard tannic acid (20-100 mg/L) calibration curve.

Vitamin C content

The estimation of Vitamin C Content in fresh pomegranate aril and peel was carried out as per Janghel *et al.* (2012). One ml juice and 4-5g thin slice of peel were homogenized with 100-150 ml of Oxalic acid as soon as possible to avoid oxidation of ascorbic acid. Then 1ml of EDTA solution was added to it and centrifuged. Supernatant of this mixture was dilute to a suitable volume with distilled water for analysis. An aliquot of 1ml sample solution was transferred into a 25 ml graduated flask. Then 0.4 ml Potassium iodide (0.1 mol/L) - Potassium iodate (0.2 mol/L) mixture solution (5:1) was added to it followed by 1ml Hydrochloric acid (0.02 mol/L) solution was added. Then the mixture was gently shaken until appearance of yellow color that indicates liberation of iodine. After that 1ml of auramine O dye

(0.01%) solution was added to it followed by 2ml of sodium acetate (2.0 mol/L) solution and the mixture was shaken for 2 minutes. The mixture was made up 25 ml with distilled water and mix well. It was kept for 10-15 minutes for completion of reaction. The absorbance of the mixture was read at 405 nm using T60 UV-Visible Spectrophotometer against blank. L-Ascorbic acid was used as standard in calibration curve preparation and vitamin C content in samples as mg/100g was obtained by reporting the absorbance on the equation from calibration curve.

Statistical analysis

The data procured were presented as means \pm standard deviation (SD). All measurements were performed in triplicate and the data analyzed by ANOVA using SPSS 17.0. The significant difference was considered at the level of $p < 0.05$.

Results and Discussion

Biochemical properties

The biochemical properties such as ash, crude fiber, lipid, moisture, pH, titratable acidity, total soluble solid and total sugar content of three different pomegranate cultivars are presented in Table 1. The ash content of aril, peel and seed ranged from 0.26% to 0.93%, 1.0 to 2.03% and 1.58% to 2.04% respectively. Bangladesh pomegranate showed the highest ash content (0.93% and 2.03%) in its aril and peel respectively, it also showed lowest ash content (1.58%) in its seed among the three cultivars. Mridula showed the highest ash content (2.04%) in its seed and lowest ash content (1.00%) in its peel among the three cultivars. Egypt pomegranate showed the lowest ash content (0.26%) in its aril. The study (Ullah *et al.*, 2012) recorded ash content in peel powder of pomegranate as 5% which is higher than the values found in this study.

The crude fiber content in peel and seed of three different cultivars were evaluated and the results showed that the fiber content ranged from 8.88% to 11.24% in peel and 37.19% to 64.96% in seed. The highest fiber content in peel was showed by Mridula (15.61%) which was significantly different from others. The lowest fiber content in peel was showed by Bangladesh pomegranate (8.88%) where Egypt pomegranate showed 11.24% fiber. The seed of Bangladesh pomegranate showed highest fiber content (64.96%) and was significantly different from others, followed by 52.72% and 37.19% by Egypt pomegranate and Mridula respectively. The study (Ullah *et al.*, 2012) recorded fiber content in peel powder of pomegranate as 21%. The results of

Table 1. Biochemical composition of different parts of three pomegranate cultivars

Cultivar	Ash* (%)		Crude fiber* (g/100g)				Lipid* (g/100g)		Moisture* (%)		pH*		Titratable acidity* (% Citric acid)		Total soluble solid* (°Brix)		Total sugar* (g DE/100g)	
	Aril	Peel	Seed	Seed	Peel	Seed	Peel	Aril	Peel	Aril	Peel	Aril	Peel	Aril	Aril	Peel		
Bangladesh pomegranate	0.93	2.03	1.58	8.88	64.96	8.04	20.47	75.43	71.69	3.54	4.12	1.53	1.87	13.03	9.02	21.14		
	± 0.03	± 0.01	± 0.25	± 0.22	± 1.53	± 0.67	± 3.45	± 0.81	± 1.77	± 0.25	± 0.02	± 0.19	± 0.14	± 0.05	± 2.67	± 5.06		
India Mridula	0.36	1.00	2.04	15.6	37.19	7.97	23.30	81.20	74.09	3.88	3.92	1.40	1.75	14.23	13.24	29.19		
	± 0.02	± 0.01	± 0.05	± 0.48	± 1.83	± 0.89	± 3.45	± 0.64	± 0.84	± 0.05	± 0.02	± 0.10	± 0.08	± 0.23	± 2.38	± 4.85		
Egypt pomegranate	0.26	1.51	1.64	11.2	52.72	11.76	21.18	80.96	76.65	4.01	3.53	1.87	1.88	14.53	10.12	21.95		
	± 0.04	± 0.35	± 0.21	± 0.74	± 2.43	± 0.75	± 3.36	± 0.67	± 0.79	± 0.22	± 0.02	± 0.26 ^a	± 0.08	± 0.37	± 1.68	± 3.94		

*All values are reported as mean ± standard deviation, n = 3.

*Means with the same letter in column and row are not significantly different (p < 0.05).

this study is little lower than that. A study (El-Nemr *et al.*, 1990) observed that the seeds of pomegranate are a rich source of crude fiber (35.3%). This study showed higher values than that.

Lipid content on a basis of dry matter in peel ranged from 7.97 g/100g for Mridula to 11.76 g/100g for Egypt pomegranate. Bangladesh pomegranate had 8.04 g/100g lipid in peel. The highest lipid content in seed was observed in Mridula (23.30 g/100g) which was significantly different from others where 20.47 g/100g and 21.18 g/100g lipid were observed in Bangladesh pomegranate and Egypt pomegranate respectively. The study (Ullah *et al.*, 2012) evaluated pomegranate peel powder and found 9.4 % lipid content in it. Egyptian pomegranate varieties (32) were studied for their physiochemical and antioxidant properties and found oil content in seeds ranged from 4.75 - 31.21 % (Hassan *et al.*, 2012). The values of this work also lied in this range.

The moisture content in aril of Mridula was highest (81.20%) and Bangladesh pomegranate showed the lowest (75.43%). The peel of Egypt pomegranate showed the highest moisture content (76.65%) and Bangladesh pomegranate showed the lowest moisture content (71.69%). The study (Opara *et al.*, 2009) reported moisture content in peels of Indian red (Ruby) and Egypt as 75.58% and 71.70% respectively. The studies (Fadavi *et al.*, 2005) revealed that pomegranate fruit juices from different varieties of Iran contained 74.5% moisture on average. These experiments supported the results found in this study.

The pH content of pomegranate arils and peels of three different cultivars ranged from 3.54 to 4.01 and 3.53 to 4.12 respectively. Arils of Egypt pomegranate showed the highest pH content (4.01) but it was not significantly different from others. The peel of Bangladesh pomegranate showed the highest

pH Content (4.12) followed by Mridula (3.53) where peels of Egypt showed lowest pH content (3.53). The study (Opara *et al.*, 2009) reported pH content in arils of Indian red and Egypt as 3.53 and 3.32 respectively. They also reported pH content of peels of Indian red and Egypt as 4.32 and 3.60 respectively. The US Food and Drug Administration (FDA 2007) reported a pH range of 2.93–3.20 for edible portions (arils) of pomegranate in the natural state, which is slightly lower (more acidic) than the pH of arils in our present study. Several factors such as fruit variety, maturity status, and postharvest handling will contribute to the differences in pH values.

The titratable acidity ranged from 1.40% to 1.87% in aril and from 1.75% to 1.88% in peel. The highest titratable acidity was showed by Egypt pomegranate (1.87%) followed by Bangladesh (1.53%) and Mridula (1.40%). The peel's highest titratable acidity was recorded by Egypt (1.88 %) followed by Bangladesh (1.87%) and Mridula (1.75%). The study (Ullah *et al.*, 2012) recorded titratable acidity in peel powder of pomegranate as 4.86%. A study (Melgarejo *et al.*, 2011) reported titratable acidity (TA) content in juices of nine Spanish pomegranate cultivars within the range of 0.24% to 1.89%. Ozgen *et al.* (2008) studied pomegranate cultivars grown in the Mediterranean region of Turkey. The highest acidity in that study was 3.8%, compared to 1.87% in this study. The acidity content definitively plays an important role in the perception of fruit quality. Data from other collections around the world suggested that the TA content and pomegranate taste depend on climate and growing conditions (Dafny-Yalin *et al.*, 2010).

The Total Soluble Solid (TSS) content of different cultivars was Bangladesh pomegranate (13.03°Brix), Mridula (14.23°Brix) and Egypt (14.53°Brix). This

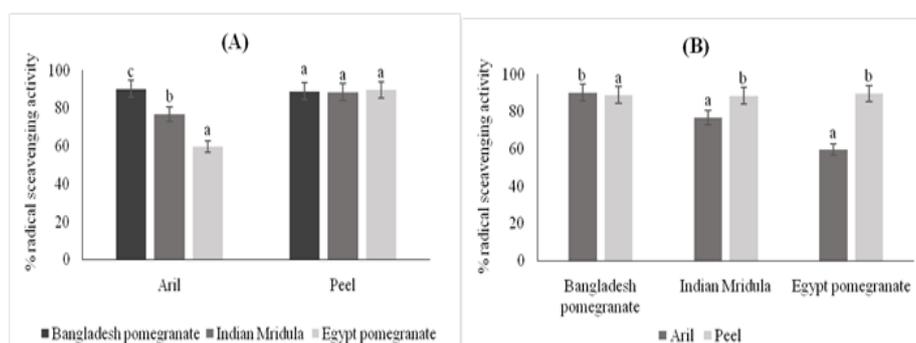


Figure 1. DPPH radical scavenging activity (mean \pm standard deviation) of peel and arils of pomegranate cultivars. For each type of fruit fraction, means followed by different letters are significantly different at $p < 0.05$. (A). Comparison among arils and peels of three pomegranate cultivars. (B). Comparison between aril and peel of individual cultivar.

TSS was in accordance with those reported from other collections grown in different regions around the world. The study described that total soluble solids ranged from 20.33 to 12.27 ($^{\circ}$ Brix) among 32 Egyptian pomegranate Accessions (Hassan *et al.*, 2012). Fadavi *et al.* (2005) reported that the pomegranate fruit juices from different varieties of Iran contained TSS within the range of 12.0 $^{\circ}$ Brix to 16.5 $^{\circ}$ Brix. TSS assessment is not only important for juice quality evaluation, but for determining also the suitability of cultivars for pomegranate wine making (Seser *et al.*, 2007).

Total sugar content of three different cultivars was ranged from 9.02% to 13.24% in aril and 21.14% to 29.19% in peel. The highest sugar content in aril was observed in Mridula (13.24%) which was statistically significant from others where lowest was observed in Bangladesh pomegranate (9.02%). The peel of Mridula showed the highest sugar content (29.19%) and was significantly different from others. Bangladesh pomegranate showed the lowest sugar content (21.14%) where Egypt pomegranate showed (21.95%) in peel. Fadavi *et al.* (2005) found total sugar content within the range of 7.20% to 12.36% in pomegranate juices grown in Iran. A study (Ullah *et al.*, 2012) recorded total sugar content in peel powder of pomegranate as 31.38%. Ozgen *et al.* (2008) reported sugar content in pomegranate juices grown in Turkey as 13.20% on average. The total sugar content found in aril and peel of three cultivars were more or less similar to the results reported by these studies.

DPPH radical scavenging activity

The DPPH radical scavenging activity is widely used method to measure antioxidant activity in food sample. The degree of discoloration indicates the scavenging potentials of the antioxidant

extract. DPPH radical scavenging activity of three pomegranate cultivars are showed in Figure 1. In this study, the differences in antioxidant activity among arils of the pomegranate cultivars were statistically significant and the values ranged from 90.13 to 59.61% but no significant difference found in peels. It can be seen that the radical scavenging activity was higher in aril of Bangladesh pomegranate and lower in Egypt pomegranate. Akhavan *et al.* (2015) evaluated juices from ten Iranian Pomegranate cultivars and found radical scavenging activity varies from 18.8% to 71.3% among cultivars which is lower than the values found in this study. Manukumar and Thribhuvan (2014) recorded 48% DPPH radical scavenging activity in pomegranate juice. Singh *et al.* (2002) found that the methanol extract of peels showed 81% antioxidant activity at DPPH model system which was close to present study.

Total phenolic content

The Folin and Ciocalteu's assay is the widely used method to determine total phenolic in plant materials. The basic mechanism is an oxidation reaction with the phenolic group where metal ion reduced forming blue color compounds. Much attention has given on polyphenol research recently because of its antioxidant activity and other beneficial effect on human health such as in the prevention and treatment of cardiovascular diseases, cancer and other antimicrobial activities (Zzaman *et al.*, 2013; Abedin *et al.*, 2015). The total phenolic content (TPC) in aril and peel powder for different pomegranate cultivars is shown Table 2. TPC significantly varied among the three evaluated cultivars in aril and peel. Bangladesh pomegranate gave the highest TPC in aril (75.36 mg GAE/g) and Mridula in peel (328 mg GAE/g) while Egypt pomegranate showed the lowest in both aril (3.77 mg GAE/g) and peel (222.73 mg

Table 2. Bioactive properties of three cultivars of pomegranate fruit fractions

Cultivar	Polyphenols mg GAE/g		Flavonoids mg QE/g		Tannins mg TAE/g	
	Aril	Peel	Aril	Peel	Aril	Peel
Bangladesh pomegranate	75.36 ± 3.05 ^{ac}	323.48 ± 1.94 ^{bs}	22.17 ± 1.17 ^{sc}	64.64 ± 2.89 ^{bc}	14.00 ± 1.20 ^{sc}	110.59 ± 1.82 ^{bc}
India Mridula	26.38 ± 3.50 ^{ab}	328.12 ± 3.04 ^{bc}	19.80 ± 1.02 ^{sb}	60.00 ± 3.99 ^{bs}	7.29 ± 0.95 ^{sb}	104.46 ± 3.08 ^{bs}
Egypt pomegranate	3.77 ± 1.81 ^{aA}	222.73 ± 2.09 ^{bA}	14.89 ± 1.55 ^{AA}	45.28 ± 4.96 ^{BA}	3.35 ± 0.74 ^{AA}	78.93 ± 3.91 ^{BA}

*All values are reported as mean ± standard deviation, n = 3.

*Means with the same letter in column and row are not significantly different (p < 0.05).

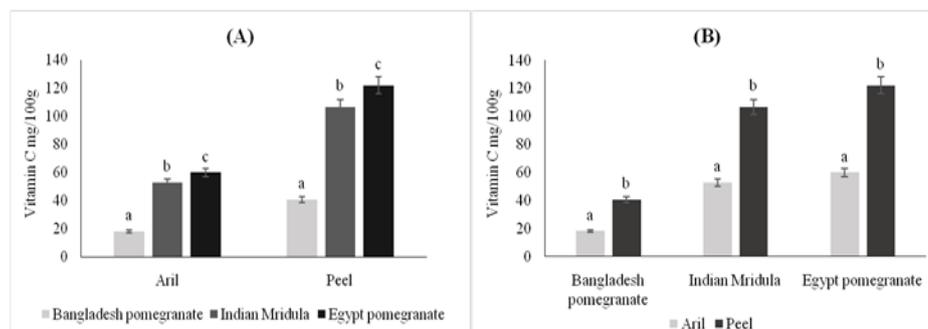


Figure 2. Vitamin C (mean ± standard deviation) of peel and arils of three pomegranate cultivars. For each type of fruit fraction, means followed by different letters are significantly different at p<0.05. (A). Comparison among arils and peels of three cultivars. (B). Comparison between aril and peel of individual cultivar.

GAE/g). Li *et al.* (2006) found that TPC in pulp extract 24.4 mg TAE/ g and in peel extract 249.4 mg TAE/ g which were close to this work. The reported levels of pomegranate TPC widely ranged from 23.7 mg/100 g up to 930.4 mg/100 g (Legua *et al.*, 2012). Furthermore, Gil *et al.* (2000) reported TPC of 2117 ± 95 and 2566 ± 131 mg/L for pomegranate juice from fresh arils and for a commercial pomegranate juice, respectively. The variation in TPC among cultivars may be due to growing seasons, agricultural practices, climate and soil condition etc.

Total flavonoid content

Flavonoids are the largest group of polyphenolic compounds found in higher plants and synthesized from the shikimic acid and malonic acid pathways. Flavonoids possess free radical scavenging activities which prevent oxidative cell damage, have anti-inflammatory, anticancer activities as well as protection against the different levels of carcinogenesis (Manukumar and Thribhuvan 2014). Total flavonoid content (TFC) in aril and peel of three pomegranate cultivars are shown in Table 2. The TFC was higher in peel extract than aril extract. There was statistical significant different among TFC of arils and peels of different cultivars which was higher in both aril (22.17 mg QE/g) and peel (64.64 mg QE/g) of Bangladesh pomegranate and also lower in both aril (14.89 mg QE/g) and peel (45.28 mg QE/g) of Egypt pomegranate. Li *et al.* (2006) also reported that flavonoids account for only a small part of total

phenolic present in the peel extract of pomegranate. They found 17.2 mg RE/g TFC in pulp extract and 59.1 mg RE/g in peel extract. This study was quite similar with values of present work. Kar *et al.* (2011) studied juices of 9 ecotypes of Tunisian pomegranate and found a range of 145 to 636 mg QE/L TFC.

Total tannin content

Tannin is one of the phenolic compounds that gives the same reaction with Folin-Ciocalteu phenol reagent. It can be also determined by the Folin and Ciocalteu's assay. As it is one of the phenolic compounds, it has same health benefits as other polyphenols. Total tannin content (TTC) of three pomegranate cultivars are shown in Table 2. There were significant differences in TTC of arils and peels of different cultivars. The TTC was higher in both aril and peel of Bangladesh pomegranate 14.00 mg TAE/g and 110.59 mg TAE/g respectively, while it was significantly lower in both aril and peel of Egypt pomegranate 3.35 mg TAE/g and 78.93 mg TAE/g respectively. These results were slightly lower than those obtained by Cam and Hisil (2010) in pomegranate peel 260.2 ± 12.6 mg TAE/g in methanolic extract and 82.6 ± 5.6 mg TAE/g in aqueous extract. TTC varies with solvent and extraction methods. Cam and Hisil (2010) reported that 262.7 ± 11.5 mg TAE/g can be recovered from dry pomegranate peel as hydrolyzable tannins using pressurized water extraction. Elfalleh *et al.* (2011) showed that juice and peel of six Tunisian

Pomegranate varieties ranges from 1.97 ± 0.48 mg TAE/ml to 3.38 ± 0.67 mg TAE/ml and 111.23 ± 10.22 mg TAE/g to 140.55 ± 7.37 mg TAE/g respectively which was very close to present work. Furthermore, Gil *et al.* (2000) reported lower total hydrolyzable tannins in juice from fresh arils (0.539 mg/mL).

Vitamin C content

The results for vitamin C content in aril and peel of pomegranate from three different cultivars are shown in Figure 2. It indicated that Egypt pomegranate showed high value for vitamin C in both fresh aril (59.91 mg/100g) and peel (122.03 mg/100g) while Bangladesh pomegranate showed lowest value both in fresh aril (18.13 mg/100g) and peel (40.71 mg/100g). Irrespective of the cultivar, the fruit peel contained significantly ($p < 0.05$) higher vitamin C than the aril. The higher vitamin C content in fruit peel was in agreement with the reports of other investigators, which showed stronger antioxidant activity in different fruit peels than aril fractions (Guo *et al.*, 2003; Li *et al.*, 2006). Opara *et al.* (2009) found that fresh aril content lower amount of vitamin C than peel which varies from approximately 55 mg/100g to 75 mg/100g in aril and 80 mg/100g to 120 mg/100g in peel. It was little similar to present work. But they found higher vitamin C content in Indian varieties than Egypt one which was totally opposite to values of present study.

Conclusion

In this study, biochemical and bioactive properties of three pomegranate cultivars namely, Bangladesh pomegranate, Indian-Mridula and Egypt pomegranate were evaluated and significant differences were observed among the cultivars. For all cultivars total acidity and sugar were higher in peel than aril while fiber and lipid content were higher in seed. All three cultivars showed outstandingly high bioactivity such as total phenolic content, total flavonoid content, total tannin content and vitamin C in peel than aril. The DPPH radical scavenging activity was very high in Bangladesh pomegranate both in aril and peel. Therefore, the combination of aril (juice) and peel had higher antioxidant activity than these parts (aril and peel) separately. This study provides important data for composition information of the fruits (e.g. vitamin C, titratable acidity, antioxidant activity etc.) highlighting that the pomegranate fruit can be a good source of nutrients which can be used to treat various chronic diseases. Besides, there are a number of criteria that should receive more attention in future study such as evaluation of antimicrobial properties

of pomegranate fruit fractions, its color component separation and use it in food products, seed oil extraction etc. Antioxidant activity of seed oil can be also estimated and finally several novel products can be developed from its aril, peel or dried seed and incorporate those in different products to increase their potentiality.

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