Natural Products Chemistry & Research

ISSN: 2329-6836

Hasan et al., Nat Prod Chem Res 2018, 6:4
DOI: 10.4172/2329-6836.1000332

Research Article Open Access

Phytochemical Investigations of Pomegranate (*Punica granatum*) Rind and Aril Extracts and their Antioxidant, Antidiabetic and Antibacterial Activity

Awatef M Hasan[1*], Ali Ali Redha[1] and Qaher Mandeel[2]

[1]*Department of Chemistry, College of Science, University of Bahrain, Sakheer-32038, Kingdom of Bahrain*

[2]*Department of Biology, College of Science, University of Bahrain, Sakheer-32038, Kingdom of Bahrain*

[*]**Corresponding author:** Awatef M Hasan, Department of Chemistry, College of Science, University of Bahrain, Sakheer-32038, Kingdom of Bahrain, Tel: 00973-17437460; E-mail: drawatefmahdi@gmail.com

Received: June 15, 2018; **Accepted:** June 20, 2018; **Published:** July 09, 2018

Abstract

Rind and aril of pomegranate (*Punica granatum*) were freeze-dried and extracted using solvents of varying polarity: petroleum ether, dichloromethane, ethyl acetate, methanol and water. Phytochemical investigations included qualitative detection of phytochemicals including phenols and tannins, flavonoids, anthocyanins, coumarins, quinones, saponins, steroids, triterpenoids, and alkaloids. Total phenolic and flavonoid content of each extract were determined quantitatively. Methanolic and aqueous pomegranate rind extracts showed highest amount of phenolic and flavonoid content. The presence of gallic acid in pomegranate rind and aril was determined by GC-MS.

Medicinal studies comprised of evaluating the antioxidant, antidiabetic and antibacterial potential of the prepared extracts. According to 1,1-diphenyl-2-picrylhydrazyl (DPPH) assay for antioxidant potential, methanolic and aqueous extracts of pomegranate rind and methanolic extract of pomegranate rind showed antioxidant activity of above 80%. Aqueous extract of pomegranate aril showed highest inhibition of alpha-amylase which was taken as antidiabetic activity according to 3,5-dinitrosalicylic acid assay (DNSA assay). Methanolic and aqueous extracts of pomegranate rind were most effective in inhibiting the growth of a number of bacteria according to the disc diffusion method.

Keywords: Pomegranate; Phenols; Flavonoids; Antioxidant; Antidiabetic; Antibacterial

Introduction

Pomegranate is an ancient fruit native to Persia which has been cultivated in the Mediterranean region through years [1,2]. This ancient fruit has been widely used in folk medicine. Pomegranate has been mentioned, in positive context, in significant religious texts such as the Bible, Quran and Buddhist texts [3,4]. Pomegranate also had a unique value in worldwide cultures. In ancient China, pomegranate seeds symbolised immortality and permanency, while Persians believed in its insuperability in battles, and Babylonians considered pomegranate seeds as a symbol of rebirth [1].

Pomegranate, which belongs to the family of Punicaceae, is botanically named *Punica granatum* [3]. Pomegranate trees are considered as shrubs or small trees which grow 5 to 10 meters high [2]. Its flowers are red and can occur either as single blossoms or clusters of several blossoms [5]. Pomegranate is a berrylike fruit with a leathery rind coating sweet juicy arils [5].

Pomegranate is rich in bioactive molecules, it has shown myriad medicinal properties due to its high phenolic content [1,6]. Pomegranate, specifically its rind, contains high amounts of hydrolysed ellagitannins, classified especially as 'pomegranate ellagitannins' which involve punicalins, punicalagins and pedunculagins [1,4]. Ellagitannins are esters that comprise of hexahydroxydiphenic acid and a polyol, which can be quinic acid or a glucose [1]. Besides ellagitannins, pomegranate rind also encompasses hydroxybenzoic acids (such as gallic acid, ellagic acid, and ellagic acid glycosides), anthocyanidins (such as cyanidin, pelargonidin, and delphinidin) and flavonoids (such as kaempferol, luteolin, and quercetin) [1,6].

Pomegranate arils are rich in anthocyanins, which are the source of the red pigment of the fruit, the dominant anthocyanins include cyanidin-3-O-glucoside, cyanidin-3,5-di-O-glucoside, delphinidin-3-O-glucoside, delphinidin-3,5-di-O-glucoside, pelargonidin-3-O-glucoside, and pelargonidin-3,5-di-O-glucoside [1,6]. In addition to anthocyanins, pomegranate arils also comprise of phenolic acids which include p-coumaric acid, chlorogenic acid, caffeic acid, ellagic acid and gallic acid [1,6].

Pomegranate rind and aril are rich in phenolic compounds. Phenolic compounds are known for their antioxidant potential, which can have radical scavenging power and/or transition metal chelating power [6]. Antioxidants quench free radicals by undergoing oxidation and acting as a reducing agent towards the radicals. Free radicals are highly reactive and unstable, with the capability of reacting with the human's DNA, proteins, carbohydrates and lipids causing cell damage [7]. Antioxidants can terminate the spontaneous radical reactions avoiding its harmful effect on the human cells, tissues and organs. Lipid peroxidation, carcinogenesis, cardiovascular diseases and nucleic acid oxidation are caused by the reaction of free reactive radicals with macromolecules [7]. Pomegranate, as a fruit rich in antioxidants, can intensively and positively contribute in humans' health.

Pomegranates strong historical, cultural and religious significances, besides to the researches determining the phytochemicals present in

pomegranate triggered analysing and evaluating pomegranate rind and aril antioxidant, antidiabetic and antibacterial activity.

Materials and Methods

Chemicals

Gallic acid, rutin, Folin-Cicocalteu reagent, 1,1-diphenyl-2-picrylhydrazyl (DPPH), 3,5-dinitrosalicylic acid (DNSA), trimethylchlorosilane and N,O-bis(trimethylsilyl) acetamide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). All other chemicals and solvents used were of analytical grade.

Instruments

Freeze dryer (Labconco, Kansas City, USA), rotary evaporator (Buchi, Rotavapor R-200, Switzerland), Ultraviolet/Visible spectrophotometer (PerkinElmer, Lambda XLS, USA) and (Orion AquaMate 800, Thermo Scientific, USA), gas chromatograph (PerkinElmer, Clarus 600, USA) and mass spectrometer (PerkinElmer, 600C, USA) located at the Department of Chemistry, University of Bahrain Laboratories.

Sample preparation

Fresh Iranian pomegranate fruit was bought from the local market and washed thoroughly using water. The rind and aril of the pomegranates were separated, washed with distilled water and dried using freeze dryer. The dried rind was powdered using commercial grinder. Samples were kept in sealed plastic bags and stored at -5°C in dark, until use.

Phytochemical screening (I): primary phytochemical screening of pomegranate rind and aril

Preparation of extracts: Aqueous extracts of pomegranate rind and aril were prepared by soaking 3.0 g of dried rind and 5.0 g of dried aril in 80 mL distilled water, separately, for 24 hours, followed by filtration. Alcoholic extracts of pomegranate rind and aril were prepared by soaking 3.0 g of dried rind and 5.0 g of dried aril in 80 mL methanol, separately, for 24 hours, followed by filtration. Chloroform extracts of pomegranate rind and aril were prepared by soaking 0.5 g of dried rind and 0.5 g of dried aril in 5 mL chloroform, separately, for 24 hours, followed by filtration.

Test for phenols and tannins: To 1 mL of aqueous extract, 1 mL of 10% aqueous ferric chloride was added. The presence of phenols is indicated by formation of blue or green colour. The presence of hydrolysable tannins is indicated by formation of dark blue colour, while the presence of condensed tannins is indicated by formation of green colour [8,9].

Test for flavonoids:

Test (a)-To 1 mL of aqueous extract, few magnesium turnings were added followed by few drops of concentrated hydrochloric acid. The presence of flavones is indicated by the formation of red colour [8].

Test (b)-To 1 mL of aqueous extract, 1 mL of 10% sodium hydroxide was added. The presence of flavonoids is indicated by the formation of yellow to orange colour [8,9].

Test (c)-To 1 mL of alcoholic extract, 1 mL of concentrated sulphuric acid was added. The presence of flavanones is confirmed by the formation of orange to crimson red [8].

Test for anthocyanins: To 1 mL of alcoholic extract, 1 mL of 10% sodium hydroxide was added and heated for 5 minutes in 100°C water bath. The presence of anthocyanins is indicated by the formation of blue colour [8,9].

Test for coumarins: To 1 mL of alcoholic extract, 1 mL of 10% sodium hydroxide was added. The presence of coumarins is indicated by the formation of yellow colour which fluorescence under ultra-violet light [8,9].

Test for quinones: To 1 mL of alcoholic extract, 1 mL of concentrated sulphuric acid was added. The presence of quinones is indicated by the formation of red colour [8].

Test for saponins: To 1 mL of aqueous extract, 5 mL of distilled water was added, the tube was vortexed for 2 minutes. The presence of saponins is indicated by lather formation [8,9].

Test for steroids: To 2 mL of chloroform extract, 2 mL of concentrated sulphuric acid was added slowly on the tube's wall. The presence of steroids is indicated by formation of two layers, an upper red layer and a lower yellowish-green layer [10].

Test for triterpenoids: To 2 mL of chloroform extract, 1 mL of acetic anhydride was added, followed by the slow addition of 1 mL of concentrated sulphuric acid. The presence of triterpenoids is indicated by formation of reddish white colour [10].

Test for alkaloids:

Test (a): Dragendroff's Test-To 2 mL of chloroform extract, few drops of Dragendroff's reagent was added. The presence of alkaloids is indicated by formation of orange colour [8]

Test (b): Mayer's Test-To 2 mL of chloroform extract, 2 mL of Mayer's reagent was added. The presence of alkaloids is indicated by formation of white precipitate [8].

Extraction of pomegranate rind and aril phytochemicals

Equal masses (30 g) of dried pomegranate rind and aril were extracted by Soxhlet extractor using 150 mL of petroleum ether, followed by dichloromethane, ethyl acetate and methanol for 2 hours each, with a gap of 24 hours between each extraction. The extracts were concentrated under reduced pressure using rotary evaporator. The pomegranate rind and aril used for extraction were allowed to dry completely at room temperature and were then soaked in 150 mL distilled water for 24 hours. The aqueous extracts were concentrated by lyophilisation using freeze dryer. All organic extract residues were then dissolved in equal volume of methanol, while the aqueous extract residue was dissolved in distilled water of the same volume of organic extract residue solutions. Extracts were stored in dark at 4°C until further use.

Phytochemical screening (II): phytochemical screening on pomegranate rind and aril extracts

Based on the results obtained in 'Primary Phytochemical Screening', the same tests, for the phytochemicals which were present in pomegranate rind and aril were repeated using the Soxhelt and aqueous extracts to trace and identify the presence of each phytochemical in each extract.

Determination of total phenolic content

To 0.5 mL of aliquot of each extract, 2.5 mL of 10-fold diluted Folin-Ciocalteu reagent was added and vortexed. After 6 minutes, 2 mL of 7.5% sodium carbonate was added and vortexed. After 30 minutes, the absorbance of each test sample was measured at 760 nm using a UV/VIS spectrophotometer (PerkinElmer, Lambda XLS, USA). Triplicate tests were done for each sample. Quartz cell was used for measuring the absorbance of samples by UV/VIS spectrophotometer. The amount of phenolic compounds in each extract were reported as gallic acid equivalents milligram per gram [11].

Determination of flavonoid content

To 0.3 mL of each extract, 1.5 mL of distilled water was added followed by the addition of 0.3 mL of 5% sodium nitrite. The solutions were vortexed and kept to stand for 6 minutes. After 6 minutes, 0.45 mL of 10% aluminium chloride (in ethanol) was added to the solutions and vortexed. After 5 minutes, 0.6 mL of 1 M sodium hydroxide was added and the solutions were vortexed. After standing for 10 minutes, the absorbance of the samples were measured at 510 nm using a UV/VIS spectrophotometer (PerkinElmer, Lambda XLS, USA). Triplicate tests were done for each sample. Quartz cell was used for measuring the absorbance of samples by UV/VIS spectrophotometer. The amount of flavonoids in each extract were reported as rutin equivalents milligram per gram [12].

Determination of gallic acid in pomegranate rind and aril by gas chromatography-mass spectrometry

Preparation of extract: An amount of 5 g of previously freeze dried rind or aril was added to 100 mL of 2:3 solution of methanol and water with 10 mL of 6 M hydrochloric acid. Nitrogen gas was bubbled into the extracts for 60 s, then sonicated for 15 minutes at 25°C. The extract solutions were then refluxed for 2 hours at controlled temperature of 80°C. Following that, the extracts were centrifuged at 5000 rpm for 30 minutes. The supernatant extracts were concentrated under reduced pressure (to approximately three-fifth of the volume). Concentrated extracts were extracted with 45 mL (3 × 15 mL) ethyl acetate. Organic layer was separated and concentrated under reduced pressure to approximately 20 mL of volume and dried using sodium sulphate [13].

Sample derivatization: The extracts were silylated to derivatize gallic acid into a volatile compound. A volume of 100 µL of each extract was derivatized, under nitrogen steam, by adding 100 µL of trimethylchlorosilane and 200 µL of N,O-bis(trimethylsilyl)acetamide followed by vortexing and incubating in 80°C water bath for 45 minutes. Screw-cap test tubes were deactivated and washed previously by soaking in aqua regia solution (prepared from hydrochloric and nitric acid with molar ratio of 3:1) for 4 hours, followed by washing with distilled water for three times, and rinsing the test tubes two times with toluene and three times with methanol. The test tubes were kept laboratory oven at 40°C until use [13].

Gas chromatography-mass spectrometry analysis

The silylated samples (1.0 µL) were injected into the GC-MS. The mass spectrometer was in electron impact mode with the mass range at m/z 50-600. A capillary column of a length of 30 m and diameter of 250 µL (PerkinElmer Brand, Elite-5MS), of 25 µm film thickness of coated material was used. The injector was set at 280°C and the detector at 290°C. GC was performed in the splitless mode with 1 min splitless-time. The temperature programme used was as follows: a 10 minutes hold at 70°C, then, from 70 to 135°C with 2°C min^{-1}, hold for 10 minutes, from 135 to 220°C with 4°C min^{-1}, hold for 10 minutes, from 220 to 270°C with 3.5°C min^{-1}, and then hold for 20 minutes. The rate flow of carrier gas (helium) was maintained at 1.0 mL min^{-1}. Identification of gallic acid was achieved by finding the silylated molecule of gallic acid by mass [13].

Antioxidant activity of pomegranate rind and aril extracts

A volume of 50 µL of each sample extract (extracts prepared in Part 2.5) were added to 2.95 mL DPPH solution (4.5 mg DPPH in 100 mL methanol). After 30 minutes, the absorbance (A) of each sample was measured at 517 nm using a UV/VIS spectrophotometer (Lambda XLS, PerkinElmer, UV Spectrometer). Triplicate tests were done for each sample. The percentage of scavenging activity of DPPH radical was calculated by the following equation [14].

Antidiabetic activity of pomegranate rind and aril extracts

Constructing maltose standard curve: To construct a standard curve of maltose, a stock solution of 2 mg/mL maltose (in distilled water) was prepared from which 5 aliquots of 0.2, 0.4, 0.6, 0.8 and 1.0 mL were added to test tubes and completed to 6 mL with distilled water respectively. To each maltose solution, 1 mL of DNSA reagent was added and immediately heated in boiling water for 5 minutes. The absorbance of each solution was measured at 540 nm using a UV/VIS spectrophotometer (Orion AquaMate 800, Thermo Scientific, USA) [15].

α-Amylase activity under optimum conditions: he α-amylase activity was determined under optimum conditions. A volume of 2.5 mL of 0.02 M phosphate buffer (pH 6.66 containing 6 mM sodium chloride), 0.5 mL of 1.0% starch solution and 0.1 mL of methanol were added into 4 test-tubes, vortexed and incubated in a 37°C water bath for 15 minutes. Then, 0.05 mL of α-amylase solution was added into each test-tube. To the first test-tube, 0.5 mL of 2 M sodium hydroxide and 1.0 mL of DNSA reagent were immediately added, vortexed and kept in boiling water for 5 minutes. After 5 minutes, the absorbance was measured by spectrophotometer at 540 nm, this was considered as base value for the concentration of maltose at 0 minute. Other three test tubes remained incubated in the water bath for 10 minutes, following that 0.5 mL of 2 M sodium hydroxide and 1.0 mL of DNSA reagent were added, vortexed and kept in boiling water for 5 minutes. After 5 minutes, the absorbance was measured by UV/VIS spectrophotometer (Orion AquaMate 800, Thermo Scientific, USA) at 540 nm, this was considered as concentration of maltose after 10 minutes in the presence of enzyme with no inhibition. Blank sample was prepared following the same procedure, however α-amylase was not added to the blank [15].

Exact absorbance of the reaction mixture was measured by subtracting the absorbance of the reaction mixture at 0 minute from that at 10 minutes, to determine maltose concentration. Enzyme activity was calculated by dividing the determined concentration of maltose by duration of reaction.

α-Amylase inhibition by acarbose (positive control) and pomegranate rind and aril extracts: The same procedure described in 2.10.2 was followed to evaluated α-amylase activity with exception of adding 0.1 mL of acarbose solution [positive control] (50 mg Glucobay® acarbose tablet solution was prepared in 100 mL methanol) or

pomegranate extracts instead of 0.1 mL of methanol at the first stage.

Percentage of relative enzyme activity was calculated by dividing the enzyme activity with the presence of extract(s) with that under optimum conditions, multiplied by hundred percent. Percentage inhibition was calculated by subtracting the relative enzyme activity from 100%.

Antibacterial activity of pomegranate rind and aril extracts

Agar well diffusion method was used for the antibacterial assay [16]. The bacteria chosen for the antibacterial assay were obtained from American Type Culture Collection (ATCC) as lyophilisates in ampoules which are: *Bacillus subtilis* (ATCC 6051) and *Staphylococcus aureus* (ATCC 6538) [as gram positive species], and *Escherichia coli* (ATCC 11775) and *Pseudomonas aeruginosa* (ATCC 10145) [as gram negative species]. The cultures of the chosen bacteria were grown on 25 mL nutrient agar (Oxoid) plates and incubated for 24 hours at 37°C. To ensure the highest bacterial energy potential, new cultures of lyophilised ampoules were used [16].

A volume of 1 mL of pomegranate rind and aril extracts (dissolved in methanol) were kept to dry in a desiccator under vaccum to concentrate, the aqueous extracts were lyophilised to concentrate. After drying the solvents, the residues were dissolved in 1 mL of dimethyl sulfoxide.

Overnight nutrient broth cultures of bacteria (10^5 per 0.5 mL) were aseptically mixed with 20 mL of nutrient agar cooled down to 50°C in plastic Petri dishes. On the next day, the plates were removed and kept at room temperature for 10 minutes, following that, wells of 7 mm diameter were made in the solidified agar medium using a sterilised steel cork borer. A volume of 50 µL of each extract was slowly loaded into the wells using micropipettes of sterilised tips, the plates were then incubated for 24 hours at 37°C. After 24 hours, the diameter of inhibition zone surrounding the agar well for each plate was measured. Triplicate tests were done for each extract [16].

Results and Discussion

Sample preparation

Fresh pomegranate rind and aril were separated and washed carefully by distilled water to remove any stains from their surface. The rind and aril were then dried by freeze drying to conserve the phytochemical component present in them as water is sublimed from solid state directly to gaseous state without going through the liquid state at which liquid water acts as a medium for many chemical reactions which if occur, may affect the nature of the phytochemicals. According to Calín-Sánchez et al. freeze drying is one of the drying treatments which conserve the chemical composition, antioxidant capacity and sensory quality of pomegranate rind and aril [17]. Due to the presence of a wide range of phytochemicals in pomegranate rind and aril, it can be predicted that they have significant medicinal values.

Phytochemical screening (I): phytochemical screening of pomegranate rind and aril

Based on primary phytochemical screening done, pomegranate rind and aril contained phenols, tannins, flavonoids, quinones, coumarins, steroids, triterpenoids and alkaloids. Anthocyanins were only present in pomegranate aril (Table 1).

Phytochemicals Tested		Rind	Aril
Phenols		+	+
Tannins		+†	+‡
Flavonoids	Test (a): Flavones	+	+
	Test (b): Flavonoids	+	+
	Test (c): Flavanones	+	+
Anthocyanins		-	+
Coumarins		+	+
Quinones		+	+
Anthraquionones		-	-
Saponins		-	-
Steroids		+	+
Triterpenoids		+	+
Alkaloids	Dragendroff's Test	+	+
	Mayer's Test	+	+
Key:(+)=Present, (-)=Absent, (+†)=Presence of Hydrolysable Tannins and (+‡)=Presence of Condensed Tannins			

Table 1: Primary phytochemical screening of pomegranate rind and aril.

Extraction of pomegranate rind and aril phytochemicals

A range of solvents with increasing in polarity (petroleum ether, dichloromethane, ethyl acetate, methanol and water) were used to extract the components of dry pomegranate rind and aril. The extraction was associated with separation of components based on their polarity and solvent. The least polar were extracted in the least polar solvent (petroleum ether) with gradual increase in polarity up to water, which extracted the most polar components of the dry material.

Phytochemical screening (II): phytochemical screening of pomegranate rind and aril extracts

Extraction by Soxhelt led to separation of the components of pomegranate rind and aril in different solvents. The separation of phytochemicals by solvents, were traced by conducting the same phytochemical tests to detect their presence in the extracts.

According to the test for tannins of pomegranate rind extract, the rind contains hydrolysable tannins and condensed tannins, this was not clear from the primary phytochemical screening done. Although in the primary phytochemical screening anthocyanins were detected, however none of the extracts (A-E) contained anthocyanins. The anthocyanins may have decomposed by Soxhlet extraction (heating of solvent was involved) due to their labile characteristic (Table 2).

Phytochemical screening (II) was helpful in determining the presence of both hydrolysable tannins and condensed tannins in pomegranate rind which was not shown by primary phytochemical screening. Anthocyanins were absent in phytochemical screening (II). Decomposition of anthocyanins by the heat involved in Soxhelt extraction is possible (Table 3).

Total phenolic content of pomegranate rind and aril extracts

Total phenolic content of pomegranate rind and aril extracts was determined by Folin-Ciocalteu method, a colourimetric method, based on oxidation-reduction reaction of Folin-Cicocalteu reagent with phenolic compounds (Graph 1).

According to the overall results obtained, the total phenolic content of pomegranate rind was higher than that of pomegranate aril. The most significant results obtained were of the methanolic and aqueous extracts of pomegranate rind which contained 44.82 and 20.78 mg GAE/g respectively. As expected, according to the background research done, the pomegranate rind is rich in ellagitannins (phenolic compounds) and thus showed a high phenolic content. The polar property of the ellagitannins lead them to be extracted in methanol and water, two polar solvents (Table 4).

Phytochemicals Tested		A	B	C	D	E
Phenols		+	+	+	+	+
Tannins		+‡	+‡	+‡	+†	+†
Flavonoids	Test (a): Flavones	-	-	-	+	+
	Test (b): Flavonoids	+	-	+	+	+
	Test (c): Flavanones	-	+	-	+	+
Coumarins		+	-	+	+	+
Quinones		-	-	-	+	-
Steroids		-	-	-	+	-
Triterpenoids		-	-	-	+	-
Alkaloids	Dragendroff's Test	-	+	-	-	-
	Mayer's Test	-	+	-	-	-
Key for extracts: A=Petroleum Ether, B=Dicholoromethane, C=Ethyl acetate, D=Methanol and E=Water						
Key: (+)=Present, (-)=Absent, (+†)=Presence of Hydrolysable Tannins and (+‡)=Presence of Condensed Tannins.						

Table 2: Phytochemical screening (II) of pomegranate rind extracts.

Phytochemicals Tested		A	B	C	D	E
Phenols		-	-	-	+	+
Tannins		-	-	-	+‡	+‡
Flavonoids	Test (a): Flavones	-	-	-	+	+
	Test (b): Flavonoids	+	-	+	+	+
	Test (c): Flavanones	-	-	-	+	+
Anthocyanins		-	-	-	-	-
Coumarins		+	-	+	+	+
Quinones		-	-	-	+	+
Steroids		-	-	-	+	+
Triterpenoids		-	-	-	+	+
Alkaloids	Dragendroff's Test	-	-	-	+	-
	Mayer's Test	-	-	-	+	-
Key for extracts: A=Petroleum Ether, B=Dicholoromethane, C=Ethyl acetate, D=Methanol and E=Water						
Key: (+)=Present, (-)=Absent, (+†)=Presence of Hydrolysable Tannins and (+‡)=Presence of Condensed Tannins.						

Table 3: Phytochemical screening (II) of pomegranate aril extracts.

Extracts	Average Total Phenolic Content of Pomegranate Rind (mg GAE/g)	Average Total Phenolic Content of Pomegranate Aril (mg GAE/g)
Petroleum ether	0.75 ± 0.08	0.45 ± 0.07
Dichloromethane	0.22 ± 0.00	0.24 ± 0.09
Ethyl acetate	0.34 ± 0.03	0.56 ± 0.16
Methanol	44.82 ± 1.25	1.35 ± 0.16
Water	20.78 ± 3.49	1.53 ± 0.14

Table 4: Total Phenolic Content of Pomegranate Rind and Aril Extracts as Gallic Acid Equivalents (GAE).

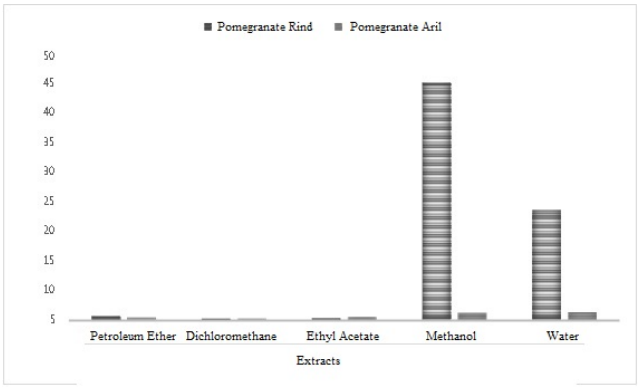
Flavonoid content of pomegranate rind and aril extracts

Total flavonoid content of pomegranate rind and aril extracts was estimated by aluminium chloride colorimetric method as described by Hajimahmoodi et al. [12]. The principle of this colorimetric method is based on the formation of acid stable aluminium chloride complexes with hydroxyl groups and keto group of flavanols or flavones [18].

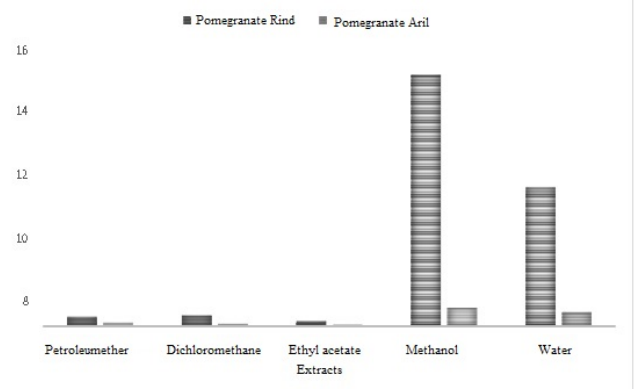
Highest amount of flavonoids were present in methanol extracts of pomegranate rind and aril, and in the aqueous extract of pomegranate rind. Overall, the flavonoid content of pomegranate rind was higher than that of pomegranate aril (Table 5). The results also suggest that pomegranate flavonoids are extracted efficiently with methanol and water due to their polarity (Graph 2).

Extracts	Flavonoid Content of Pomegranate Rind (mg RE/g)	Flavonoid Content of Pomegranate Aril (mg RE/g)
Petroleum ether	0.50 ± 0.04	0.22 ± 0.01
Dichloromethane	0.59 ± 0.06	0.15 ± 0.03
Ethyl acetate	0.23 ± 0.04	0.10 ± 0.02
Methanol	14.58 ± 1.12	1.09 ± 0.01
Water	8.35 ± 0.71	0.81 ± 0.03

Table 5: Flavonoid content of Pomegranate rind and Aril extracts as rutin equivalents (RE).



Graph 1: Total phenolic content of Pomegranate rind and Aril extracts as gallic acid equivalents (GAE).



Graph 2: Flavonoid content of pomegranate rind and aril extracts as rutin equivalents (RE).

Detection of gallic acid in pomegranate rind and aril

Gallic acid is the building unit of several ellagitannins including punicalin, punicalagin and pedunculagin. The presence of gallic acid in pomegranate rind and aril was confirmed by detecting the silylated gallic acid based on its molar mass (458.92 g mol^{-1}) using GC-MS Silylated gallic acid from rind extract was detected at 65.072 ± 0.035 min, while that from aril extract detected at 65.025 ± 0.000 min (Figures 1 and 2).

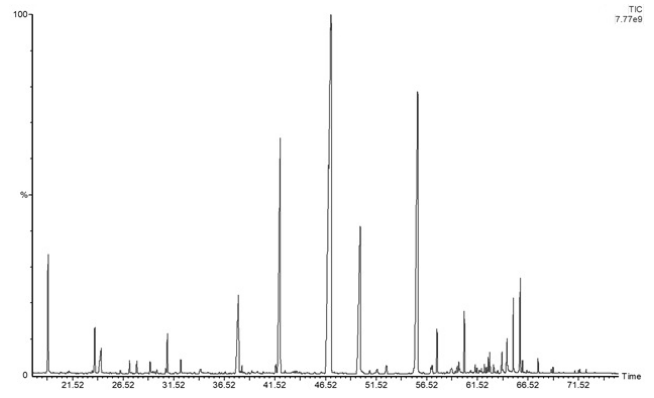


Figure 1: GC Chromatograph of pomegranate rind extract.

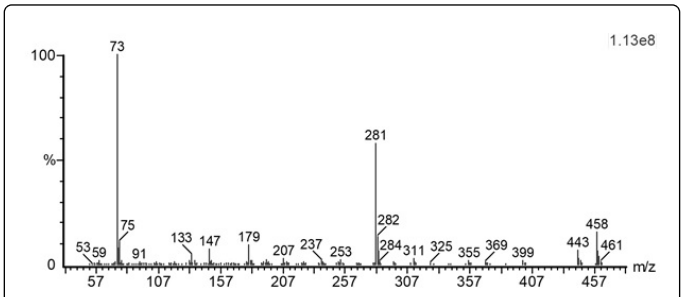


Figure 2: Mass Spectra of Silylated Gallic Acid.

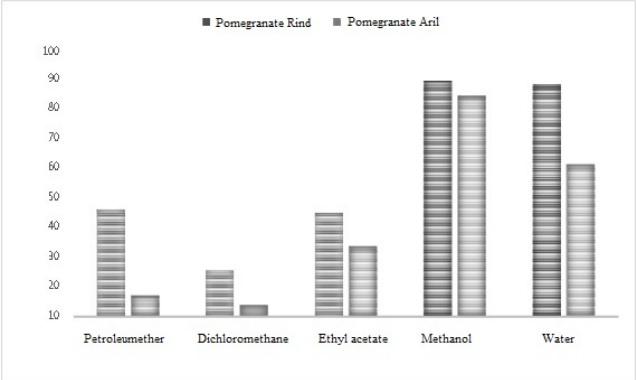
Antioxidant activity of pomegranate rind and aril extracts

DPPH assay, as developed by Blois, was used to measure the antioxidant power of pomegranate extracts. DPPH solution is purple in colour, when DPPH is stabilised by accepting an electron from phenolic compounds, it becomes oxidised and loses its purple colour, turning into yellow (Table 6).

Methanol extract, followed by water extract, of pomegranate rind showed the highest and most significant radical scavenging power. Those two extracts have previously shown the highest amount of phenolic and flavonoid content. Thus, as expected both extracts had a valuable antioxidant power towards DPPH radical. Overall, the pomegranate rind extracts had higher antioxidant activity compared to the aril extracts (Graph 3).

Extracts	DPPH Radical Scavenging Activity of Pomegranate Rind (%)	DPPH Radical Scavenging Activity of Pomegranate Aril (%)
Petroleum ether	40.16 ± 2.02	8.08 ± 5.70
Dichloromethane	17.43 ± 0.64	4.42 ± 0.73
Ethyl acetate	38.91 ± 1.13	26.49 ± 8.35
Methanol	88.57 ± 2.76	83.24 ± 2.40
Water	87.19 ± 1.47	57.41 ± 0.85

Table 6: Percentage DPPH Radical Scavenging Activity of Pomegranate Rind and Aril Extracts.



Graph 3: Percentage DPPH Radical Scavenging Activity of Pomegranate Rind and Aril Extracts.

Antidiabetic activity of pomegranate rind and aril extracts

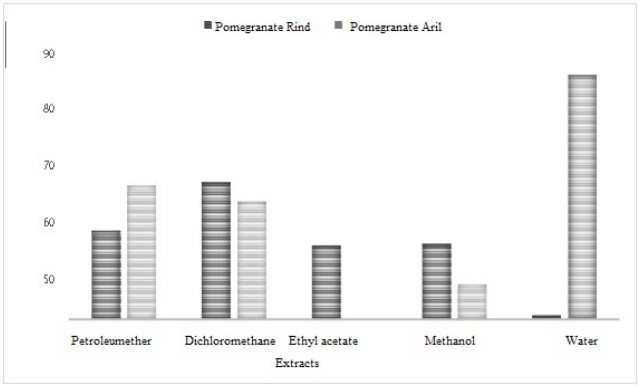
Antidiabetic activity of pomegranate rind and aril extracts was determined by 3,5- dinitrosalicylic acid assay. The hydrolysis of starch to smaller oligosaccharides molecules such as maltose, maltotriose and other α-(1-6) and α-(1-4) oligoglucans requires the aid of α-amylase to catalyse the initial step of the hydrolysis reaction. The oligosaccharides then require α- glucosidases to further degrade them into glucose molecules. Inhibiting α-amylase, which is the first enzyme required in starch hydrolysis, can delay the digestion of carbohydrates taken in by man, reducing the speed of glucose release in blood. Zinjarde et al. found that inhibition of α- amylase reduces post-prandial serum glucose levels. The positive control, acarbose, was able to inhibit α-amylase activity by 64.07 ± 1.43%.

Based on the experimental work done, average enzyme activity was 0.033 ± 0.001 mg/mL min. According to the results, the aqueous aril extract showed the highest inhibition of α-amylase (83.01 ± 5.74%) compared to all the other extracts. The petroleum ether and dichloromethane extracts of pomegranate rind and aril showed similar inhibition rate, 30.13 ± 18.20, 46.64 ± 10.90, 45.17 ± 2.06 and 39.78 ± 9.63% respectively. The ethyl acetate and methanolic extracts of pomegranate rind also showed a similar effect on α-amylase with 25.04 ± 6.12 and 25.70 ± 4.79% inhibition respectively. The lowest enzyme inhibition was by methanolic extract of pomegranate aril followed by the aqueous extracts of pomegranate rind, which were 11.71 ± 12.79 and 1.25 ± 15.34% respectively (Table 7).

The results suggest that pomegranate aril extracts were more effective in inhibiting of α- amylase, which is practically beneficial for humans who usually consume pomegranate aril. Nevertheless, the capability of the methanolic and aqueous extracts could have been higher in inhibiting the enzyme if the extracts were sugar-free. The sugars present in the extracts acted as interferences for this assay (Graph 4).

Extracts	Percentage Inhibition of α-Amylase by Pomegranate Rind (%)	Percentage Inhibition of α-Amylase by Pomegranate Aril (%)
Petroleum ether	30.13 ± 18.20	45.17 ± 2.06
Dichloromethane	46.64 ± 10.90	39.78 ± 9.63
Ethyl acetate	25.04 ± 6.12	0.00 ± 6.73
Methanol	25.70 ± 4.79	11.71 ± 12.79
Water	1.25 ± 5.34	83.01 ± 5.74

Table 7: Percentage Inhibition of α-Amylase Activity by Pomegranate Rind and Aril Extracts.



Graph 4: Percentage Inhibition of α-Amylase Activity by Pomegranate Rind and Aril Extracts.

Antibacterial activity of pomegranate rind and aril extracts

Antibacterial activity of pomegranate rind and aril extracts was determined by disc diffusion method. *E. coli* growth was only inhibited by the methanolic and aqueous extracts of pomegranate rind and the aqueous extract of pomegranate aril. The methanolic extract of pomegranate rind was the most effective in inhibiting the growth of *E. coli* by 24.00 ± 1.00 mm. Nevertheless, the pomegranate rind aqueous extract was also significantly effective in inhibiting its growth by 19.67 ± 0.58 mm. The growth of *P. aeruginosa* was again only inhibited by the methanolic and aqueous extracts of pomegranate rind, while the pomegranate aril extracts did not show inhibition against this bacterium. *S. aureus* growth was inhibited by three of pomegranate rind extracts: methanol, water and ethyl acetate extracts, with the highest inhibition by methanolic extract. This was the only bacterium affected by the ethyl acetate extract of pomegranate rind. Also, three extracts of pomegranate aril inhibited the growth of *S. aureus*: water, methanol and dichloromethane extracts, with the highest inhibition by methanolic extract. This was the only bacterium affected by the dichloromethane extract of pomegranate aril. The growth of *B. subtilis* was inhibited by the methanolic and aqueous extracts of pomegranate rind only, the methanolic and the methanolic, aqueous and petroleum ether extracts of pomegranate rind showed antibacterial activity against this bacterium (the methanolic extracts being the highest in each case). This was the only bacterium affected by the petroleum ether extract of pomegranate aril (Table 8).

Overall, the most effective extract in inhibiting the growth of the chosen bacteria was the methanolic extract of pomegranate rind. According to the phytochemical screening (II), this extract comprises of phenols, tannins, flavonoids (including flavones and flavanones), coumarins, quinones, steroids and triterpenoids. Moreover, this extract showed the highest amount of phenolic and flavonoid content in the quantitative assays. Nevertheless, the aqueous extract of pomegranate rind also had significant antibacterial activity. On the other hand, the petroleum ether and dichloromethane extracts of pomegranate rind and the ethyl acetate extract of pomegranate aril did not show any antibacterial activity against the chosen bacteria. Generally, the antibacterial potential of pomegranate rind seems to be stronger than that of pomegranate aril. The results also suggest that the bacterial inhibition is due to polar components of pomegranate rind and aril (Table 9).

Extracts	Mean E. coli Inhibition Zone Diameter (mm)	Mean P. aeruginosa Inhibition Zone Diameter (mm)	Mean S. aureus Inhibition Zone Diameter (mm)	Mean B. subtilis Inhibition Zone Diameter (mm)
Petroleum ether	NI	NI	NI	NI
Dichloromethane	NI	NI	NI	NI
Ethyl acetate	NI	NI	9.00 ± 1.00	NI
Methanol	24.00 ± 1.00	22.33 ± 0.58	24.67 ± 0.58	27.67 ± 2.52
Water	19.67 ± 0.58	20.33 ± 1.53	21.00 ± 1.73	19.67 ± 2.08
NI-No Inhibition.				

Table 8: Mean Inhibition Zone Diameter of Bacteria by Pomegranate Rind Extracts.

Extracts	Mean E. coli Inhibition Zone Diameter (mm)	Mean P. aeruginosa Inhibition Zone Diameter (mm)	Mean S. aureus Inhibition Zone Diameter (mm)	Mean B. subtilis Inhibition Zone Diameter (mm)
Petroleum ether	NI	NI	NI	12.33 ± 0.58
Dichloromethane	NI	NI	7.33 ± 0.58	NI
Ethyl acetate	NI	NI	NI	NI
Methanol	NI	NI	10.67 ± 0.58	18.33 ± 0.58
Water	13.67 ± 1.53	NI	13.00 ± 1.00	14.67 ± 0.58
NI-No Inhibition.				

Table 9: Mean Inhibition Zone Diameter of Bacteria by Pomegranate Aril Extracts.

Conclusion

The present study provides more evidence on the importance and value of pomegranate fruit, especially pomegranate's rind which is usually considered as a waste product. According to the phytochemical screening done both pomegranate rind and aril contain phenols and tannins, flavonoids, quionones, coumarins, steroids, triterpenoids and alkaloids. Anthocyanins were only present in pomegranate aril. The quantitative assays showed that the total content of phenolic compounds and flavonoids in pomegranate rind is higher than that of the aril. Antioxidant activity of polar extracts of pomegranate rind and aril were the most significant. The aqueous extract of pomegranate aril showed the highest antidiabetic activity according to the 3,5-dinitrosalicylic acid assay. The methanolic and aqueous extracts of pomegranate rind were the most effective in inhibiting the growth of a number of bacteria according to the disc diffusion method. Further studies could be conducted on the antibacterial capacity of pomegranate rind components, which may led to the discovery of new antibacterial agents.

Acknowledgment

This work was conducted at University of Bahrain. All chemicals and instruments were provided for use by University of Bahrain.

References

1. Viuda-Martos M, Fernández-López J, Pérez-Alvarez J (2010) Pomegranate and its Many Functional Components as Related to Human Health: A Review. Comprehensive Reviews in Food Science and Food Safety 9: 635-654.
2. Silva J, Rana T, Narzary D, Verma N, Meshram D, et al. (2013) Pomegranate biology and biotechnology: A review. Science Horticulturae 160: 85-107.
3. Chandra R, Babu K, Jadhav V, Silva J (2010) Origin, History and Domestication of Pomegranate. Fruit, Vegetable and Cereal Science and Biotechnology, pp: 1-6.
4. Baliga M, Shivashankara A, Shetty C, Thilakchand K, Periera N, et al. (2013) Antidiabetic Effects of Punica granatum L (Pomegranate): A Review. Bioactive Food as Dietary Inventions for Diabetes, pp: 355-369.
5. Stover E, Mercure E (2007) The Pomegranate: A New Look at the Fruit of Paradise. HortScience 42: 1088-1092.
6. Akhtar S, Ismail T, Fraternale D, Sestili P (2015) Pomegranate peel and peel extracts: Chemistry and food features. Food Chemistry 174: 417-425.
7. Lobo V, Patil A, Phatak A, Chandra N (2010) Free radicals, antioxidants and functional foods: Impact on human health. Pharmacognosy Reviews 4: 118-126.
8. Firdouse S, Alam P (2011) Phytochemical investigation of extract of Amorphophallus campanulatus tubers. International Journal of Phytomedicine 3: 32-35.
9. Sangeetha R, Jayaprakash A (2015) Phytochemical Screening of Punica granatum Linn. Peel Extracts. Journal of Academic and Industrial Research 4: 160-162.
10. Hossain MA, Al-Raqmi KAS, Al-Mijizy ZH, Weli AM, Al-Riyami Q (2013) Study of total phenol, flavonoids contents and phytochemical screening of various leaves crude extracts of locally grown Thymus vulgaris. Asian Pacific Journal of Tropical Biomedicine 3: 705-710.
11. 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. Food Chemistry 96: 254-260.
12. Hajimahmoodi M, Moghaddam G, Ranjbar AM, Khazani H, Sadeghi N, et al. (2013) Total Phenolic, Flavonoids, Tannin Content and Antioxidant Power of Some Iranian Pomegranate Flower Cultivars (Punica granatum L.). American Journal of Plant Sciences 4: 1815-1820.
13. Proestos C, Sereli D, Komaitis M (2006) Determination of phenolic compounds in aromatic plants by RP-HPLC and GC-MS. Food Chemistry 95: 44-52.
14. Basiri S, Shekarforoush SS, Aminlari M, Akbari S (2015) The effect of pomegranate peel extract (PPE) on the polyphenol oxidase (PPO) and the quality of Pacific white shrimp (Litopenaeus vannamei) during refrigerated storage. Food Science and Technology 60: 1025-1033.
15. Sudha P, Zinjarde SS, Bhargava SY, Kumar AR (2011) Potent α-amylase inhibitory activity of Indian Ayurvedic medicinal plants. BMC Complementary and Alternative Medicine 11: 5.
16. Mandeel Q, Hasan A, Al-Nafea H, Abbas A (2010) Antibacterial Activity of Extracts from Selected Marine Algae in Bahrain. Arab Gulf Journal of Scientific Research 28: 147-162.
17. Calín-Sánchez Á, Figiel A, Hernández F, Melgarejo P, Lech K, et al. (2013) Chemical Composition, Antioxidant Capacity and Sensory Quality of Pomegranate (Punica granatum L.) Arils and Rind as Affected by Drying Method. Food Bioprocess Technol 6: 1644-1654.
18. Bhaigyabati T, Devi PG, Bag GC (2014) Total Flavonoid Content and Antioxidant Activity of Aqueous Rhizome Extract of Three Hedychium Species of Manipur Valley. Research Journal of Pharmaceutical, Biological and Chemical Sciences 5: 970-976.