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Original Article

Chemical Composition and Antioxidant Activity of Lebanese *Punica Granatum Peels*

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Objective: The use of traditional medicines and medicinal plants in most developing countries as a normative basis for the maintenance of good health has been widely observed. The present study was undertaken first, to determine the chemical composition, the total phenolic and total flavonoids content of the Lebanese *Punica granatum* peels; second to quantify some of its bioactive contents and finally to evaluate its antioxidant capacity.
Methods: Chemical composition was determined using a standard phytochemical screening. The antioxidant capacity has been evaluated using the DPPH *in vitro* test. Total phenolic and total flavonoids contents have been determined using the Folin-Ciocalteau reagent and the aluminum chloride method respectively.
Results: The obtained results of the phytochemical screening indicated the presence of various secondary metabolites such phenols, flavonoids among others. Also, methanol extract from peels of *P. granatum* has the highest phenolic content (185.43 mg/ml) than that of ethanol (80.01 mg/ml) and water (14.91 mg/ml) extracts. Also, these peels were rich in flavonoids (3.86 mg/ml). On the other hand, ethanol extract has exerted a higher antioxidant capacity reaching 95 % at the concentration 50 µg/ml than that of methanol extract (45 %) at the same concentration.
Conclusion: *Punica granatum*'s peels are considered as good source of different antioxidant and bioactive compounds.

Keywords: *Punica granatum* peels, antioxidant capacity, phytochemical screening

1. INTRODUCTION

Medicinal plants play an important role in the development of potent therapeutic agents. Natural products from plant, animal and minerals have been the basis of the treatment of human disease. Today about 80 % of people in developing countries still relay on traditional medicine based largely on species of plants for their primary care. Herbal medicines are

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currently in demand and their popularity is increasing day by day [1].

Punica granatum belongs to the family of Punicaceae. It is commonly known as pomegranate, grenade, granats and punica apple. Pomegranate fruits comprise the not edible exocarp and mesocarp (peel), and also the edible endocarp which contains the seeds, forming the arils. All of these contain interesting bioactive molecules such as anthocyanins in the arils, hydrolysable tannins in the peel and punicic acid in the seeds, hence pomegranate whole fruit extracts are also very interesting as dietary supplements and nutraceuticals [2,3].

P. granatum has been used extensively as a traditional medicine in many countries for the treatment of dysentery, diarrhea, helminthiasis, acidosis, hemorrhage and respiratory pathologies. In addition, it is reported to have antioxidant anti-atherosclerotic, antibacterial, and antiviral properties [4].

Pomegranate peels are characterized by an interior network of membranes comprising almost 26-30% of total fruit weight and are characterized by substantial amounts of phenolic compounds, including flavonoids (anthocyanins, catechins and other complex flavonoids) and hydrolysable tannins (punicalin, pedunculagin, punicalagin, gallic and ellagic acid) [4].

In the literature, there is no study on the phytochemical screening, the chemical composition and the antioxidant activity of *P. granatum* peels grown in Lebanon. For that, in the current study, for the first time, we determined the chemical composition for five extracts from Lebanese *P. granatum* peels, and we evaluated their antioxidant capacity using an *in vitro* test, 2,2- diphenyl-1-picrylhydrazyl (DPPH).

2. MATERIAL AND METHODS

Fruit collection and powder preparation

Fresh fruits were gathered from south of Lebanon at 300 meters of altitude. They were well cleaned and washed with water and then the peels were dried in the shade at room temperature, away from the sunlight. During the drying process, the peels were turned over repeatedly for homogeneous drying. After that, the dried peels were grinded by a grinder to obtain powder form, then preserved in a container away from light, heat and moisture for later use.

Preparation of crude extracts by maceration

A quantity of 15 g of *P. granatum* peels powder was placed in a beaker with 150 mL of the selected solvent (hexane, dichloromethane, water, ethanol and methanol). The solution was macerated under room temperature for 24 h with agitation. After the maceration period, the solution was filtrated and then concentrated by a rotary evaporator. The obtained extracts were stored in the refrigerator for later analysis [5].

Phytochemical Screening

In order to study the chemical composition of the different extracts, qualitative tests have been done according to Nasser et al. [6] to detect the presence of primary and secondary metabolites as shown in Table 1. These tests are useful to estimate some biological activities might be due to the presence of some secondary metabolites in the peels of the studied fruit.

Table 1 : Detection of primary and secondary metabolites

Metabolites	Added reagent	Expected result
Alkaloids	Dragendorff reagent	Red or orange precipitate
Tanins	$FeCl_3$ (1%)	Blue coloration
Resines	Acetone + water	Turbidity
Saponines	Agitation	Formation of foam
Phenols	$FeCl_3$ (1%) + $K_3(Fe(CN)_6)$ (1%)	Green-blue coloration
Terpenoids	Chloroform + H_2SO_4 conc	Reddish brown coloration
Flavonoids	KOH (50%)	Yellow color
Carbohydrates	-naphtol + H_2SO_4	Purple ring
Reducing sugar	Fehlings (A+B)	Brownish-red precpitate
Quinones	HCl conc	Yellow precpitate
Sterols & Steroids	Chloroform + H_2SO_4 conc	Red color (surface) + fluorescence Greenish-yellow
Cardiac glycosides	Glacial acetic acid + $FeCl_3$ (5%) + H_2SO_4 conc	Ring
Diterpenes	Copper acetate	Green color
Anthraquinones	HCl (10%) + Chloroform + Ammonia (10 %)	Pink color
Proteins & aminoacids	Ninhydrin 0.25%	Blue color
Lignines	Safranine	Pink color
Phlabotannins	HCl (1%)	Blue color
Anthocyanines	NaOH (10%)	Blue color
Flavanones	H_2SO_4 conc	Bluish-red color
Fixed oils and fats	Spot Test	Oil stain

Determination of total phenolic content (TPC)

The method of Folin-Ciocalteau reagent has been used to estimate the TPC [7,8]. 100 µL of each used extract and 0.5 mL of Folin-Ciocalteau (1/10 dilution in water) were mixed with 1.5 mL of Na_2CO_3 (2%). The mixture was incubated in the dark at room temperature for 30 min. The absorbance of the solution was measured at 765 nm using a Gene-Quant 1300 UV-Vis spectrophotometers.

The blank was composed of 0.5 mL of selected solvent and 1.5 mL of Na_2CO_3.

The results were expressed as milligram of gallic acid equivalent (GAE) per gram extract (mg GAE g^{-1}).

Determination of total flavonoids content (TFC)

The aluminium chloride method [9] was used for the determination of TFC of all studied extracts. 1 mL of various

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concentrations of all crude extracts was mixed with 1 mL of 2 % methanolic aluminium chloride solution. After an incubation period for 1 h at room temperature in the dark, the absorbance of all samples was determined at 415 nm using a Gene-Quant 1300 UV-Vis spectrophotometers. The results were expressed as milligram of rutin equivalent (RE) per gram extract (mg RE g^{-1}).

Determination of total alkaloid content

The determination of alkaloids content has been done according to the method of Harborne [10]. 100 mL of 10% acetic acid in ethanol was added to 1 g of dry powdered peels and then the extract was covered and allowed to stand for 4 h. After that, the extract has been filtrated and concentrated on a water bath to 25 mL of its original volume. Droplets of concentrated ammonium hydroxide were added to the extract until the precipitation of the whole solution. Then the precipitates were washed with dilute ammonium hydroxide and filtered using filter paper whatman N1 0.45µm. The residue was dried in the oven at 40 ºC and weighted. The alkaloid content was determined using the following formula:

% Alkaloid = [final weight of the sample / initial weight of the extract] ×100

DPPH Radical scavenging activity

The antioxidant activity was practiced according to the method of Rammal et al. [11,12] using free radical DPPH. Increasing concentrations of extracts (0.05, 0.1, 0.2, 0.4, 0.5 mg mL^{-1}) were prepared. 1 mL of each prepared dilution of each extract was added to 1 mL of DPPH reagent. The solutions were incubated in the dark at room temperature for 30 min and the absorbance was measured at 517 nm by a Gene-Quant 1300 UV-Vis spectrophotometer. The DPPH scavenging ability of peels extracts was calculated according to the following equation:

% Scavenging activity = [(Abs control-Abs sample) / Abs control] ×100

Control was prepared by mixing 1 mL DPPH with 1 mL of selected solvent.

The blank was composed of 1 mL of the selected solvent.

Determination of total lipids

The method described by Aberoumand [13] was used for the determination of total lipids. 2 g of peels powder was placed on a thimble which is placed in the chamber of extraction of Soxhlet apparatus containing petroleum ether for 8 h under heat. After Soxhlet, the extract was taken and placed in a beaker; the beaker was heated in the oven at 100 ºC to evaporate the solvent and then cooled in a desiccator and weighted. After that, the lipid weight was calculated.

Determination of minerals content

An acid digestion was carried out to determine the minerals content. 1 g of powder was put in the oven at 80 ºC for 24 h. Then, 10 mL of HCl were added at 80 ºC with agitation. The beaker was covered by a watch glass. From time to time, few drops of H_2O_2 (35 %) were added. The beaker was left to be heated for 15 h. After the evaporation of HCl, 10 mL of HNO_3 were added. A filtration was carried out for the obtained mixture followed by another filtration by syringe.

Followed minerals, iron, calcium, magnesium, lead, copper, cadmium, chromium, zinc and manganese are proportioned by the atomic absorption spectrometry.

Determination of total saponin [14]

Powdered peels (1 g) have been added to 100 mL of ethanol (20 %) and then heated over a hot water bath for 4 h at 45 ºC with continuous stirring. The mixture was filtered and the residue was again extracted with another 100 mL of ethanol (25%). The combined extracts were concentrated by using rotary evaporator in 40 ºC to get 40 mL approximately. The concentrate was transferred into separator funnel and extracted twice with 20 mL diethyl ether. The ether layer was discarded while the aqueous layer was kept and the re-extracted with 30 mL n-butanol was added. The n-butanol extracts were washed twice with 10 mL of sodium chloride (5%). The remaining solution was evaporated. After evaporation, the samples were dried in the oven at 40 ºC to a constant weight. The saponin content was calculated using the following formula:

% Saponin = [final weight of sample / initial weight of extracts] × 100

Determination of humidity content [15]

Powdered peels (1 g) were transferred into a thin porcelain dish already weighed. It was dried in an oven at 100 ºC for 1 h, then cooled in a desiccator for half hour and again weighed. Before and after heating the difference in mass was calculated and expressed in percent of the moisture content using the following formula:

% Moisture = [(initial weight-final weight) / weight of sample] × 100

Estimation of ash proportion

1 g of dried peels powder was placed in and burned in a furnace burning (muffle furnace) at 550 ºC for 5 h till obtaining an ovary gray color of the powders. Then, the residues have been weighted and the percentage of ash has been estimated according the essential dry weight of plant powder [16].

3. RESULTS AND DISCUSSION

Phytochemical screening

The present study conducted on five extracts from *P. granatum* peels showed the presence of active components. Bioactive compounds of peels were qualitatively evaluated. The obtained results are reported in Table 2. All extracts tested positive for phenolic compounds are known to have antioxidant, anti-carcinogenic, anti-inflammatory, anti-apoptosis, anti-aging, anti-atherosclerosis, and cardiovascular protection effects [17,18].

On the other hand, the screening revealed the presence of alkaloids in ethanolic extract only. They are known to exhibit analgesic and antibacterial activities [18].

Terpenoids known to have significant pharmacological activities such as anti-viral, anti-bacterial, anti-malarial, anti-

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inflammatory, inhibition of cholesterol synthesis and anti-cancer activities [19] were found in ethanol, methanol and aqueous extracts.

Tannins, known to have anti-carcinogenic and antibacterial activities [20], were found in ethanol, methanol and aqueous extracts.

Also, flavonoids and flavanones, known to have antioxidant and anti-inflammatory effects [21], were found in ethanol and methanol.

Cardiac glycosides, known to lower pressure [22], were found in ethanol, methanol and aqueous extracts.

Lignins, known to make vegetables firm and crunchy, and gives us what we call “fiber” in our food [23], were found in methanol and aqueous extracts.

Carbohydrates were found in ethanol, methanol and aqueous extracts.

Finally, fixed oils and fat were found in hexane and dichloromethane extracts.

Table 2: Phytochemical screening of *P. granatum* peels

	Hexane	dichloromethane	Water	Ethanol	Methanol
Alkaloids	--	--	--	+	--
Tannins	--	--	+	+	+
Resines	--	--	--	--	--
Saponins	--	--	--	--	--
Phenols	+	+	+	+	+
Terpenoides	--	--	+	+	+
Flavonoids	--	--	--	+	+
Carbohydrates	--	--	+	+	+
Proteins	--	--	--	--	--
Quinones	--	--	--	--	--
Sterols	--	--	--	--	--
Cardiac glycosides	--	--	+	+	+
Diterpenes	--	--	--	--	--
Anthraquinones	--	--	--	--	--
Reducing sugars	--	--	+	+	+
Lignins	--	--	+	--	+
Phlabotannins	--	--	--	--	--
Anthocyanins	--	--	--	--	--
Flavanones	--	--	--	+	+
Fixed oils/fat	+	+	--	--	--

(--) sign indicates the absence of chemical constituents
(+) sign indicates the presence of chemical constituents.

Total phenolic content & total flavonoids content

The obtained results showed that methanol extract has the highest phenolic content (185.43 mg mL^{-1}). However, ethanol and aqueous extracts have respectively 80.01 mg mL^{-1} and 14.91 mg mL^{-1}, as a total phenolic content. So we can say that methanol is the best solvent for the extraction of phenolic compounds from dried plant material.

On the other hand, the obtained results showed that peels are rich in flavonoids compounds and the TFC was equal to 3.86 mg mL^{-1}.

Comparing these obtained results with those obtained by others (Table 3) we can see that Lebanese *P. granatum* peels contain more phenolic compounds that those from Oman and Tunisia but less than those from Turkey. On the other hand, the TFC of the Lebanese peels are less than those from Tunisia and Turkey but higher than those from Oman. This difference may be due to various factors such climate, soil, irrigation among other [24–26].

Table 3: TPC and TFC values

Country	TPC mg GAE/g dry powder	TFC mg RE/g dry powder
Lebanon	185.4	3.9
Oman	64.2	1.4
Tunisia	85.6	51.5
Turkey	264.3	18.1

Antioxidant activity

An antioxidant can be defined as any substance that, when present in low concentration compared to an additional substrate, significantly delays or inhibits the oxidation of the substrate. Therefore, the physiological role of antioxidants, to avoid damage of cells which is a consequence of chemical reactions involving free radicals.

Antioxidant reacts with DPPH, which is a stable free radical, and convert it to , -diphenyl- -picryl hydrazine. The degree of discoloration indicates the scavenging potentials of the antioxidant extract. The results reported in Table 4 demonstrated that antioxidant activity of ethanol extract is higher than that of methanol extract. Also note that the antioxidant activity of both extracts increases with the increase of concentration.

Table 4: Antioxidant activity of ethanol and methanol extracts

	C_5=0.05 mg/ml	C_4=0.1 mg/ml	C_3=0.2 mg/ml	C_2=0.4 mg/ml	C_1=0. 5 mg/ml
Ethanol	94.7	95.7	96.01	96.13	96.25
Methanol	44.8	47.1	75.1	75.9	76.5

Comparing our obtained results with those obtained by Singh et al. [27], we can see that ethanol extract from peels of Lebanese *P. granatum* has exerted a higher antioxidant capacity reaching 95 % at the concentration 50 µg/ml. However, methanol extract from Lebanese peels at the same concentration (50 µg mL^{-1}) has exerted an antioxidant capacity (45%) smaller than that obtained by Singh et al. [27] which was 81 %.

Minerals content

The results reported in Table 5 showed that we can ensure that *P. granatum* peels don't contain heavy metals having side effect, but they are rich in minerals beneficial to human health. We found a high calcium content (259.57 mg L^{-1}), a good level as calcium is a key structural element of bones and teeth, it acts as a signal for vital physiological processes, including vascular contraction, blood clotting, muscle contraction and nerve transmission. Also we found magnesium (38.87 mg L^{-1}) which is involved in protein and nucleic acid synthesis and is needed for normal vascular tone and insulin sensitivity [28].

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There is also iron (3.92 mg L^{-1}) which has several functions such as the transport of oxygen, the formation of red blood cells and it is a cofactor of enzymes and other proteins involved in energy and the proper functioning of cells [29].

Also we found zinc (0.67 mg L^{-1}) which is an essential of more than 300 metalloenzymes participating in the synthesis and degradation of carbohydrates, lipids, proteins, and nucleic acids as well as in the metabolism of other micronutrients [30].

Table 5: Minerals content in the dried peels of *P. granatum*

Minerals	Ca	Mg	Fe	Zn	Pb	Cd	Cr	Mn	Cu
[mg/L]	259.57	38.87	3.92	0.67	0	0	0	0	0

Total active content

Quantitative estimation of the percentage of the crude chemical components in *P. granatum* peels was determined. The obtained results show that peels contained a high percentage of alkaloid (1.4%) and low percentages of saponin (0.46%), humidity (5.3%), ash (1%) and lipid (0.11%). These results correspond to those provided by the phytochemical screening tests presented above.

4. CONCLUSION

According to the results obtained in this work, *Punica granatum* peels contain high levels of minerals and bioactive compounds, additionally they possess significant antioxidant activity dependent on the nature of the extract and the its concentration.

They contain no heavy metals, so there is no toxic effect that may be related to their use; also they contain beneficial minerals to the human health.

In addition, several bioactive compounds were isolated and identified, these compounds have several activities such as: antibacterial, antioxidant, anti-inflammatory, preventive and anti-cancer.

These results justify the use of *Punica granatum* peels, so they can be used as a source of multi drug resistant in the future.

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