J. Agr. Sci. Tech. (2013) Vol. 15: 1393-1403

Phenolic Compounds, Antioxidant, and Antibacterial Activities of Peel Extract from Tunisian Pomegranate

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ABSTRACT

Pomegranate tree possesses a vast ethnomedical history and represents a phytochemical reservoir of heuristic medicinal value. In the present study, total phenolics, antioxidant, and antibacterial activities of pomegranate peel were determined by Folin–Ciocalteu, 2,2-diphenyl-1-picrylhydrazyl (DPPH) and disk-diffusion methods, respectively, and compared among the accessions. Methanolic extract gives higher total phenolics than the water extract. Six phenolic compounds were identified and quantified in pomegranate peel using the HPLC/ultraviolet method. The predominant compound was gallic acid, followed by ellagic acid, caffeic acid, *p*-coumaric acid, quercetin, and vanillic acid. Antioxidant activity expressed as IC_{50} varied among the cultivars and between solvents and was highly correlated with the total phenolics. All extracts were efficient against the five tested bacteria. Statistical analysis revealed three groups of accessions. The first group showed a high polyphenol compound that had both high antioxidant and antibacterial properties. These findings support the improvement and the selection for obtaining high products with well-defined functional properties.

Key words: DPPH, Disc diffusion method, Polyphenols, *Punica granatum* L.

INTRODUCTION

The pomegranate is one of the oldest edible fruits (Evreinoff, 1949). It is considered native of Persia and surrounding areas. It is well adapted to Mediterranean climate and arid zones (Salaheddin and Kader, 1984). In Tunisia, its cultivation spread throughout the country, except areas above sea level where growers feared the frost. The main production centers are the oasis of Gabes and Gafsa, Cap Bon, the region of Bizerte and Sousse in the Sahel.

In the world, the production and consumption of pomegranate have increased because it is used in various fields. Indeed, besides its fresh use, it is used for making refreshing drinks, aromas, jam, and other preparations such as cakes, wines, etc. (Evreinoff, 1949; Aviram and Dornfeld, 2001). Apart from its nutritional value, the pomegranate has been used in traditional medicine as natural astringent to treat diarrhea and internal parasites (Asish *et al.*, 1999). During the last decade, the pomegranate has attracted the interest of researchers because of its medicinal value and many works have been undertaken. Most studies have aimed to assess the neutraceutical qualities of pomegranate, while its valorization in food industry are focused only on the fruit juice and fruit pulp from edible part (Mokbel and Hashinaga, 2006). The peel of the pomegranate, which is about 50% of the total weight, has been used extensively in the folk medicine of many cultures (Reddy *et al.*, 2007). Although several studies have shown that this byproduct is an important source of bioactive compounds such as phenolic

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[DOR: 20.1001.1.16807073.2013.15.7.2.1]

compounds, which are secondary plant metabolites and possess anti-inflammatory, antiatherosclerotic, antitumor, antimutagenic, anticarcinogenic, antibacterial, or antiviral activities, its use remained very limited and traditional (Cai *et al*., 2004; Abdel Motaal and Sherif, 2011; Li *et al.*, 2006).

Considering that peels are not consumed and rarely approached, the high amount of bioactive compounds present in these non-edible parts could be used for different purposes in the food industry such as enrichment or development of new products. The aim of this work was to evaluate the antioxidant and the antibacterial activities and to quantify the phenolic compounds among peels extract for 21 Tunisian pomegranate accessions, by using HPLC in order to valorize these accessions and to provide information related to their peel characteristics, which may be of both industrial and nutritional interest.

MATERIALS AND METHODS

Plant Material

Twenty one pomegranate accessions cv. Gabsi were collected from mature trees in October 2010 in the region of Gabes in the South-East of Tunisia, which is characterized by an arid bioclimate of Mediterranean type with a mild winter. Fruits were transferred to +4°C store room on the harvest day. Table 1 presents information about geographical origin of the accessions.

Preparation of Pomegranate Peel Extracts (PPE)

Fresh pomegranate fruits were peeled manually and collected peels were then rinsed with distilled water. Peels were air dried in the dark at room temperature (+27°C) and then ground into a powder using a mechanical grinder. According to Owen and Johns (1999), several organic solvents can be used to extract phenolic compounds. In this study, the rough extracts were obtained by successive extractions with solvents of increasing polarities i.e. we used methanol then water. The conventional Soxhlet extraction apparatus consisting of a condenser, a Soxhlet chamber, and an extraction flask were used (Negi and Jayaprakasha, 2003). Prior to solvent extraction study, 15 g of dried and ground peel were placed in a Whatman cellulose thimble (25×100 mm). The cotton was then placed into the thimble. The purpose of using cotton was to ensure the presence of samples inside the thimble during the experiment. Samples were extracted in a Soxhlet extraction system (BUCHI Extraction System Model B-811) using 150 ml of methanol. Later, the thimble containing the sample was placed into the extraction chamber. Lastly, the condenser was placed on top of the extraction flask and all the parts were fixed vertically. The extraction was carried out for four hours**.** The crude extract solutions obtained were filtered through Whatman (No. 41) filter paper for removal of peel particles. The filtrate obtained was named methanolic

Table 1. Accessions of *Punica granatum* L. (cv. Gabsi), their codes and their places of origin.

Accessions	Codes	Origin	Latitude (N)	Longitude (E)	Altitude (m)
GME1, GME2, GME3	1, 2, 3	Metouia	33°57’	10°00’	22
GO1, GO2, GO3	4, 5, 6	Ouedhref	33°59’	9°58’	26
GG1, GG2, GG3	7, 8, 9	Gabès ville	33°52’	10°04’	12
GC1, GC2, GC3, GC4, GC5	10, 11, 12,13, 14	Chenini	33°52’	10°04’	24
GM1, GM2, GM3, GM4	15, 16, 17, 18	Mareth	33°37’	10°17’	48
GK1, GK2, GK3	19, 20, 21	Kettana	33°45’	10°11’	20

[DOR: 20.1001.1.16807073.2013.15.7.2.1]

extract. After exhaustion with methanol, residual marcs were dried. The powder obtained was recovered in 150 ml of water and the same operation was repeated to get the water extract. The obtained extracts were kept at -20°C until further use.

Determination of Total Phenolics (TP)

The content of phenolic compounds in methanolic and water extracts was determined according to the method of Jayaprakasha *et al*. (2001). The extracts were dissolved in water and 0.5 ml of diluted samples was mixed with 0.5 ml of 10-fold-diluted Folin–Ciocalteu reagent. After 3 min, 4 ml of 7.5% sodium carbonate was added. The mixture was allowed to stand for 30 minutes in the dark at room temperature before the absorbance was measured at 765 nm using a spectrophotometer. The final results were expressed as mg gallic acid g^{-1} dry weight (DW). All samples were analyzed in triplicate.

Antioxidant Activity

The method of Okonogi *et al*. (2007) was used for the determination of the antioxidant activity. Different concentrations of each methanolic and water extracts were prepared from a stock solution (1 mg ml^{-1}). DPPH (100 µM) was dissolved in water and mixed with a 100 µl of each concentration. After vigorously shaken, the mixture was left to stand for 30 minutes in the dark at room temperature. The absorbance was measured at 517 nm using a spectrophotometer. All measurements were performed in triplicate. The radical-scavenging activity was calculated as % inhibition (I%) from the following equation:

$I\% = [(A_c - A_s)/A_c] \times 100$

Where, A_c is the absorbance of the control reaction (containing all the reagents except the test sample) and A_s is the absorbance of the sample.

Antimicrobial Activity

Microorganisms and Growth Conditions

Table 2 lists microorganisms that were used to evaluate the antimicrobial activity. The strains were stored at 5°C in slants of Muller-Hinton broth. Working cultures were activated at 37°C for 24 hours.

Antimicrobial Activity

The antibacterial activity of the methanolic extracts was tested using the disk-diffusion method (Rios and Recio, 2005). 100 µl of a bacterial suspension was swabbed uniformly across Miller Hinton agar contained into petri dishes and then left to dry for 30 minutes. Discs of 6 mm in diameter (Whatman filter paper No. 3) were placed onto the surface. Each disc was soaked with 15 µl of extracts. The plates were left at room temperature for 30 minutes to allow diffusion of the materials.

Plates were incubated at 37°C for 24 hours, until visible growth of test microorganisms was evident in the control plates. Inhibition zones in mm (including disc diameter) around discs were measured. The antimicrobial activity was expressed as the diameter of inhibition zones produced by the extracts against test microorganisms. The experiment was repeated three times.

High-Performance Liquid Chromatography Analysis

The individual phenolic compounds content from methanolic extract of pomegranate peel was determined using the method of Chaira *et al*. (2009). The High-performance liquid chromatography (HPLC) system consisted of Knauer Wellchrom « Asteris » model (Knauer, Germany), with a column of Eurospher 100 C_{18} (250×4.6 mm, 17 µm). Detection was monitored by a UV Detector (Knauer, Germany). Prior to

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Table 2. Inhibition zone (mm) created by the methanolic extracts of different accessions on the studied microorganisms[a].

Accessions	Staphylococcus aureus ATCC 25923	Salmonella typhimurium ATCC 1408	Escherichia coli ATCC 25923	Enterococcus faecalis ATCC 29212	Staphylococcus epidermidis CIP 106510
GME1	23.0 ± 1.0	22.3 ± 0.6	26.3 ± 2.1	19.3 ± 2.1	21.3 ± 2.1
GME2	23.0 ± 1.7	20.3 ± 0.6	20.7 ± 2.1	26.3 ± 1.1	24.3 ± 1.1
GME3	22.3 ± 1.5	25.3 ± 2.3	21.0 ± 1.0	19.3 ± 1.5	21.3 ± 3.2
GO1	24.0 ± 1.7	23.7 ± 1.5	24.0 ± 1.0	25.3 ± 1.5	26.0 ± 2.6
GO2	31.7 ± 0.6	25.7 ± 1.1	34.0 ± 1.0	29.3 ± 3.8	27.7 ± 2.5
GO3	17.3 ± 0.6	16.3 ± 1.1	15.0 ± 2.0	14.3 ± 0.6	12.0 ± 1.7
GG1	28.0 ± 2.6	24.3 ± 2.5	28.0 ± 4.0	32.0 ± 2.0	28.7 ± 3.2
GG2	31.0 ± 1.7	27.0 ± 4.4	28.7 ± 1.5	32.3 ± 0.6	28.3 ± 2.1
GG3	29.3 ± 1.1	30.7 ± 2.9	29.7 ± 6.4	28.7 ±1.5	35.0 ± 2.0
GC1	19.7 ± 1.5	20.7 ± 0.6	23.3 ± 2.1	24.0 ± 1.0	23.3 ± 0.1
GC2	26.3 ± 1.1	29.0 ± 2.0	28.3 ± 1.5	27.0 ± 2.6	23.7 ± 1.5
GC3	34.7 ± 4.0	25.0 ± 0.0	32.3 ± 2.5	25.0 ± 5.0	27.3 ± 4.0
GC4	23.3 ± 5.1	24.0 ± 1.7	25.7 ± 4.0	19.7 ± 2.5	20.7 ± 1.1
GC5	26.3 ± 1.1	26.7 ± 0.6	27.7 ± 0.6	27.7 ± 1.5	26.0 ± 1.7
GM1	31.3 ± 2.3	30.0 ± 2.0	24.0 ± 1.7	25.0 ± 1.7	33.3 ± 2.9
GM2	25.3 ± 2.9	23.3 ± 1.5	20.3 ± 3.5	25.0 ± 4.4	23.3 ± 2.9
GM3	24.3 ± 2.5	24.3 ± 0.6	25.7 ± 1.1	24.7 ± 3.0	23.7 ± 1.5
GM4	24.7 ± 2.5	27.3 ± 2.5	30.3 ± 5.0	33.7 ± 1.5	32.0 ± 2.0
GK1	24.7 ± 1.5	29.3 ± 0.6	30.0 ± 1.0	31.3 ± 2.3	26.3 ± 2.3
GK2	27.0 ± 1.0	26.7 ± 2.5	25.3 ± 0.6	23.3 ± 1.1	24.0 ± 3.6
GK3	25.0 ± 3.0	22.0 ± 1.7	24.3 ± 4.6	22.7 ± 1.1	20.7 ± 1.1
Mean±SD (n= 21)	25.8 ± 4.1	24.95 ± 3.5	25.9 ± 4.4	25.5 ± 4.9	25.9 ± 5.0
F_{obs}[b]	10.0	10.3	7.4	13.0	13.5
P values[c]	<0.001	<0.001	<0.001	<0.001	<0.001

[a] Values are average of three individual samples each analyzed in duplicate ±SD.
[b] Snedecor-Fisher Factor, [c] P values were determined by Fisher's exact test, significantly different (P= 0.05).

use, solvents were filtered over a 0.45 µm membrane filter and degazed for 15 min in an ultrasonic bath Cleaner Model SM 25E-MT (Branson Ultrasonics Corporation, Dambury, USA). The mobile phase consisted of methanol/ acetonitrile 50/50 (A) and acetic acid in ultra pure water pH 3.2 (B). While flow-rate and the injection volume during the experiment were 1.0 ml min^{-1} and 20 µl, respectively, the pumps gradient during analyses was: 5 to 30% (A): 0-25 minutes; 30 to 38% (A): 25-35 minutes; 38 to 45% (A): 35-45 minutes; 45 to 52% (A): 45-50 min.

The integrator was calibrated with external standards consisting of caffeic, gallic, ellagic, vanillic, *p*-coumaric acids and quercetin solutions. The concentration of each standard was 330 µg ml^{-1} (in methanol). Simple polyphenols were identified by comparison of their retention times with standards. They were quantified by comparing the peak area against the standard curve obtained specifically for the reference solutions containing that compound. All samples were analysed in triplicate and the calculation was done using the rules of three and the following equation:

$C_S = C_{St} \times (P_S/P_{St})$

Where, C_s is the concentration of the sample; C_{St} is the concentration of the

[DOR: 20.1001.1.16807073.2013.15.7.2.1]

standard; P_S is the peak area of the sample and P_{St} is the peak area of standard.

Statistical Analysis

All the analyses were performed in triplicate. Results were expressed as means±standard deviation. Analysis of variance procedure (ANOVA) was performed. Results were significant when $P< 0.05$. Mean values recorded for each parameter were used to perform factor analysis and clustering of genotypes into similarity groups using Ward's method. Correlations between total phenolic content and the tested activities were established using the test of Pearson. Data processing was performed using SPSS software (version 18.0) and StatBox (version 6.40).

RESULTS AND DISCUSSION

Total Phenolics and Antioxidant Activity

Results for the total polyphenols and antioxidant activity of methanol and water extracts of pomegranate peel are given in Figure 1. The variance analysis showed a highly significant difference between accessions (P< 0.001). Total peel polyphenols ranged from 82.0±0.1 as mg gallic acid g^{-1} DW in the accession 3 from Mareth (GM3) to 230.4±0.6 as mg gallic acid g^{-1} DW in the accession 3 from Gabes (GG3) in methanolic extract and from 40.8±0.2 in the accession 2 from Mareth (GM2) to 59.8±0.6 mg gallic acid g^{-1} DW in GG3 in water extract. These values were comparable to those reported by other researchers (Li *et al*., 2006), though lower than those of Negi and Jayaprakasha (2003) and Sultana *et al*. (2008), and higher than those obtained by Abdel Monein (2012).

The radical-scavenging activity on DPPH was expressed as IC_{50}±*SD* (n =3). This value was the concentration of the extract required to inhibit 50% of the initial DPPH free radical. Variation of the IC_{50} was significant (P< 0.001). Figure 1 shows that the methanolic extract gives higher activity than the water extract, mean values ranged, respectively, from 1.9 to 4.3 µg ml^{-1} and from 10.2 to 13.1 µg ml^{-1}. These values are comparable with those obtained by Negi and Jayaprakasha (2003), who, while working on the antioxidant activity using four different solvents including EtOAc, acetone, MeOH and water, found that methanol gave the maximum antioxidant yield and water gave the lowest one. The same result was also obtained by Singh *et al.* (2002). Methanolic extract is usually used to determine the antioxidant activity (Ayoughi *et al.,* 2011; Mazidi *et al*., 2012).

However, methanol is an effective solvent for polyphenols and it is commonly used in the laboratory and in industrial extraction process (Wang *et al*., 2004). Usually, ordinary people use water when they prepare traditional extract. Indeed, water is not an effective solvent for the extraction of phenols compared to methanol. But, results obtained with water also show reasonable quantities of natural antioxidants. Similar peel DPPH antioxidant capacities were reported by Elfalleh *et al*. (2009) in Tunisian pomegranate peel.

Antimicrobial Activity

Table 2 shows the antimicrobial activity methanolic extract of pomegranate peel as evaluated by the disc diffusion method via determination of the surrounding zones of inhibition. The PPE is efficient against the five tested bacteria. Results show a significant difference between the accessions (P< 0.001). Abdollahzadeh *et al.* (2011), Naz *et al.* (2007), Vasconcelos *et al*. (2003), and Singh *et al*. (2002) also reported that extracts of *Punica granatum* peel in different concentrations were effective against *S. epidermidis*, *S. aureus*, *S. mutans*, *S. sanguinis* and *S. salivarius*. McCarrell *et*

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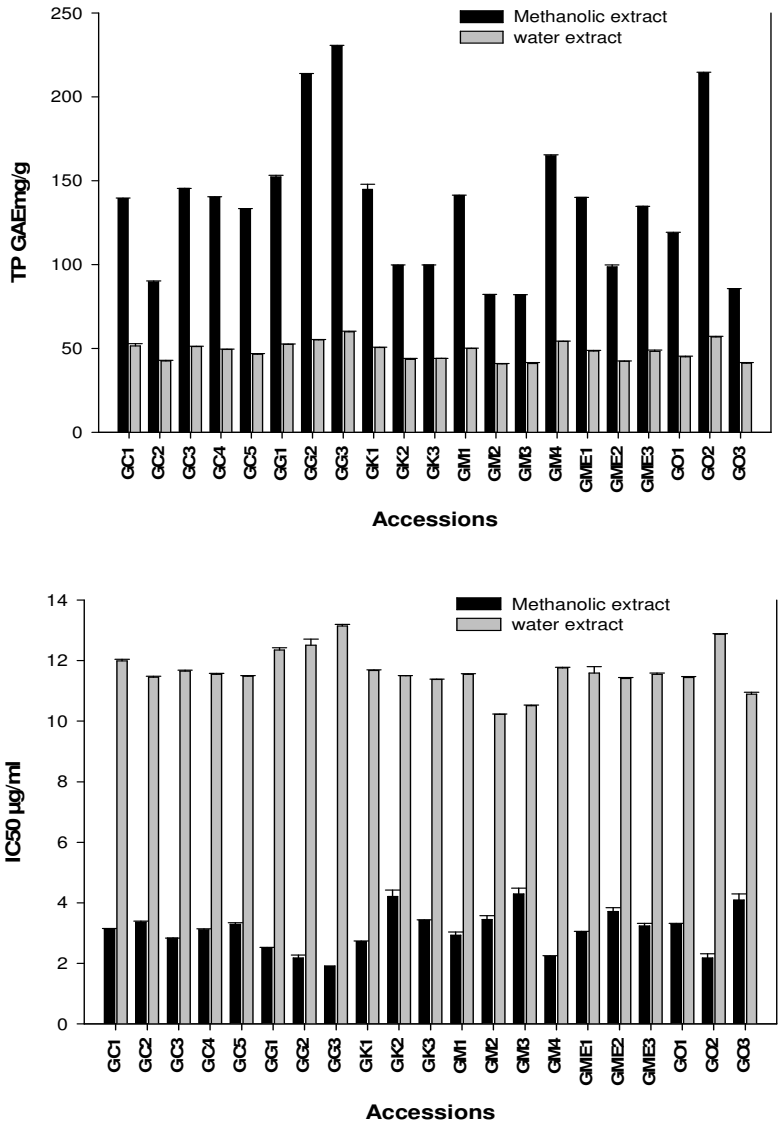

Figure 1. Total polyphenols and diphenyl-1-picrylhydrazyl (DPPH) of 21 pomegranate Peel Extract (PPE).

al. (2008) also demonstrated antibacterial activity of autoclaved pomegranate peel extract against *Staphylococcus aureus* and *B.subtilis*.

Individual Phenolic Compounds Content of the Peel

The observed antioxidant and antimicrobial activities of pomegranate peel methanol extract in the present study might be attributed to the presence of polyphenols, such as ellagic acid and gallic acid (Gil *et al*., 2000). Hence, in order to investigate phenolic compounds from the PPE, the HPLC analysis of methanolic extract was performed. These compounds included 3 hydroxybenzoic acids (vanillic, gallic and ellagic acids), 2 hydroxycinnamic acids (caffeic and *p*-coumaric acids), and one flavonol (quercetin). Table 3 gives the concentrations of individual phenolic compounds (mean±SD) identified in

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Table 3. Individual phenolic compounds (mg/100 g DW) in peels of studied pomegranate accessions[a].

Accessions	Gallic acid	Vanillic acid	Caffeic acid	p-Coumaric acid	Ellagic acid	Quercetin
GME1	140.4 ± 1.1	1.3 ± 0.1	18.1 ± 0.1	4.9 ± 0.2	35.5 ± 1.3	1.8 ± 0.1
GME2	87.6 ± 1.2	1.3 ± 0.1	17.9 ± 0.3	4.8 ± 0.1	34.8 ± 1.7	1.8 ± 0.1
GME3	125.7 ± 2.9	1.5 ± 0.1	22.7 ± 0.4	4.1 ± 0.2	34.4 ± 0.4	2.1 ± 0.1
GO1	110.7 ± 0.6	1.7 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	36.9 ± 0.5	2.1 ± 0.0
GO2	122.3 ± 0.3	1.4 ± 0.1	0.0 ± 0.0	0.0 ± 0.0	36.6 ± 0.8	2.1 ± 0.0
GO3	138.3 ± 0.2	1.5 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	34.9 ± 1.1	2.6 ± 0.0
GG1	106.2 ± 2.4	0.0 ± 0.0	22.9 ± 0.4	4.5 ± 0.3	37.2 ± 2.3	1.4 ± 0.2
GG2	98.7 ± 1.0	0.0 ± 0.0	22.1 ± 0.2	4.6 ± 0.0	28.8 ± 2.1	2.9 ± 0.0
GG3	138.7 ± 2.3	0.0 ± 0.0	23.7 ± 0.7	4.7 ± 0.1	28.6 ± 0.2	1.5 ± 0.3
GC1	102.8 ± 0.3	1.0 ± 0.0	20.5 ± 1.1	4.6 ± 0.0	36.1 ± 2.5	1.9 ± 0.1
GC2	109.1 ± 0.8	1.6 ± 0.0	22.6 ± 0.7	4.5 ± 0.0	29.1 ± 0.5	1.3 ± 0.0
GC3	139.1 ± 1.9	1.3 ± 0.1	19.2 ± 0.2	4.5 ± 0.3	34.8 ± 1.5	1.4 ± 0.3
GC4	120.1 ± 1.5	1.2 ± 0.0	24.7 ± 0.6	4.7 ± 0.0	35.8 ± 1.8	1.4 ± 0.0
GC5	117.2 ± 1.4	1.5 ± 0.0	25.9 ± 1.0	5.3 ± 0.0	35.0 ± 1.5	1.4 ± 0.2
GM1	148.1 ± 0.2	1.4 ± 0.0	0.0 ± 0.0	4.8 ± 0.1	38.5 ± 0.0	2.3 ± 0.0
GM2	131.6 ± 0.1	1.6 ± 0.1	0.0 ± 0.0	4.5 ± 0.1	35.8 ± 0.8	2.0± 0.1
GM3	129.0 ± 0.9	1.2 ± 0.0	0.0 ± 0.0	3.2 ± 0.1	35.3 ± 0.4	1.4 ± 0.0
GM4	139.2 ± 0.4	1.6 ± 0.1	0.0 ± 0.0	4.6 ± 0.0	39.4 ± 0.0	1.2 ± 0.0
GK1	138.8 ± 0.7	0.0 ± 0.0	22.4 ± 0.4	4.4 ± 0.0	35.3 ± 1.3	2.1 ± 0.0
GK2	124.1 ± 0.2	0.0 ± 0.0	23.4 ± 0.6	4.5 ± 0.1	31.4 ± 5.2	2.3 ± 0.1
GK3	139.0 ± 0.4	0.0 ± 0.0	22.4 ± 0.8	4.5 ± 0.0	34.6 ± 1.7	2.4 ± 0.0
Mean±SD (n= 21)	124.1 ± 16.5	1.0 ± 0.7	14.7 ± 10.8	3.9 ± 1.7	34.7 ± 2.9	1.9 ± 0.5
F_{obs}[b]	501.2	289.8	1401.5	560.2	8.6	44.6
P values[c]	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001

[a] Values are average of three individual samples each analyzed in duplicate ±SD.
[b] Snedecor-Fisher Factor, [c] P values were determined by Fisher's exact test, significantly different (P= 0.05).

pomegranate peel of different accessions. Variations of the individual phenolic compounds content were significant between the accessions (P< 0.001). The studied pomegranate accessions were rich in gallic acid with an average concentration of about 124.14±16.52 mg 100 g^{-1} DW. GM1 accession showed the highest level (148.1±0.2 mg 100 g^{-1}) and GME2 contained the least amount of gallic acid (87.6±1.2 mg 100 g^{-1}). Vanillic acid and quercetin were present only in small quantities equal to 1.0±0.7 and 1.9±0.5 mg 100 g^{-1} (Table 3). In literature, many authors proved the presence of quercetin and vanillic acid in pomegranate peel (Artik, 1998; Cai *et al*., 2004; Van Elswijk *et al*., 2004). For *p*-coumaric acid, as analyzed by HPLC, its average content was only 3.9±1.7 mg 100 g^{-1} of dry matter. Significant differences were found among the caffeic acid levels of different accessions. It ranged from 0 mg 100 g^{-1} (accessions of Ouedhref and Mareth) to 25.9±1.0 mg 100 g^{-1} (GC5) with an average of 14.7±10.8 mg 100 g^{-1}. Our results are greater than those reported by Ben Nasr *et al*. (1996) in Tunisian pomegranate peel, who reported 11.7 ± 0.1 mg 100 DW^{-1} ellagic acid and 3.0 ± 0.1 mg 100 DW^{-1} gallic acid. But, our results corroborate those obtained by Elfalleh *et al*. (2011) who reported that gallic acid as the major phenolic compound (123.8±9.6 mg 100 g^{-1}), followed by ellagic acid (35.9±2.4 mg 100 g^{-1}), caffeic acid (20.6±1.5 mg 100 g^{-1} and *p*-coumaric acid (4.5±0.4 mg 100 g^{-1}).

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The accessions were divided into three main groups based on the combination of total phenolics, antioxidant and antibacterial activities (Figure 2). The first group consisted of three accessions (GO2, GG2 et GG3), characterized by the highest phenolic content (an average of 219.4 mg g^{-1}), antioxidant (an average IC50 of 2.1 µg ml^{-1}) and antibacterial activities (an average inhibition zone of 29.9 mm). The second group comprised the majority of the accessions (10). They had average values. The third group held eight accessions characterized by the lowest TP (an average of 94.5 mg g^{-1}), antioxidant (an average of 3.7 µg ml^{-1}), and antibacterial activities (an average of 23.2 mm).

The highest negative correlation coefficient was observed between the total phenolic content and the IC50 (r= -0.91). A negative correlation was also noticed between the antimicrobial activity and the IC50. The total phenolic content was positively correlated with the antimicrobial activity (r= 0.66). Therefore, the higher total phenolic content resulted in higher total antioxidant and antimicrobial capacity, thus, the large amount of phenolics contained in peel extract may account for its strong activities (Huang *et al*., 2005).

CONCLUSIONS

Pomegranate has an important role in folk medicine. It is known as a rich source of pharmacological properties. The results of this study seemed to make pomegranate peel, which are the agro-industrial waste of this fruit, an attractive candidate as a nutritional supplement for cattle feed and outlines of valorization can be advanced. The analysis of results allows distinguishing some genotypes. Among the twenty one accessions studied, peels of

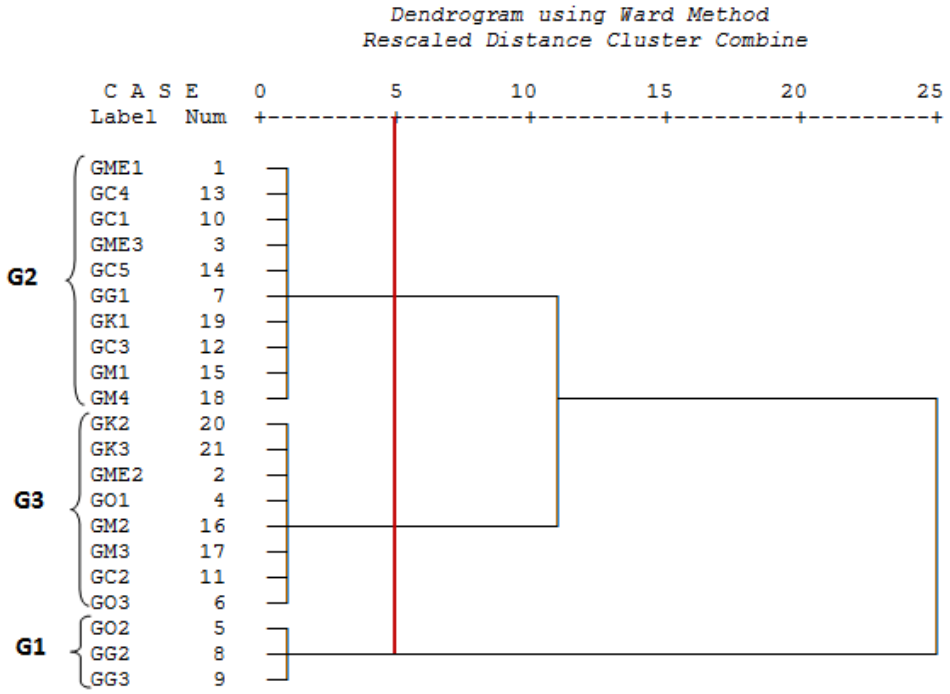

Figure 2. Cluster analysis of studied accessions according to total phenolic content, DPPH radical scavenging activity and antimicrobial activity. G1: Group 1; G2: Group 2, and G3: Group 3.

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GO2, GG2 and GG3 that showed high content of total phenolics and high antioxidant and antibacterial activities can be used as natural food additives or supplements with high nutritional value to fulfill the requirements of consumers for natural and preserved healthy food.

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ترکیبات فنلی وفعالیت های ضد اکسید کنندگی و ضد باکتریایی عصاره پوست انار تونسی

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چکیده

درخت انار سابقه ای طولانی در تاریخ طب سنتی دارد و مخزنی از مواد شیمیایی گیاهی را میماند که ارزش آموزشی-اکتشافی پزشکی دارند. در تحقیق حاضر، کل مواد فنلی و فعالیت های ضد اکسید کنندگی و ضد باکتریایی پوست انار به ترتیب با روش DPPHو روش پخشیدگی دیسک تعیین شدند

[DOR: 20.1001.1.16807073.2013.15.7.2.1]

و بین نمونه های بانک ژن مقایسه شدند. نتایج نشان داد که عصاره گیری با متانولیک در مقایسه با عصاره گیری با آب مقدار بیشتری مواد فنولی کل به دست می دهد. با استفاده از روش طیف سنجی مایعی کارآ / فرابنفش شش ترکیب از مواد فنلی در پوست انارشناسایی شد. ترکیب غالب گالیک اسید بود و به دنبال آن الاجیک اسید، اسید کافئک، اسید پ-کوماریک،quercetin و اسید وانیلیک بودند. مقدار فعالیت ضد اسید کنندگی که با IC50 بیان می شود بین کالتیوارها و بین حل کننده ها متغیر بود ولی رابطه زیادی با کل مواد فنلی داشت. تمام عصاره ها بر علیه ۵ باکتری آزمون شده موثر بودند. تحلیل آماری داده ها، سه گروه از نمونه های بانک ژن را آشکار ساخت. گروه نخست دارای ترکیبات فنلی زیادی بود که خاصیت های ضد باکتریایی و ضد اکسید کنندگی بالایی داشتند. این یافته ها کارهای اصلاح نژادی و انتخابی برای دستیابی به موادی دارای اثرات و خاصیت هایی که به خوبی تعریف شده اند را تایید می کند.

[DOR: 20.1001.1.16807073.2013.15.7.2.1]