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Introduction of the Peel of Iranian Pomegranate as a Potential Natural Additive in Food by Phytochemical-based Characterization of Different Genotypes

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Abstract

Over the past decades, the use of natural additives has increased as an alternative to artificial ingredients in the food industry. The purpose of this study was to investigate the potential of pomegranate peel (PP) as a natural food additive. Many factors, including genotype, could affect the quality of PP as a by-product of juice production with many nutritional, functional and anti-infective properties. In this study, the most significant phytochemical characters of thirty Iranian pomegranate peels (IPP) from different genotypes, including total phenolic (TPC) and flavonoid content (TFC), and nine phenolic compounds were determined. The HPLC-DAD-MS results of PPEs revealed nine phenolic compounds in the IPP extracts. Punicalagin β, punicalagin α, and ellagic acid were the main components constituting 20.8–48.7, 13.9–30.1, and 1.6–13.4 µg/mg DW, respectively. The peel of IPP23 (Kabdar-Shirin-e- Behshahr) contained the highest quantity of polyphenolic compounds. Also, TPC and TFC of the peel extracts ranged between 66.38 and 181.41 mg GAE/ g DW and 38.5 to 144.13 mg RE/ g DW, respectively. Eventually, antioxidant potential estimated by the DPPH assay ranged between 4.1 and 14.4 µg/ml. The results showed that the antioxidant property of pomegranate peel extracts is significantly higher than the standard of gallic acid. Also, the peel of the genotypes that had high phenolic compounds were introduced as superior genotypes. The results of HCA showed that, among the studied genotypes, the peel of IPP23 can be introduced as a potential source of natural preservatives in the food industry.

Keywords: Antioxidant, Food preservative, Phytochemical, Polyphenol, Pomegranate

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Introduction

In recent years, natural food additives have been one of the most exciting and widely used areas in the food industry, and consumer demand for healthy food products has increased. To increase the nutritional value, improve the organoleptic properties and increase the shelf life of foods, food additives are used (Kaderides, Kyriakoudi, Mourtzinos, & Goula, 2021). Until now, synthetic antioxidants, like butylated hydroxytoluene (BHT), have been used as additives to delay or inhibit the oxidation of foods. Due to the toxic, and carcinogenicity effect of synthetic antioxidants, the interest in using natural antioxidants has been increased. Natural antioxidants include phenolic compounds of plants such as oregano, rosemary, sage, agricultural wastes, and by-products such as grape pomace, olive leaves, rice husks, etc. (Drevelegka & Goula, 2020; Mourtzinos *et al*., 2016; Nenadis, Kyriakoudi, & Tsimidou, 2013). For example, in the European Union, rosemary extract is used as an additive in several food groups such as seafood, meat and dairy products, edible oils and frying fats (Al-Moghazy, El-Sayed, & Abo-Elwafa, 2022; Kaderides *et al*., 2021).

Pomegranate (*Punica granatum* L.), a medicinal and ancient fruit that belongs to the Punicacea family, is a well-known horticultural crop that is widely grown in semi-arid mild temperate to subtropical regions with hot summers and cold winters (Eikani, Golmohammad, & Homami, 2012). Iran is one of the largest genetic resources and producers of pomegranates in the world. More than 790 genotypes and varieties of pomegranate are distributed across Iran (Zeinalabedini *et al*., 2012). Today, all parts of pomegranate fruit, including peel (exocarp and mesocarp), pulp, and seeds, are used in the food, medicinal, and cosmetic industries (Dhumal, Karale, Jadhav, & Kad, 2014). Annually about 25% of the harvested pomegranate fruit is used for the juice industry and other food products. After juice extraction, a considerable amount of waste materials such as peel and seed are produced (Russo *et al*., 2018; Sood & Gupta, 2015). Pomegranate peel (PP) contain more bioactive compounds such as tannins, flavonoids, and phenolic acids than the edible parts of pomegranate fruit (Fernandes *et al*., 2015; Hernández, Melgarejo, Martínez, Martínez, & Legua, 2011; Parashar, 2010).

PP is a rich resource of bioactive compounds, specifically punicalagin and ellagic acid isomers, which belong to the ellagitannins group. Antioxidant activity and therapeutic effect of PPEs against diabetes, cancer, cardiovascular diseases, inflammation, etc., have proved with scientific evidence (Du *et al*., 2019; Stojanović *et al*., 2017). The presence of natural compounds such as tannins, phenolic acids, and flavonoids in PP acts as antimicrobial and antioxidant agents which prevent the growth of microorganisms, the process of lipid peroxidation, and the elimination of free radicals. It can be used as an additive in food, which increases the stability of food during processing, storage or gastrointestinal digestion conditions. In most studies, no negative effect on sensory properties was observed in foods by adding PPE (Giri, Gaikwad, Raigond, Damale, & Marathe, 2023).

The high area under cultivation of diverse genotypes of pomegranate in Iran leads to access to high volume of PP as by-product. Furthermore, genotype and environmental conditions can influence the chemical composition of PP. The objectives of this study were to determine the highest percentage of polyphenolic compounds and antioxidant activity, and comprehensive phytochemical profiling of thirty well-known Iranian pomegranate genotypes.

Material and Methods

Solvents and reagents

The solvents and reagents used in this study were HPLC or analytical grade. Ethanol, methanol, and trifluoroacetic acid (TFA) used for the extraction and HPLC analyses were purchased from Chemopharma (Vienna, Austria). The reference compounds, such as ellagic acid, punicalagins, rutin, and gallic acid

were purchased from Phytopurify (Chengdu, China). Folin–Ciocalteu, DPPH (2,2-diphenyl-1-picrylhydrazyl), sodium carbonate, sodium nitrite, aluminum chloride, and sodium hydroxide were obtained from Merck (Darmstadt, Germany).

Collection site and plant materials

In the second week of October 2018, thirty different Iranian pomegranate fruit genotypes (Table 1 and Fig. 1) were collected from Yazd Pomegranate Collection, central Iran. In this collection, approximately 790 genotypes from different regions of Iran were collected and planted. From each genotype, five fully mature fruits were manually harvested. Peels were manually separated and then dried in the shade at room temperature for two weeks.

Extraction method

Air-dried, powdered fruit peel (exocarp+mesocarp) of IPPs were extracted by ultrasound-assisted extraction (UAE) method as described by (Pan, Qu, Ma, Atungulu, & McHugh, 2012). For this purpose, 200 mg powdered samples and 10 mL of ethanol-water (70:30, v/v) were placed in test tubes and then were immersed in an ultrasonic bath (Elmasonic P, Germany) under optimal conditions for extraction (4×30 min) with temperature, ultrasonic frequency, and power, at 25°C, 37 kHz and 100% respectively (Rifna & Dwivedi, 2022). The tubes were then centrifuged for 10 min at 4000 rpm and the supernatant was used for further analysis.

Table 1- Thirty different Iranian pomegranate genotypes listed regarding their code

No.	Pomegranate genotype	Code
1	Poust-siyah-dastjerd-shirin-e-Isfahan	IPP1
2	Shirin-Nar-e-Behshahr	IPP2
3	Bavasi-Poust-Sefeed-e-Lorestan	IPP3
4	Poust Sorkh-Daneh-Sefeed-Torsh-e-Khuzestan	IPP4
5	Atabaki-poust-Ghermez-e-Sarvestan	IPP5
6	Siyah-Nar-e-Behshahr Toursh Mazandaran	IPP6
7	Shahvar-poust-Ghermez-e-Shirin	IPP7
8	Torsh-Poust-Coloft-e-Izeh	IPP8
9	Vahshi-Torsh-e-Guilan	IPP9
10	Shirin-Poust-Sefeed-e-Chaharmahal and Bakhtiari	IPP10
11	Goroch-Shahvar-e-Yazdi	IPP11
12	Poust-Ghermez-Chak Chak-e-Ardakan	IPP12
13	Aban-Mahi-Abrandabad-e-Yazd	IPP13
14	Shirin-Pishras-e-Najafabad	IPP14
15	Shoor-Poust-Nazok-Saaghand-e-Yazd	IPP15
16	Vahshi-Kan-Shirin-e-Tehran	IPP16
17	Poust-Ghermez-Torsh-e-Gorgan	IPP17
18	Kodro-Poust-Coloft-e-Kazerun-e-Fars	IPP18
19	Shirin-Poust-Sefeed-e-Shahreza	IPP19
20	Ghermez-Shirin-e-Koohdasht-e-Lorestan	IPP20
21	Sakoli-Sidun-Malas-e-Marvdasht	IPP21
22	Malas-Shahpar-Pishva-Varamin	IPP22
23	Kabdar-Shirin-e-Behshahr	IPP23
24	Togh-Gardani-e-Yazdi	IPP24
25	Agha-Mohseni-e-Gorgan	IPP25
26	Sefeed-Poust-Khosk-e-Bafgh	IPP26
27	Yek-Kilo-Malas-e-Sistan	IPP27
28	Berit-Poust-Ghermez-Malas-e-Fars	IPP28
29	Vashik-Toursh-e-Sistan	IPP29
30	Galou-Koutah-e-Yazdi	IPP30

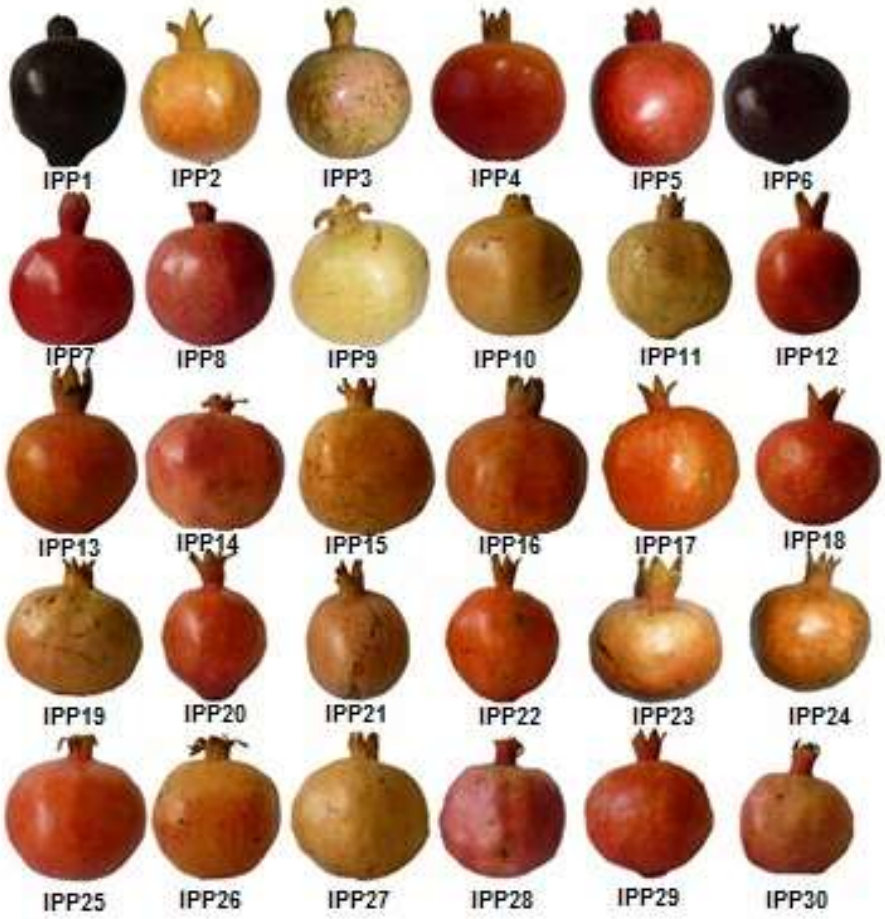

Fig. 1. Thirty Iranian pomegranate genotypes collected from the Agriculture and Natural Resources Research Center of Yazd

Determination of total flavonoid content (TFC) and total phenolic content (TPC)

The method of Shirazi *et al*. (Shirazi, Khattak, Shukri, & Nasyriq, 2014) was employed to determine TFC. 25 µL of extract (1000 ppm), 125 µL of Folin–Ciocalteu solution, and 100 µL of 7.5% sodium carbonate solution were mixed together and placed in the dark at room temperature. After two hours the absorbance of the samples was read at 760 nm. The results were displayed as mg rutin (RE)/ g DW as a mean of five replicates.

A modified method of Folin–Ciocalteu was used for the assessment of TPC in PPE. (Singleton, Orthofer, & Lamuela-Raventós, 1999). 7.5 µL of sodium nitrite (5%) was added to 25 µL of the extract (1000 ppm). Then 7.5 µL of aluminum chloride solution (10%) and 100 µL of sodium hydroxide solution (4%) were added. After 15 minutes, the absorbance of the solutions was read at 510 nm. The reference standard was gallic acid (GAE), and the results were demonstrated as mg GAE/ g DW for five replicates.

DPPH radical scavenging assay

Antioxidant activity of different PPE was measured with DPPH according to the method reported by Ganesan *et al*. (Ganesan, Kumar, & Rao, 2011). In order to measure the antioxidant activity, 1000 µg/ml solution of gallic acid and peel extract of each genotype was prepared. Five different concentrations of extract and standard were prepared in a 96-well plate and DPPH reagent was added to each one. The plates were placed in the dark at room temperature for 30 minutes. The absorbance of each well was read at 517 nm. The mean value

of three replicate was used for the calculation of EC50 (Effective Concentration of 50%, interpreted as a concentration required for 50% scavenging activity) from the dose-response curve. For positive control, a standard of gallic acid was used.

HPLC-DAD and LC-MS analysis of pomegranate peel extracts

20 µL of each sample was injected into a Waters high-performance liquid chromatography (USA) coupled with a photodiode array (PDA) detector. The separation was carried out with a C_{18} Column (Waters SunFire C_{18} Column, 100Å, 3.5 µm, 4.6 mm×150 mm). Data acquisition was made with a DAD detector in the range from 200 to 700 nm, and analytes were recorded at 258, 280, 360 and 520 nm. A gradient elution at 0.5 mL/min to analyzing phenolic compounds in the standards and examined samples was used. The mobile phases, including water with 0.02 % TFA as eluent A and methanol with 0.02 % TFA as eluent B. The samples were eluted by following the gradient program starting with 98% A and 2% B for 5 min, 50% A and 50% B until 30 min, 0% A and 100% B at 38 min, and finally, 98% A and 2% B until 42 min. The standards used for quantitative analysis were: ellagic acid, punicalagins α and β. A mixture of two standard compounds (punicalagin anomers and ellagic acid). The peak areas were plotted versus ppm concentration, with good correlation coefficients (R^2=0.989 and 0.999). PPE samples were also spiked with standards before injection.

LC-MS was used for the characterization of phenolic compounds in PPE using an Agilent HPLC 1200 system (Agilent, Germany) equipped with ChemStation software. The separation was carried out with a C18 Column (Waters SunFire C18 Column, 100Å, 3.5 µm, 4.6 mm×150 mm). The mobile phases, including water with 0.02 % TFA as eluent A and methanol with 0.02 % TFA as eluent B. The samples were eluted by following the gradient program starting with 98% A and 2% B for 5 min, 50% A and 50% B until 30 min, 0% A and 100% B at 38 min, and finally, 98% A and 2% B until 42 min. 20 µL of each sample was injected .A gradient elution at 0.5 mL/min to analyzing phenolic compounds in the standards and examined samples was used. To acquire mass spectra, the HPLC system was coupled to the mass spectrometer (Finnigan™ LCQ™ DECA ion trap). An electrospray ionization device was used for sample analyses (sheath gas: 40 mL min^{-1}, auxiliary gas: 20 mL min^{-1}, spray voltage: 5 kV, capillary temperature: 150°C, capillary voltage: 15 kV, and tube lens: 30 kV). The Xcalibur 2.0 SR2 software (copyright Thermo Electron Corporation 1998–2006) was used for spectra acquisition and processing.

Statistical analysis

To measure the significance of differences among 30 IPPs regarding individual phenolic acids composition, multiple-range tests and one-way analysis of variance (ANOVA) was utilized. Tukey's HSD (Honestly Significant Difference) test was used to discriminate among the means. SPSS 25.0 for Windows were performed for statistical analyses. Hierarchical clustering analysis (HCA) with heatmap based on complete method was performed using "gplots" package in the R program, respectively.

Results and Discussion

Determination of antioxidant activity, TFC, and TPC

TFC and TPC of thirty IPPs are shown in Table 2. The ethanol extract of the PPE showed TPC ranged between 66.4 and 181.4 mg GAE/ g DW. Also, TFC ranged between 38.5 to 144.1 mg RE/ g DW. Among the analyzed genotypes, peel extracts of IPP24 (172.1 ± 0.6 mg GAE/ g DW, 133.5 ± 2.4 mg RE/ g DW), IPP2 (170.1 ± 1.7 mg GAE/ g DW, 144.1 ± 3.1 mg RE/ g DW) and IPP23 (161.5 ± 0.7 mg GAE/ g DW, 131.6 ± 3.1 mg RE/ g DW) showed the highest TPC and TFC content (Table 2). Our results were higher than (89.7- 179.92 mg GAE/g FW) the genotypes reported by Russo *et al*. They reported total phenolic compounds of different

PPE with different methods of extraction (*Russo et al*., 2018). Li *et al*. reported that TPC in PP was between 205.1 and 261.7 mg GAE/g (Li *et al*., 2006). Although a comparison of the values reported in other studies is difficult because they are related to different genotypes, analytical methods, environmental conditions and maturity stages. Our results are similar to values reported by Young *et al*. (52.9-134.2 mg GAE/g) for American genotypes (Young *et al*., 2017), Diamanti *et al*. (150.6 mg GAE/g) for Greek pomegranates (Diamanti, Igoumenidis, Mourtzinos, Yannakopoulou, & Karathanos, 2017), Ali *et al*. (TPC: 103.2 mg GAE/g and TFC: 132.4 mg RE/g) for methanolic peel extract of pomegranate (Ali, El-Baz, El-Emary, Khan, & Mohamed, 2014), while it is lower than the value measured by Hasnaoui *et al*. (208.3-276.3 mg GAE/g) for PPEs of 12 genotypes grown in Tunisia (Hasnaoui, Wathelet, & Jiménez-Araujo, 2014). Antioxidant capacity has been linked to a reduced risk of developing many chronic diseases, such as cancer, diabetes, obesity, and cardiovascular diseases. An effective concentration that requires to increase the initial DPPH concentration by 50 % is defined as EC50 value and better protection has come from a lower EC50 value. (Konsoula, 2016). DPPH scavenging based antioxidant activity was tested for all PPE and GA was used as a reference antioxidant compound. The results expressed as EC_{50} (µg/ml) for studied PPE are summarized in Table 2. There are significant differences in the antioxidant activity between PPEs. DPPH values ranged between 4.1 and 14.4 µg/ml for PPEs. These are in agreement with previously reported studies in comparison with GA (26.9 µg/ml). Among the analyzed genotypes, peel extracts of IPP23 (4.1 µg/ml) and IPP21 (4.4 µg/ml) showed the highest free radical scavenging activity. Our results showed that the peel extracts of the studied Iranian genotypes show an antioxidant capacity comparable to synthetic antioxidants such as gallic acid. Also, PPEs can be used as an alternative antioxidant to protect food against oxidative degradation. These results are close to that of Panichayupakarananta *et al*. work that reported the antioxidant activity (EC_{50}) for two different varieties from Israel and Italy at 3.1 µg/ml and 3.6 µg/ml, respectively (Panichayupakarananta, Issuriya, Sirikatitham, & Wang, 2010). Masci et al. reported that the antioxidant activity of peel extract of the Chinese genotype was 5.8 µg/ml (Masci et al., 2016). Also, Kazemi *et al*. reported higher yield and antioxidant activity of PPEs, and found 5.5 µg/ml as the highest potency of antioxidants by optimization of a pulsed ultrasound-assisted extraction method (Kazemi, Karim, Mirhosseini, & Hamid, 2016). Our finding differs from Indian pomegranate (16.8 µg/ml) reported by Jag Pal. Differences might be due to growing conditions, harvesting, and region (Pal *et al*., 2017). Also, Okonogi *et al*. (Okonogi, Duangrat, Anuchpreeda, Tachakittirungrod, & Chowwanapoonpohn, 2007) reported that PPE had the highest antioxidant activity (IC_{50} of 3µg/mL) among the other eight fruit peel extracts. Thus, it could be concluded that the difference between our obtained data and literature (in terms of TFC, TPC, and antioxidant activity) might be due to differences in fruit ripening, soil composition, latitude, temperature changes, rainfall, and light of different regions. However, this achievement can be used for breeding purposes of this plant (Parcerisa *et al*., 1995).

The highest antioxidant activity of PPE may occur due to its highest polyphenolic compounds, such as ellagitannins, ellagic acids, and gallic acids. Many studies reported that extracts prepared from PP have a phenolic content of 10–45 fold higher than that found in the pulp. The contents of flavonoids and antioxidants were also higher in PPE than in pulp extract. Also, the results of several studies confirm that the antioxidant property of PPE is more than pulp extract (Ali *et al*., 2014; Hasnaoui *et al*., 2014; Russo *et al*., 2018).

Table 2- Total Phenolic content (TPC), Total Flavonoids Contents (TFC) and DPPH Radical Scavenging Assays of PP from different genotypes

Samples	TPC (mg GAE/g)	TFC (mg RE/g)	DPPH EC_{50} (µg/ml)
IPP1	129.8 ± 1.1	96.4 ± 2.6	6.7 ± 0.2
IPP2	170.1 ± 1.7	144.1 ± 3.1	6.1 ± 0.1
IPP3	112.2 ± 2.4	59.3 ± 3.0	10.8 ± 0.1
IPP4	100.1 ± 2.9	56.4 ± 3.6	10.5 ± 0.1
IPP5	89.8 ± 1.3	61.0 ±2.5	9.5 ± 0.1
IPP6	134.4 ± 1.0	84.8 ± 1.9	4.5 ± 0.1
IPP7	86.47 ± 1.0	40.4 ± 3.3	8.0 ± 0.1
IPP8	134.2 ± 0.7	62.7 ± 3.0	7.2 ± 0.1
IPP9	113.3 ± 1.1	71.6 ±1.9	13.6 ± 0.3
IPP10	181.4 ± 2.9	82.3 ± 3.7	14.4 ± 0.3
IPP11	158.8 ± 2.2	95.4 ± 3.3	10.7 ± 0.2
IPP12	89.9 ± 1.0	85.4 ± 0.6	7.5 ± 0.2
IPP13	107.7 ± 0.8	89.8 ± 3.5	7.4 ± 0.2
IPP14	126.9 ± 0.9	77.3 ± 3.1	9.5 ± 0.2
IPP15	114.4 ± 1.0	91 ± 1.8	7.4 ± 0.2
IPP16	112.4 ± 0.9	79.8 ± 2.5	9.5 ± 0.2
IPP17	66.4 ± 0.9	38.5 ± 2.5	5.6 ± 0.3
IPP18	110.1 ± 0.8	83.5 ± 0.1	6.3 ± 0.2
IPP19	113.1 ± 1.4	75.4 ± 0.6	5.5 ± 0.3
IPP20	122.1 ± 0.7	75.2 ± 1.6	6.3 ± 0.2
IPP21	136.5 ± 0.7	66.0 ± 1.0	4.4 ± 0.3
IPP22	112.8 ± 1.7	69.1 ± 1.9	9.3 ± 0.2
IPP23	161.5 ± 0.7	131.6 ± 3.1	4.1 ± 0.3
IPP24	172.1 ± 0.6	133.5 ± 2.4	5.6 ± 0.3
IPP25	87.2 ± 0.4	40.4 ± 3.1	6.5 ± 0.3
IPP26	94.1 ± 1.4	60.4 ± 0.6	6.4 ± 0.2
IPP27	154.6 ± 1.4	97.9 ± 1.9	5.8 ± 0.2
IPP28	103.0 ± 0.5	54.8 ± 1.8	11.2 ± 0.2
IPP29	117.5 ± 1.1	58.9 ± 3.3	5.4 ± 0.3
IPP30	81.2 ± 1.2	53.9 ± 0.6	4.7 ± 0.3
Gallic acid	-	-	26.9 ± 0.3

Chromatographic profiling of pomegranate peel polyphenolic constituents

HPLC-DAD and LC-MS were used for the quantification and metabolite profiling of active ingredients in PPE from various genotypes. Fig. 2 presents data related to the characteristic peaks of PPE. The HPLC-DAD chromatograms and retention time order and their corresponding mass spectrum in the negative mode of ionization were used for peak annotation. Also, punicalagin α/β and ellagic acid are confirmed by authentic reference material.

Based on LC-MS and HPLC-DAD data, nine phenolic compounds, including ellagitannin α/β (1 and 2), two corresponding pedunculagin I isomers (3 and 4), punicalin (5), ellagic acid derivatives (ellagic acid (6), ellagic acid glucoside (7), ellagic acid deoxyhexoside (8), and ellagic acid pentoside (9) were identified (Fig. S1 & S2). The major compounds in the peel extract were quantified in the examined genotypes (Table 3 and S1). Gullon *et al*. determined the antibacterial activity and polyphenolic profile of PP. The HPLC analysis of PP showed eight phenolic compounds that punicagalin and ellagic acid were the main components (Gullon, Pintado, Pérez-Álvarez, & Viuda-Martos, 2016). Identification and quantification of phenolic compounds in different parts of pomegranate were reported by Fischer *et al*. In their study, based on their HPLC-PAD and ESI/MS^n, they

detected 48 compounds. Among them, ellagitannins, gallotannins, hydroxybenzoic acids, gallagyl esters, hydroxycinnamic acids, and dihydroflavonol were identified (Fischer, Carle, & Kammerer, 2011).

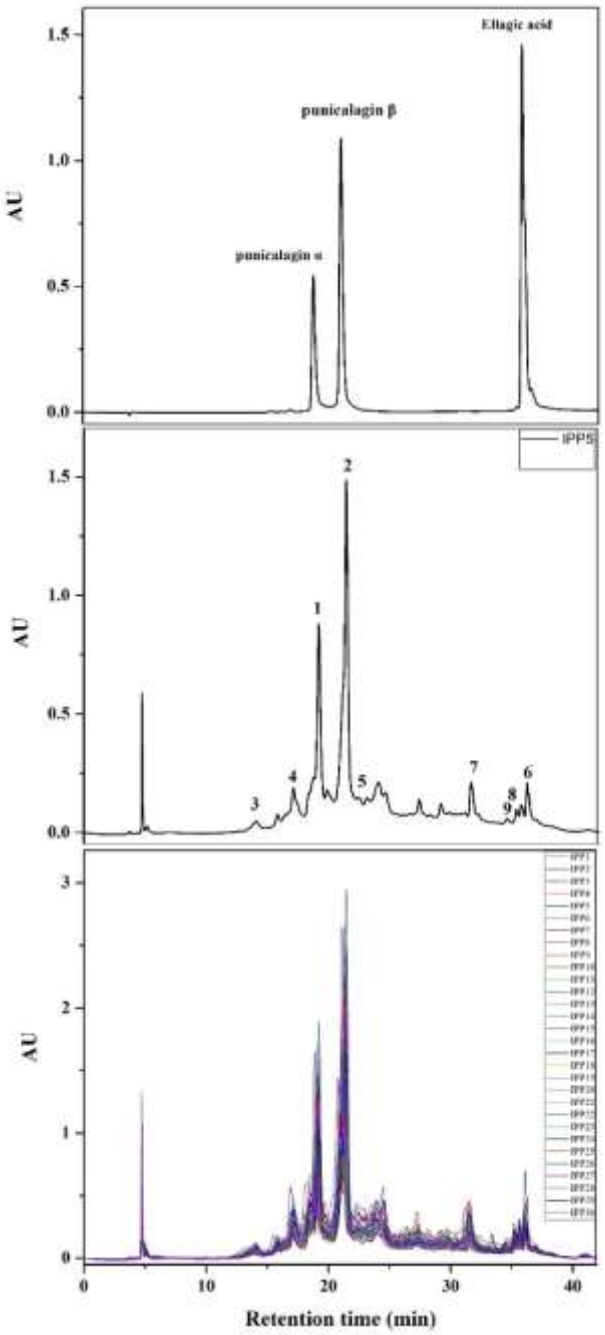

Fig. 2. HPLC-DAD chromatograms of standards and PP extracts in 280 nm

Table 3- Concentration variation of polyphenolic compounds among the peel of thirty studied pomegranate genotypes

Id.	Compound	R_t (min)	λ_{max} (nm)	m/z ([M - H]$^-$)	Concentration (µg/mg dry sample)		
					Range	Average	SD
1	punicalagin α	19.13	258/377	1083	13.9 – 30.1	20.8	4.5
2	punicalagin β	21.30	258/377	1083	20.8 – 48.7	30.6	6.3
3	pedunculagin I isomer	14.04	258/377	783	1.7 – 7.0	3.5	1.3
4	pedunculagin I isomer	18.53	258/377	783	5.3 – 16.9	9.7	2.6
5	Punicalin	22.30	258/377	783	3.4 – 15.7	6.7	3.1
6	Ellagic acid	36.07	254/364	301	1.6 – 13.4	5.9	2.5
7	Ellagic acid glucoside	31.41	254/364	463	2.7 – 20.8	8.8	4.3
8	Ellagic acid deoxyhexoside	35.17	254/364	447	0.7 – 5.1	2.7	1.2
9	Ellagic acid pentoside	34.43	254/364	433	0.1 – 5.9	2.1	1.4

Significant differences were found among genotypes in the studied phenolic compounds ($P<0.01$). Metabolomics as complete metabolite fluctuation found punicalagin β (20.8–48.7 µg/mg DW) followed by punicalagin α (13.9–30.1 µg/mg DW) and ellagic acid (1.6–13.4 µg/mg DW) as main components in PPE samples (Table S1). These results showed that PPE is a rich source of ellagitannins, especially two isomers of punicalagin (α/β). However, the differences with ellagic acid content were extremely high (8-fold). The total content of punicalagins and the content of each isomer was determined by Lu *et al*., and their results showed that the mean value of punicalagin content is 82.4 mg /g for 16 pomegranate genotypes (Lu, Ding, & Yuan, 2008). Our findings also are consistent with the literature (Aqil *et al*., 2012; Kazemi *et al*., 2016; Russo *et al*., 2018).

Similar to our results, many studies have also reported that ellagic acid and its derivatives are spread in PP (Gullon *et al*., 2016; Nuncio-Jáuregui *et al*., 2015; Russo *et al*., 2018). Russo *et al*. reported that about 39.7 to 84.2 percent of the main components of PP samples are ellagitannins, which are found at lower percentages also in pomegranate juice and pulp. More than 70% of PP ellagitannins are related to two anomers of punicalagin (α and β) and two isomers of ellagic acid glucoside, which are very close to our results (Russo *et al*., 2018). The content of punicalagin (116.6 mg/g), ellagic acid, and the other ellagic acid derivatives (4.5 mg/g) in PP extracted by pressurized water were reported by Çam and Hışıl (Çam & Hışıl, 2010). Our results for punicalagin correspond to these results for most genotypes, but the sum of ellagic acid was higher than that of the values reported by Çam and Hışıl. It may be attributable to the low solubility of ellagic acid in water compared to ethanol/water, which is used as an extraction solvent in our study. Peel extract of IPP23 showed the highest amount of polyphenolic compounds, such as punicalagin anomers and ellagic acids, than the other studied pomegranate genotypes .

According to several studies, the biological activities of PPE, such as antibacterial or antioxidant properties, anticarcinogenic and antimutagenic properties, are validated by these bioactive compounds (Al-Zoreky, 2009; Wu, Ma, & Tian, 2013). However, the concentration and type of these valuable components depend on different parameters such as genotype analyzed, environmental conditions, maturity stages, etc.

Hierarchical clustering analysis (HCA) with heatmap

A heatmap is a way to visualize hierarchical clustering where data values are transformed to color scale. Also, heatmaps allow us to simultaneously visualize clusters of samples and measured traits. In the present study of HCA with heatmap, the row tree represents the thirty pomegranate genotypes, the column tree represents the measured phenolic compound, and the colors represent the intensities or values

of the data set (Fig. 3)(Shameh, Alirezalu, Hosseini, & Maleki, 2019)

As shown in Fig. 3, the measured phenolic compounds are divided into three groups. The first group includes punicalin, pedunculagin I isomer 1, and ellagic acid glucoside. Punicalagin α and β are in the second group. The third group includes pedunculagin I isomer 2, ellagic acid, ellagic acid pentoside, and ellagic acid deoxyhexoside. The examined genotypes were divided into four groups. The first group had one accession, IPP23 resulting in clustering analysis presented in Figure 3, demonstrating clear discrimination between IPP23 and different genotypes and falls into a separate group. As mentioned in the previous sections, IPP23 has a higher content of polyphenolic compounds like punicalagin α/β, Ellagic acid glucoside, and other punicalagin derivatives. Also, the amount of punicalin in this genotype is low. The second group consisted of 11 genotypes, IPP1, IPP2, IPP6, IPP10, IPP11, IPP15, IPP19, IPP22, IPP24, IPP26, and IPP27. Among the genotypes of this group, IPP1, IPP2, IPP6, IPP15, IPP24, IPP26, and IPP27 are in the separate subgroup. These genotypes have a high content of polyphenolic compounds such as punicalagin and ellagic acid and their derivatives. The genotypes in another subgroup have similar amounts of punicalin and punicalagin β.

The third group included five genotypes, IPP12, IPP13, IPP21, IPP25, and IPP28. The genotypes of this group have the lowest levels of punicalagin α and β, as shown in Table S1. The two genotypes IPP21 and IPP28, which are in a separate subgroup, have high and the same amounts of punicalin and ellagic acid. The last group consisted of IPP3, IPP4, IPP5, IPP7, IPP8, IPP9, IPP14, IPP16, IPP17, IPP18, IPP20, IPP29, and IPP30. The genotypes in this group have the lowest values of punicalin, punicalagin α/β, pedunculagin I isomer1, ellagic acid, ellagic acid and its derivatives and are in a separate cluster. Genotype IPP18, which is in a separate subgroup, has more punicalin than other genotypes.

The results of these classifications showed that punicalagin α/β, ellagic acid had an important role in grouping and differentiation between 30 genotypes.

Conclusion

Following quantitative analysis of the main phenolic compounds in thirty Iranian pomegranates, significant differences in their amount and ratio were observed. Our results showed that IPPs are important sources of phenolic compounds. Among all pomegranate peels, IPP23 (Kabdar Shirin Behshahr) showed the highest quantity of polyphenolic compounds. Considering the parameters measured in the genotypes, IPP23, which has a high amount of measured phenolic compound, can be introduced as the superior genotype. Also, according to the results of this study, PPE had higher antioxidant properties against DPPH than gallic acid standard. It should be noted that the IPP23 extract showed the most significant free radical scavenging capability among other examined genotypes. Here, as all plants were grown at the same location and under the same agrotechnical approach, we can attribute most of the diversity in secondary metabolites to genetic background, even if mediated by the varied susceptibility to the same environmental influences. Eventually, using PP as a valuable natural substance that may act as supplement, prebiotic, food preservative, food additive, stabilizer, and quality-enhancing agent is a new and practical approach to preventing some chronic diseases. Therefore, based on this study, PP can be recommended as a strong source of antioxidants to stabilize food systems.

Conflict of Interest

Authors has no any conflict of interest.

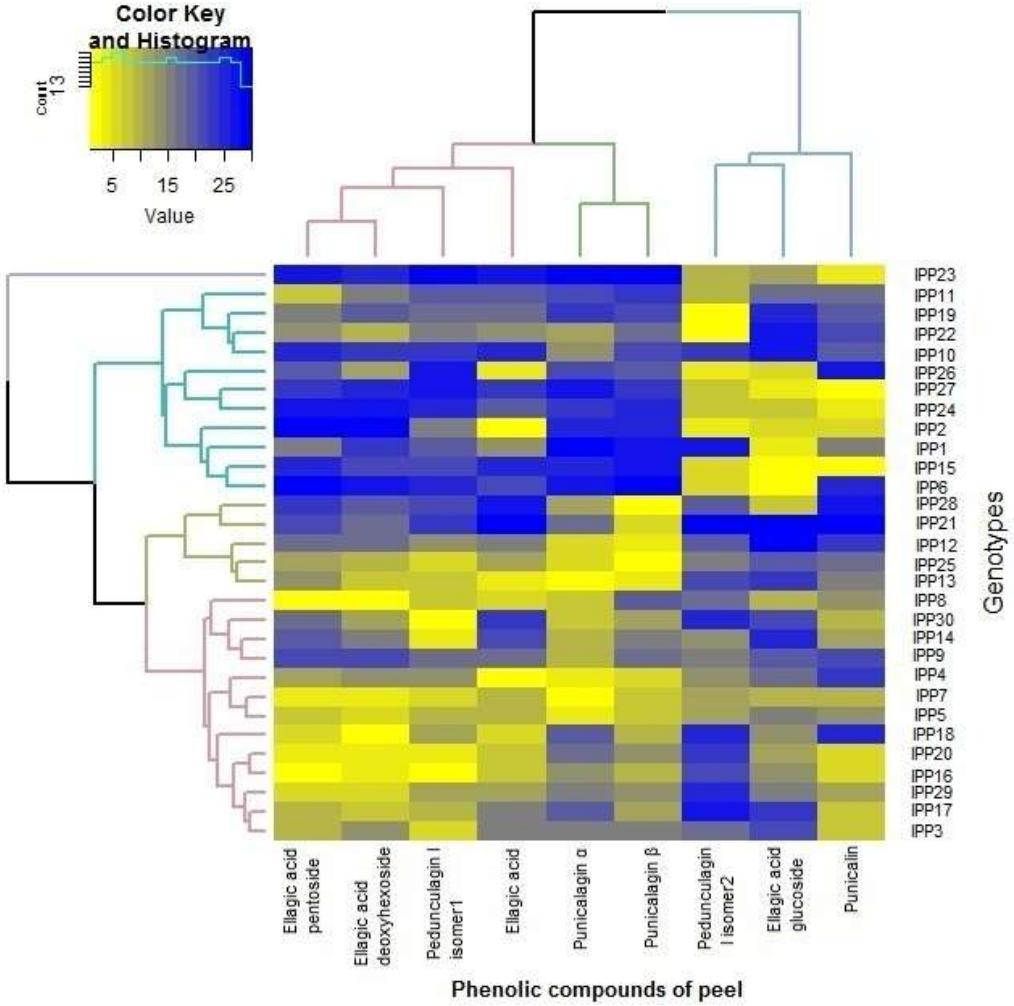

Fig. 3. Hierarchical cluster analysis (HCA) of pomegranate genotypes based on phenolic compounds in peel

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بررسی خصوصیات فیتوشیمیایی پوست ژنوتیپ‌های انار ایرانی و معرفی آن به‌عنوان افزودنی غذایی طبیعی

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چکیده

امروزه، استفاده از افزودنی‌های طبیعی به‌عنوان جایگزینی برای افزودنی‌های سنتزی در صنایع غذایی افزایش یافته است. در این مطالعه به بررسی پتانسیل عصاره پوست انار به‌عنوان یک افزودنی طبیعی غذایی پرداخته شد. پوست انار (*Punica granatum* L.) محصول جانبی میوه انار است که خواص تغذیه‌ای، عملکردی و ضد عفونی کننده‌ای آن در ژنوتیپ‌های مختلف متفاوت است. فعالیت مهار رادیکال آزاد (DPPH)، محتوای فنل و فلاونوئید کل در پوست سی ژنوتیپ انار ایرانی مورد بررسی قرار گرفت. همچنین به‌منظور یافتن تنوع در خصوصیات فیتوشیمیایی پوست ژنوتیپ‌های انار از دستگاه HPLC-DAD-MS استفاده و تعداد نه ترکیب فنلی شناسایی و تعیین مقدار شدند. ترکیبات اصلی پوست انار شامل پونیکالاژین β (۴۸/۷-۲۰/۴۸ میکروگرم بر میلی‌گرم)، پونیکالاژین α (۳۰/۱-۱۳/۹ میکروگرم بر میلی‌گرم) و الاژیک اسید (۱۳/۴-۱/۶ میکروگرم بر میلی‌گرم) می‌باشند. ژنوتیپ IPP23 (کابدار شیرین بهشهر) بیشترین مقدار ترکیبات فنلی در بین سایر ژنوتیپ‌ها را دارد. میزان ترکیبات فنلی کل (۱۸۱/۱- ۶۶/۴ میلی‌گرم گالیک اسید به ازای یک گرم پودر خشک گیاه)، ترکیبات فلاونوئیدی کل (۱۴۴/۱-۳۸/۵ میلی‌گرم روتین به ازای یک گرم پودر خشک گیاه) و خاصیت آنتی‌اکسیدانی عصاره‌های مختلف (۱۳/۹-۳/۸ (میکروگرم در میلی‌لیتر) تعیین شدند. نتایج نشان می‌دهد که خاصیت آنتی‌اکسیدانی عصاره‌های پوست انار به‌طور قابل توجهی بالاتر از استاندارد گالیک اسید است. در نهایت در بین ژنوتیپ‌های مورد بررسی، ژنوتیپ IPP23 به‌عنوان منبع مهم افزودنی‌های طبیعی تعیین شد.

واژه‌های کلیدی: آنتی‌اکسیدان، پلی‌فنل، فیتوشیمیایی، میوه انار، نگهدارنده مواد غذایی

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