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Original Article

Diversity of Phenolic Profiles in Peel of an Iranian Pomegranate Cultivar (*Punica granatum* L.)

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Abstract

Pomegranate (*Punica granatum* L.) is one of the oldest known fruits native to Iran. In addition to multiple studies carried out on different parts of pomegranate, the peels are noted due to various phytochemicals in different colors and regions. In the present study, major anthocyanins and non-anthocyanins of an Iranian black pomegranate cultivar (i.e., pomegranate samples of Ghaemshahr, Iran) were analyzed using high-performance liquid chromatography-mass spectrometry. In total, 30 compounds were obtained from pomegranate peels. Among these compounds, 5 anthocyanins, 1 gallotannin, 15 ellagitannins, 4 hydroxybenzoic acids, 2 gallagyl esters, 1 dihydroflavonol, and 7 hydroxycinnamic acids were identified based on their fragmentation patterns and ultraviolet spectra. Among the varieties of Iranian pomegranates, the anthocyanins of peonidin-hexoside and cyanidin-pentoside were identified in this species for the first time. Peonidin-hexoside, caffeic acid, coumaric acid-hexoside, and galloyl-hexoside were major phenolic compounds. In addition, the antioxidant activity of different fractions was tested using DPPH free radical scavenging *in vitro* assay (IC_{50}: 133.13-557.23 µg/mL). The obtained results of this study highlighted that this cultivar can be an important candidate for future new drug discoveries.

Keywords: Punica granatum, peel, anthocyanin, non-anthocyanin phenolic compounds, HPLC-MS/MS, antioxidant.

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1. Introduction

Pomegranate (*Punica granatum* L.) is consumed as a popular fruit and juice due to the potential health benefits and phytochemicals. It is native to the Mediterranean region and is cultivated in subtropical and tropical regions around the world, such as Iran, California, Turkey, Egypt, Italy, India, Chile, and Spain [1]. Pomegranate peels (PPs) remain as byproducts after juice extraction. At present, fruit peels have become attractive due to beneficial phytochemicals and are utilized in pharmaceutical, food, and cosmetic industries [2, 3]. In PPs, the main phenolic compounds (i.e., one class of

bioactive phytochemicals) previously reported in the literature include phenolic acids (i.e., chlorogenic, syringic, caffeic, sinapic, ferulic, p-coumaric, ellagic, cinnamic, and gallic acids), tannins (i.e., ellagic acid derivatives, such as punicalin, punicalagin, pedunculagin, and ellagitannins), and flavonoids (i.e., anthocyanins, such as delphinidin, pelargonidin, cyanidin, and their derivatives, and anthoxanthins, such as epicatechin, catechin, and quercetin) [3-5]. Pomegranates have a large variety of colors from white to red and dark red. The red color and mild astringency are the characteristics of pomegranates related to polyphenol constituents [6]. The color of PPs is due to the presence of anthocyanins (i.e., one class of flavonoids). Cultivars with a dark red color possess higher antioxidant activity than other portions [4, 7]. The identification and characterization of secondary metabolites in this fruit are important due to their role in reducing the risk of diseases, such as cancer, inflammation, and cardiovascular and neurodegenerative diseases [8-10]. Previous studies have reported a number of methods for the identification of phenolic compounds in pomegranates [11]. Recently, particular attention has been given to high-performance liquid chromatography (HPLC) with mass spectrometric detection due to the limited availability of reference standards and difficult identification of compounds only based on ultraviolet spectra [12, 13]. Although there have been many studies carried out on the evolution of phenolic compounds in pomegranate cultivars grown in Iran and other countries, there are a limited number of studies are on the profiles of these compounds in the Iranian cultivar. In this paper, considering the structural diversity of the active compounds of pomegranates, a cultivar of *p. granatum* with black color was selected to investigate the diversity of anthocyanins and non-anthocyanins in PPs using the HPLC and mass spectrometry (MS) techniques. Firstly, different fractions of PPs (i.e., methanol, ethanol acetone, and ethyl acetate) were evaluated for antioxidant activity using 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging *in vitro* assay. Among different fractions of PPs, the methanolic and ethanolic extracts were chosen for the characterization of phenolic compounds due to the highest amount of antioxidant activity.

2. Materials and Methods

2.1. Solvents and Reagents

All reagents and solvents used for HPLC-MS separation and identification were purchased (Merck & Co., Inc., Germany) with analytical or HPLC grade. Moreover, all other solvents for extraction were also purchased (Merck & Co., Inc., Germany).

2.2. Plant Materials

Iranian pomegranate fruits of unknown cultivar obtained from Ghaemshahr, Iran, were washed and steamed (5 min) for enzyme inactivation. A voucher number (803885) was deposited in the Herbarium of Gonbad Kavous University, Gonbad, Iran.

2.3. Extracts Preparation

At first, the peels were lyophilized and ground with a blender. Then, each extract was obtained as it will be described. Subsequently, the dried powder was separately extracted with ethyl acetate, acetone, methanol, and ethanol for the evaluation of the antioxidant activity.

2.4. Quantification of Antioxidant Activity

The antioxidant potential of pomegranate fractions (i.e., methanol, ethanol acetone, and ethyl-acetate) was determined by DPPH free radical scavenging assay. The DPPH reduced by an antioxidant, which can donate hydrogen. The changes in color were measured from deep violet to light yellow using an ultraviolet-visible light spectrophotometer. Firstly, dried powder of peel was separately extracted with ethyl acetate, acetone, methanol, and ethanol. In order to determine the radical scavenging capacity of the different concentrations of extracts against stable DPPH, a slightly modified method was spectrophotometrically used as it will be described [14]. Briefly, 50 µl of different concentrations of the obtained extracts (35-250 µg/ml) were mixed with 50 µl of the 0.1 mM solution of methanolic DPPH. The 50-µL methanol was employed as a control in the experiment. After 30 min of incubation at 37ºC, the absorbance of the samples using a microplate reader was spectrophotometrically read at 517 nm. Butylated hydroxyl toluene (BHT) within the range of 35-250 µg/ml was used as a positive control. The antioxidant activity as percentage inhibition was calculated using the following equation:

$$\%\text{DPPH scavenging} = [A_c\text{-}A_s/A_c] \times 100$$

where A_s is the absorbance of the sample, while A_c is the absorbance of control. Different concentrations of extracts inhibiting 50% DPPH (IC_{50}) were calculated by the concentration of the extracts against the inhibition (%) of DPPH.

2.5. Extraction of Anthocyanins

The peel powder (1.0 g) was sonicated in 30 mL of methanol (containing 0.1 % HCl) for 30 min. The obtained hydroalcoholic extract was centrifuged at 5,000 rpm for 30 min and dried in a rotary evaporator under vacuum at 35°C [7].

2.6. Extraction of Non-anthocyanin Polyphenolics

The plant extract was prepared as described in a study by Zahin et al. [15]. The methanolic and ethanolic extracts of *P. granatum* were gained by a continuous stirring of the dried peels at ambient temperature for 2 days. Furthermore, at the end of the extraction, the extracts were separately filtered to make methanol and ethanol fractions, and the fresh solvent was added to the plant materials. The obtained extracts were concentrated using a rotary evaporator for dryness. A crude extract was obtained after the removal of the extraction solvent and stored at 4°C in the dark until further testing. A stock solution of 1 mg/ml in the water of LC-MS grade was prepared and 2 µl was injected for LC-MS analysis.

2.7. High-Performance Liquid Chromatography-Mass Spectrometry (LC-MS)

The method of Zahin et al. [15] was used for the LC-MS analysis of the secondary metabolites. The HPLC (Shimadzu, Tokyo, Japan) system coupled with a mass spectrometer, which combines an AB SCIEX 3200 Q-TRAP with a Turbo V electrospray ionization (ESI) source, was developed in the present study. All the experiments were performed using a Thermo scientific liquid chromatography system connected to ESI. The separation was carried out on a C_{18} reversed-phase column (SUPELCO Analytical HS-C18, 4.6×150 mm, 3 µl, Sigma-Aldrich, USA). The mobile phase consisted of gradient program water and methanol within the range of 20-100% over 25 min with a flow rate of 0.5 mL/min. The MS was carried out in negative ion mode using ESI source with some conditions (i.e., a capillary voltage of −10V, capillary temperature of 200ºC, and Sheath and Auxiliary Gas flow [N_2]). The MS spectra were acquired by full range acquisition covering m/z 50-2000. MZmine analysis software package (version 2.3) was used for instrument control, data acquisition, and data analysis [16].

2.8. Statistical Analysis

Statistical analysis was carried out using Excel software with three replications. All the obtained results were presented as mean±standard deviation. Moreover, a p-value of 0.05 was considered statistically significant.

3. Results and Discussion

3.1. Antioxidant Activity

The PPs have antioxidant activities due to a good source of phenolic compounds (e.g., anthocyanins, quercetin, catechin, gallotannins, ferulic acid, ellagitannins, and ellagic and gallic acids). The obtained results of the current study are in agreement with the antioxidant activities of PPs reported in the literature from different regions. The findings of studies have shown that methanol, acetone, and aqueous extracts of Tunisian cultivars have antioxidant activity with IC_{50} values of 1.9-1900 µg/mL [5, 17-21]. The range of IC_{50} values has been reported at 15-27 µg/mL for the methanol extracts of Persian pomegranate cultivars [22-24]. The methanol and aqueous extracts of PPs collected from different regions of India showed antioxidative potential with IC_{50} values of 4.9-8730 µg/mL [15, 25-27]. The ethyl acetate and ethanol extracts of two types of PPs obtained from Italy exhibited antioxidant potential with IC_{50} of 2.430 µg/mL and 94.7% radical inhibition, respectively [28, 29]. The methanol and aqueous fractions obtained from Egyptian, South African, and Turkish cultivars displayed antioxidant capacity (range: 71.65-96.24%) [28, 30-33]. In addition, the peels of Peruvian, Chinese, Georgian, Spanish, and Mauritian pomegranates were reported to have radical scavenging activities with Trolox equivalents of 5.20-2239 mmol TE/kg [11, 34-37]. In the present study, the antioxidant potential of the peel extracts was measured using the DPPH method. The scavenging activity was evaluated at different concentrations (range:

35-250 µg/ml). These fractions inhibited DPPH absorption (methanol: 92.28%, ethanol: 86.55%, acetone: 75.1%, ethyl acetate: 21.72%, and positive controls [BHT]: 88.1%; Figure 1). Furthermore, the extracts exhibited strong activity to reduce the DPPH with IC_{50} values of 133.13-557.23 µg/ml (Table 1). The activity of methanolic and ethanolic extracts was stronger than that reported for the BHT with IC_{50} values of 141.25 µg/ml. Therefore, these fractions were selected for the analysis of phenolic compounds. The promising antioxidant activity of the extracts might be conferred by phenolic compounds identified through LC-MS analysis, mainly phenolic acids, such as hydroxycinnamic acids and ellagitannins.

3.2. LC-ESI/MS Analysis of Extracts of P. granatum Peel

The extracts of *P. granatum* peels were analyzed using Liquid Chromatography Electrospray Ionization Mass Spectrometry (LC-ESI/MS) in a negative ion mode. In the observed LC-MS profile, deprotonated molecular ions of different phenolic compounds consist of flavonoids, hydroxycinnamic acids, and phenolic acids. These polar compounds were characterized based on the mass patterns and their retention time by comparison to the data obtained from the history of pomegranate different cultivars. The obtained results of the present study are in line with the findings of previous studies carried out on phenolic compounds of PPs from different regions. Flavonoids (e.g., epicatechin, catechin, and rutin), anthocyanins (e.g., cyanidin 3,5-diglucoside and delphinidin 3,5-diglucoside), and hydrolyzable tannins (e.g., hydroxybenzoic acid, gallic acid, and ellagic acid) were identified and quantified from the methanolic extract of South African cultivars [31]. Additionally, the reverse phase-HPLC profile of Tunisian PPs showed the presence of ellagitannins of punicalagin and punicalagin derivatives as major tannin compounds and galloyl-HHDP-hexoside, pedunculagin I, pedunculagin II, granatin A, granatin B, bis-HHDP-gluconic acid (lagerstannin A), p-coumaric, and vanillic acids [17, 21]. Polyphenols of gallic and ellagic acids, punicalagin A, punicalagin B, chlorogenic, ferulic, p-hydroxybenzoic, and p-coumaric acid were reported in the extracts of Turkish PPs [38, 39]. Phenolic acids, anthocyanins, gallotannins, ellagitannins, gallagyl esters, and dihydroflavonols were identified and quantified in the peel of Peruvian pomegranate cultivar [11]. Punicalagin in pomegranate different varieties from Pakistan was reported [32]. Punicalagin, gallic and ellagic acids, punicalagin, and punicalin were the main identified compounds in the peel extracts of Serbian pomegranate [28, 40]. Punicalagin was identified as the major tannin reported in the peel of a Brazilian pomegranate cultivar [41]. Gallagic acid, ellagic acid, gallic acid and punicalagin were the main phenolic compounds reported in the peel of an Israelian pomegranate cultivar [35]. Phenolic compounds of gallagic and ellagic acid derivatives, gallic acid, caffeic acid, catechin, punicalin, punicalagin, and granatin A and B were detected in the PPs of Indian

cultivars using HPLC-MS analysis [15, 24, 25, 29, 42]. Pande and Akoh (2009) were reported caffeic, p-coumaric, ferulic acids and catechin in the peel of Georgian pomegranate [36]. Punicalagins (in alpha and beta forms) and ellagic acid were identified from the methanolic extracts of six Spanish pomegranate cultivars [37]. In addition, gallic and ellagic acids, punicalagin, punicalin, 2,3-(S)-HHDP-D-glucose, castalagin corilagin, punicalin, punicalagin, and granatin A and B were detected by HPLC-MS analysis in the PPs of Indian and Serbian cultivars [40, 42]. In the present study, 35 metabolites (i.e., anthocyanin and non-anthocyanin) were identified and are reported in tables 2 and 3.

3.2.1. Anthocyanins

Data Scan of LC-MS showed that 3, 5-diglucoside derivatives were difficult to detect due to their low concentrations and only 3-glucoside derivatives of cyanidin delphinidin and pelargonidin as cyanidin 3-glucoside, delphinidin 3-glucoside, and pelargonidin 3-glucoside were recorded. The identified anthocyanins, such as peonidin hexoside, cyanidin-pentoside, pelargonidin 3-glucoside, delphinidin 3-glucoside, and cyanidin 3-glucoside at m/z 464, 420, 434, 465, and 450 respectively, were in high-level base on mass data (Table 2). Furthermore, a flavonol, namely myricetin hexoside, with M^+ ion at m/z 481 was detected in the analysis of the anthocyanins of the peel extract. However, there were differences between the present study and previously reported studies regarding other Iranian ecotypes. Zarezadeh reported that Ardestan, Saveh, and Kerman pomegranates contained 5 anthocyanins, mainly cyanidin 3, 5-diglucoside, cyaniding 3-glucoside, pelargonidin 3, 5-diglucoside, pelargonidin 3-glucoside, and delphinidin 3-glycoside; however, cyanidin-pentoside and peonidin hexoside were not detected [43]. This variation could be due to the differences in fruit development stages. Anthocyanins' profiles in the peel demonstrate that cyanidin-pentoside, peonidin hexoside, and myricetin-hexoside have been the first reports to date among the Iranian cultivars. The total chromatograms of the LC-MS analysis of the extracts containing anthocyanins are illustrated in Fig.2. In addition, the chromatogram of the compound of peonidin hexoside as the highest intensity is depicted in Fig.3.

3.2.2. Non-anthocyanins

The qualitative identification of non-anthocyanin polyphenolics in PPs is shown in Table 3. Among the polyphenolics, 30 and 26 compounds were characterized in the methanolic and ethanolic extracts, respectively, as hydrolyzable tannins, such as ellagitannins, gallotannins, and gallagyl esters, in addition to hydroxycinnamic acids, hydroxybenzoic acids, and dihydroflavonols based on comparing their mass spectrometric data to previous data on other pomegranate varieties. According to the results, the methanolic and ethanolic extracts had almost similar constituents. Compounds of protocatechuic acid-derivative, dihydrokaempferol-hexoside, ellagic acid-hexoside, valoneic acid bilactone, ellagic acid

derivative, and galloyl-HHDP-DHHDP-hexoside (granatin B) in the methanolic extract were not detected in the ethanolic extract. Moreover, ellagic acid derivative, bis-HHDP-hexoside (pedunculagin I), and castalagin derivative in the ethanolic extract were not detected in the methanolic extract. The comparison of the results of the presents study and previous data shows that the compounds of galloyl-hexoside, ellagic acid-deoxyhexoside, ellagic acid derivative, ellagic acid dihexoside, ellagic acid derivative, protocatechuic acid, protocatechuic acid-derivative, vanillic acid-hexoside, caffeic acid hexoside, caffeic acid hexoside derivative, caffeic acid derivative, 5-O-caffeoylquinic acid, ferulic acid-hexoside, coumaric acid, and dihydrokaempferol-hexoside reported in previous studies in the juice and mesocarp of this plant, were also observed in the peel extracts. Some of the identified compounds in methanolic extract, including caffeic acid derivative, coumaric acid-hexoside, caffeic acid hexoside, 5-O-caffeoylquinic acid, coumaric acid, HHDP hexoside, protocatechuic acid-derivative, ellagic acid derivatrive, and galloyl-hexoside, were in high levels at m/z 299, 325, 341, 353, 163, 481, 425, 441, and 331, respectively. In addition, chemical compounds in ethanolic extract, including galloyl-hexoside, caffeic acid derivative, coumaric acid, coumaric acid-hexoside, HHDP hexoside, and 5-O-caffeoylquinic acid, were in high levels at m/z 331, 299, 163, 325481, and 353, respectively. The total chromatograms of the LC-MS analysis of the methanolic and ethanolic extracts are illustrated in Figure 2. Furthermore, the chromatograms of the compounds of caffeic acid derivative in the methanolic extract and galloyl-hexoside in the ethanolic extract as the highest intensity are depicted in Fig. 4. Moreover, Table 4 tabulates the structures of anthocyanin and some non-anthocyanin polyphenolics in the pomegranate peel.

4. Conclusion

In this study, the application of HPLC-MS was reported for the characterization of the phenolic compounds of one black pomegranate cultivar obtained from Iran. Firstly, the antioxidant activity of different fractions of PPs (*P. granatum*) was evaluated using DPPH free radical scavenging *in vitro* assay, and two methanolic and ethanolic extracts were selected for the analysis of phenolic compounds due to their high radical scavenging. Approximately 35 metabolites (i.e., anthocyanins and non-anthocyanins) were identified from the peel extracts. Differences in anthocyanin compounds profiles were observed among different Iranian pomegranate varieties. These fractions are rich in ellagitannins and ellagic acid, as a group of phenolics responsible for confirming antioxidant properties; therefore, they could be standardized and selected with efficacy and safety in the animal model system and pharmaceutical industry. In addition, the optimization, detection, and separation of phenolic compounds, especially anthocyanins, in pure forms is difficult, and further studies are required in this regard.

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Tables:

Table 1. Total antioxidant capacity of the *P.granatum* extracts according to the DPPH assays.

Fractions	Inhibition (%)	Calibration curve	IC_{50} (µg/ml)
Methanol	92.28	y = 0.3738x + 0.2342 R^2 = 0.9979	133.13
Ethanol	86.55	y = 0.3519x - 0.4355 R^2 = 0.9983	143.32
Acetone	75.1	y = 0.3093x - 1.1678 R^2 = 0.9955	165.43
Ethyl acetate	21.72	y = 0.0899x - 0.0952 R^2 = 0.9891	557.23
BHT	88.1	y = 0.365x - 1.5584 R^2 = 0.9917	141.25

Table 2. The anthocyanins present in pomegranant peel.

No.	Name	t_R (min)	(m/z)	Relative intensity (%)
1	Pelargonidin 3-glucoside	16.14	434	2.7
2	Cyaniding 3-glucoside	16.23	450	1.0
3	Cyaniding-pentoside	16.84	420	48.0
4	Peonidin hexoside	16.84	464	90.0
5	Delphinidin 3-glucoside	16.84	465	17.0

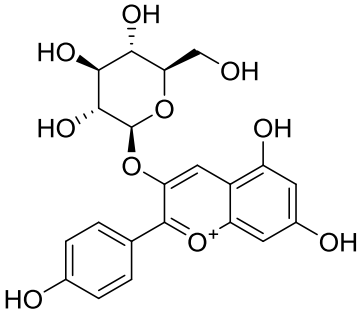
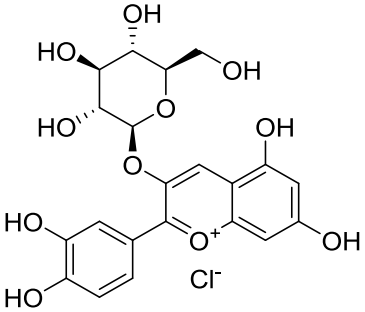
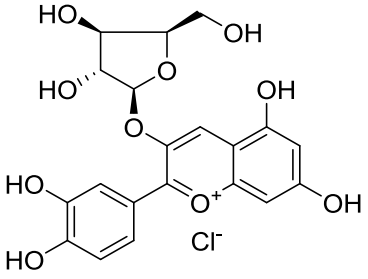
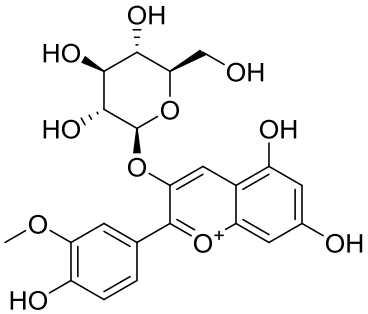
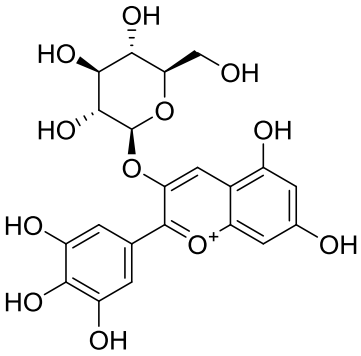
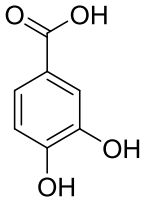
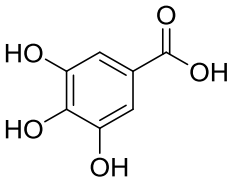
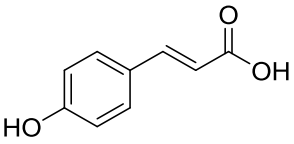
Table 3. The non-anthocyanin phenolic compounds of the peel methanolic and ethanolic extracts of black pomegranate peel.

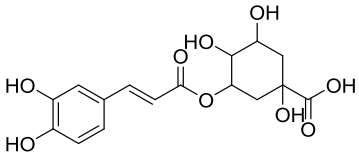
Methanolic extract					
No.	Classification	Name	t_R (min)	(m/z)	Relative intensity (%)
1	Hydroxybenzoic acids	Protocatechuic acid	22.31	153	1.7
2		Gallic acid	19.15	169	3.8
3		Vanillic acid 4-hexoside	20.21	329	15.4
4		Protocatechuic acid-derivative	14.19	425	67.0
5	Hydroxycinnamic acids	Coumaric acid	7.50	163	4.7
6		Caffeic acid derivative	27.92	299	59.0
7		Coumaric acid-hexosides	24.56	325	36.0
8		Caffeic acid hexoside	21.05	341	12.4
9		5-O-Caffeoylquinic acid	24.70	353	4.4
10		Ferulic acid-hexoside	9.75	355	1.0
11		Caffeic acid hexoside derivative	16.50	451	4.4

		derivative			
12	Dihydroflavonol	Dihydrokaempferol-hexoside	19.94	449	4.8
13	Gallagyl esters	Gallagyl-hexoside (penicillin)	7.10	781	1.8
14		HHDP-gallagyl-hexoside (punicalagin)	14.98	1083	4.2
15	Gallotannins	Galloyl-hexoside	24.70	331	49.0
16	Ellagitannins	Ellagic acid	26.35	301	10.0
17		Ellagic acid derivatrive	18.10	441	50.0
18		Ellagic acid derivative	26.40	443	5.8
19		Ellagic acid-deoxyhexoside	16.99	447	3.4
20		Ellagic acid-hexoside	23.21	463	4.8
21		Valoneic acid bilactone	15.01	469	19.6
22		HHDP-hexoside	28.41	481	69.0
23		Ellagic acid dihexoside	16.01	625	6.0
24		Galloyl-HHDP-hexoside	25.02	633	1.2
25		Digalloyl-HHDP-hexoside(pedunculagin II)	29.84	785	10.0
26		Ellagic acid derivative: granatin A (HHDP-DHHDP-hexoside) or lagerstannin A (bis-HHDP-gluconic acid)	24.41	799	19.5
27		Galloyl-bis-HHDP-hexoside (casuarinin)	25.87	935	0.31
28		Flavogalloyl-HHDP-gluconic acid (lagerstannin B)	1.64	949	45.0
29		Galloyl-HHDP-DHHDP-hexoside (granatin B)	21.34	951	5.9
30		hydroxylated derivative of lagerstannin B	28.65	967	7.0
		Ethanolic extract			
1	Hydroxybenzoic acids	Protocatechuic acid	22.29	153	2.9
2		Gallic acid	25.79	169	12.0
3		Vanillic acid 4-hexoside	11.66	329	29.0
4	Hydroxycinnamic acids	Coumaric acid	18.93	163	72.0
5		Caffeic acid derivative	28.16	299	100.0
6		Coumaric acid-hexosides	27.46	325	63.0
7		Caffeic acid hexoside	21.61	341	15.9
8		5-O-Caffeoylquinic acid	11.45	353	41.0
9		Ferulic acid-hexoside	11.31	355	78.0
10		Caffeic acid hexoside derivative derivative	17.03	451	10.3
11	Gallagyl esters	Gallagyl-hexoside (penicillin)	14.05	781	1.1
12		HHDP-gallagyl-hexoside (punicalagin)	12.51	1083	0.48
13	Gallotannins	Galloyl-hexoside	24.62	331	68/0
14	Ellagitannins	Ellagic acid	22.46	301	1.0
15		Ellagic acid derivatrive	19.05	441	13.0
16		Ellagic acid derivative	19.05	443	7.5
17		Ellagic acid-deoxyhexoside	22.40	447	1.5
18		Ellagic acid derivative	7.25	392	22.0
19		Bis-HHDP-hexoside (pedunculagin I)	16.13	783	1.0
20		HHDP-hexoside	27.93	481	55.0
21		Ellagic acid dihexoside	15.25	625	10.5
22		Galloyl-HHDP-hexoside	26.82	633	4.3
23		Digalloyl-HHDP-hexoside(pedunculagin II)	16.45	785	1.0

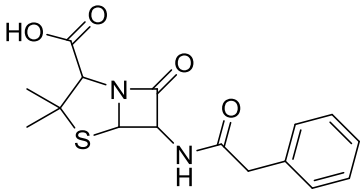
24	Castalagin derivative	26.52	965	5.5
25	Galloyl-bis-HHDP-hexoside (casuarinin)	28.33	935	1.8
26	Flavogalloyl-HHDP-gluconic acid (lagerstannin B)	18.37	949	15.0

Table 4. Chemical structure of anthocyanins and some non- anthocyanins of *P.granatum* peel.

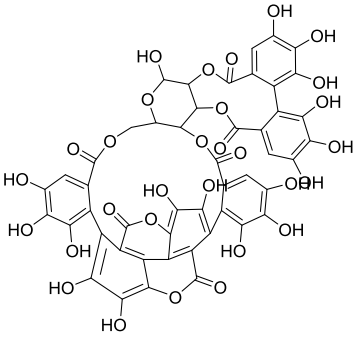
Anthocyanins		
Pelargonidin 3-glucoside	Cyaniding 3-glucoside	Cyaniding-pentoside
		
Peonidin hexoside	Delphinidin 3-glucoside	
		
Non-anthocyanins		
Protocatechuic acid	Gallic acid	Coumaric acid
		
5-O-Caffeoylquinic acid	Gallagyl-hexoside (penicillin)	HHDP-gallagyl-hexoside (punicalagin)



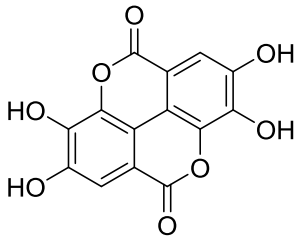
Ellagic acid

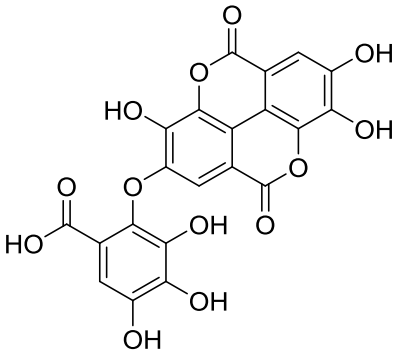


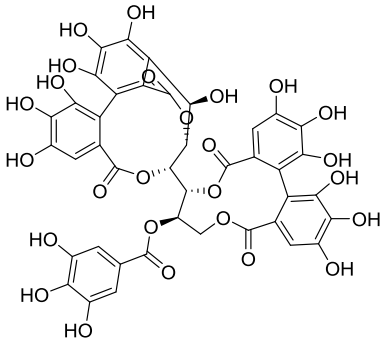
Valoneic acid bilactone



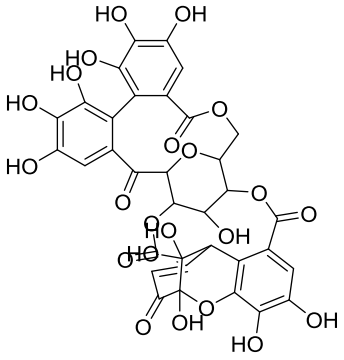
Galloyl-bis-HHDP-hexoside (casuarinin)







Galloyl-HHDP-DHHDP-hexoside (granatin B)



Figures:

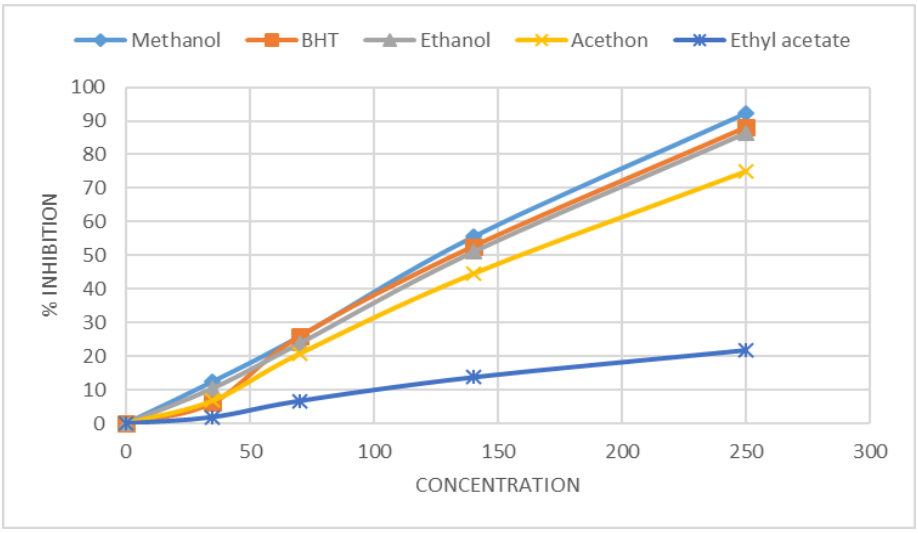


Figure 1. Radical scavenging activity of different concentrations (35–250 µg/mL) of pomegranate peel fractions (methanol, ethanol, acetone, and ethyl acetate) by DPPH method. The activity is compared with BHT.

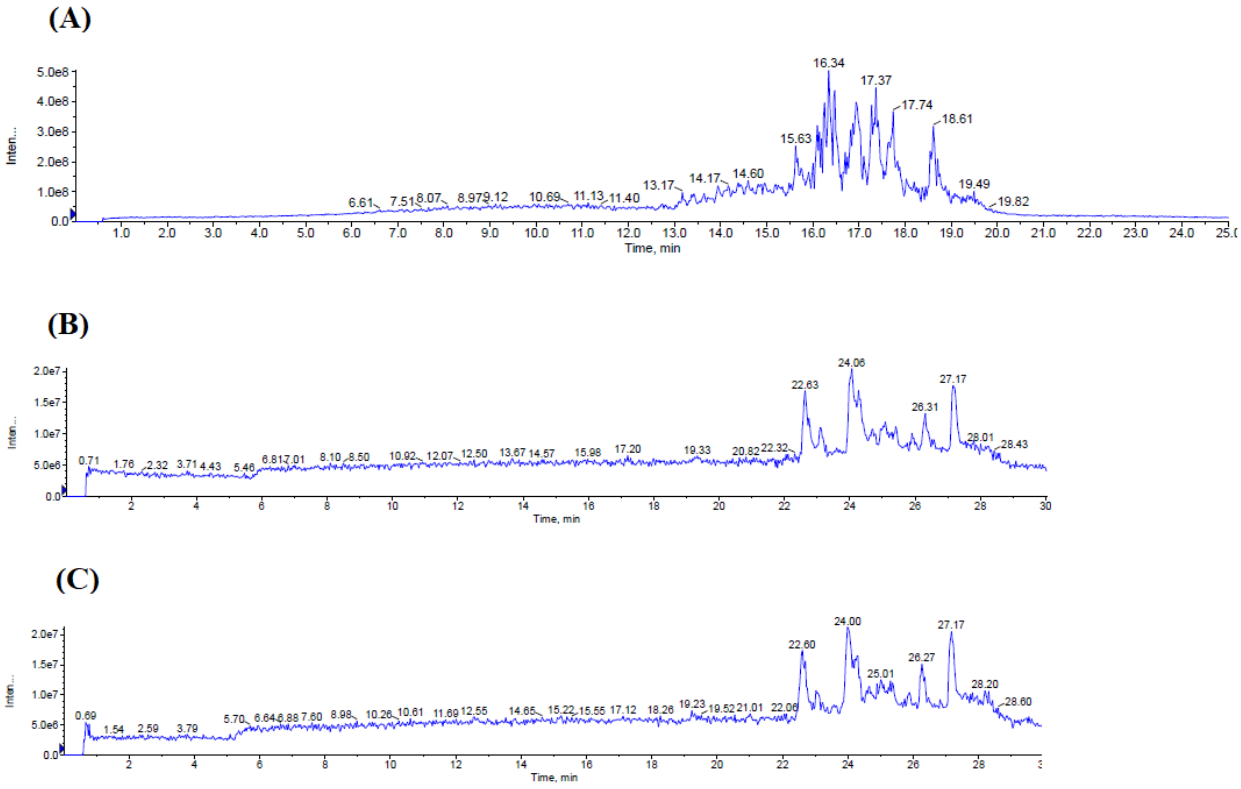


Figure 2. The HPLC-MS/MS total chromatogram of anthocyanins and non- anthocyanins phenolic compounds: (A) total chromatogram of acidic methanol extract; (B) total chromatogram of methanolic extract; and (C) total chromatogram of ethanolic extract.

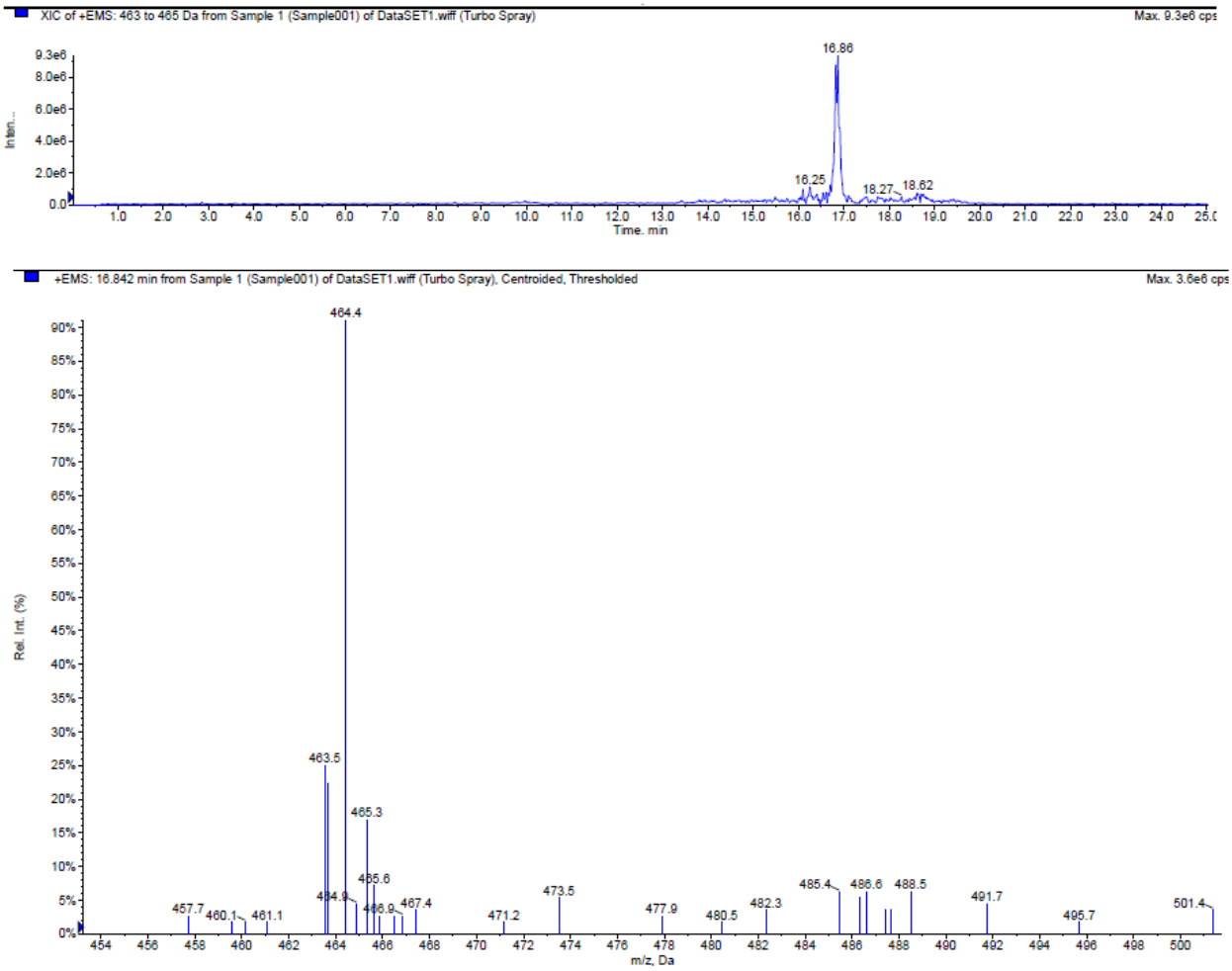


Figure 3. The HPLC-MS/MS analysis of peonidin hexoside.

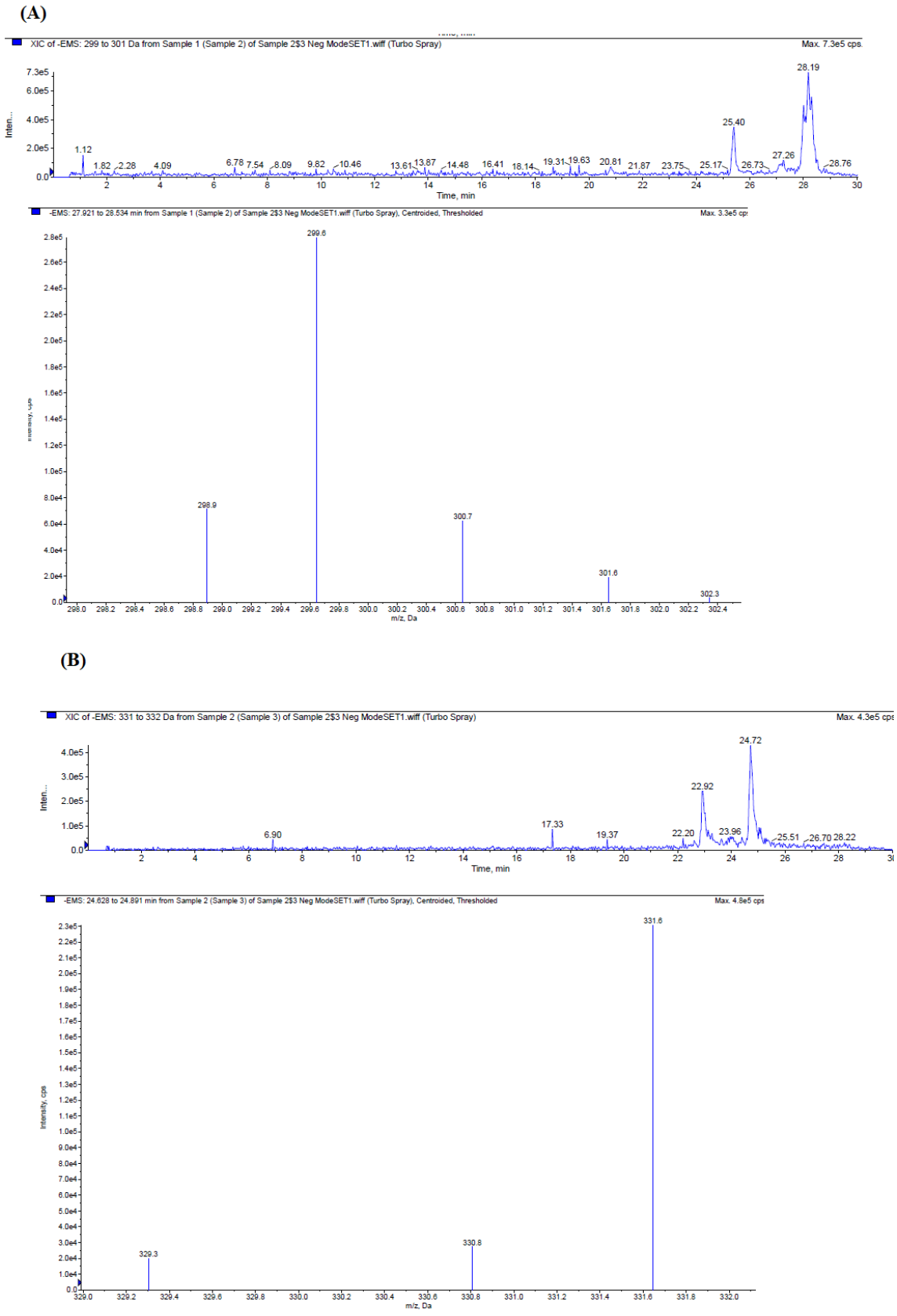


Figure 4. The HPLC-MS/MS analysis of methanolic and ethanolic extracts: (A) caffeic acid derivative chromatogram; and (B) galloyl-hexoside chromatogram.

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