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**یافته‌های نوین در علوم زیستی**
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# رویکرد نوین در تنوع فیتوشیمیایی و ریخت‌شناسی میوه و آبمیوه ارقام انار تجاری

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**چکیده.** میوه انار یکی از مهمترین محصولات باغبانی به دلیل وجود ترکیبات پلی‌فنل‌ها، آنتی‌اکسیدان و ضد قارچی است. در مطالعه حاضر تنوع ریخت‌شناسی و ترکیبات فیتوشیمیایی هشت رقم تجاری انار مورد بررسی قرار گرفت. چهارده صفت ریخت‌شناسی و ۱۰ ترکیب شیمیایی اندازه‌گیری شد. براساس آنالیز مولفه-های اصلی (PCA)، متغیرترین صفات ریخت‌شناسی میان ارقام انار مورد مطالعه شامل نوک برگ، حاشیه برگ، قدرت رشد و فرم رویشی بود. کروماتوگرافی مایع با کارایی بالا ترکیباتی شامل کلروژنیک‌اسید، کافئیک‌اسید، پاراکوماریک‌اسید، اسیدیته، محتوی فنولی، فعالیت آنتی‌اکسیدانی در آبمیوه و پوست میوه توانستند ارقام مورد مطالعه را متمایز کنند. مقایسه دندروگرام UPGMA براساس داده‌های ریخت‌شناسی و محتوی شیمیایی عدم تطابق بین آنها را نشان داد. تست مانتل بین صفات ریختی، ترکیبات شیمیایی و فاصله جغرافیایی ارتباط معنی داری را نشان نداد. این نتایج نشان دهنده تفاوت در محتوی ژنتیکی ارقام انار در مناطق مختلف ایران است و تنوعات در صفات مطالعه شده ارتباطی با فاصله جغرافیایی آنها ندارد.

**واژه های کلیدی.** پارا کوماریکاسید، تست مانتل، کافئیک اسید،کروماتوگرافی مایع HPLC، کلروژنیک اسید

# A new insight to the phytochemical and morphological diversity in commercial pomegranate (*Punica granatum*) cultivars

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**Abstract.** The pomegranate fruit is one of the most important horticultural products due to the presence of polyphenolic, antioxidant and anti-fungal compounds. In the present study, morphological and phytochemical compounds diversity was investigated in eight Iranian commercial pomegranate cultivars. Fourteen morphological characters and 10 chemical compounds were measured. Based on PCA analysis the most variable morphological characteristics among *Punica* cultivars studied were the wood surface, the leaf tip, the leaf incision, growth power and rained condition. High-performance liquid chromatograms of chlorgenic acid, caffeic acid, Para Coumaric acid as well as titrable acidity, total soluble solid, total phenolic contents, antioxidant activity in juice and peel of cultivars differentiated the cultivars studied. Comparing the two UPGMA dendrograms of *Punica* cultivars based on morphological data and chemical contents indicated certain disagreement between them. The correlation between morphological and chemical compounds and geographical distances of *Punica* cultivars was not statistically significant by the Mantel test. These findings indicate that *Punica* cultivars studied differ in their genetic content, however, this genetic difference is not correlated with their geographical distance.

**Keywords.** caffeic acid, chlorgenic acid, liquid chromatograms HPLC, Mantel test, para coumaric acid

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## INTRODUCTION

Pomegranate (*Punica granatum* L.) belongs to the family Lythraceae (Khan et al., 2018) and is widely cultivated in tropical and subtropical regions of the world (Mahajan et al., 2018). This plant is native to a geographical range from Iran to the Himalayas, and has been cultivated all over the Mediterranean region since ancient times (Khan et al., 2018).

Pomegranate is mainly cultivated in Iran, India and South East Asia, as well as in tropical Africa (Sun et al., 2018). Pomegranate adapts to different climate conditions, and can grow in light and heavy soils, and can tolerate water salinity as well as drought. Therefore, many cultivated forms of pomegranate are known which grow in different climatic and edaphic conditions (Vazifeshenas et al., 2012; Sunet al., 2018). These cultivated forms are diploid with n=8 (2n=2x=16) chromosome number (Gill et al. 1981; Xue, et al. 1992; Sheidai & Noormohammadi, 2005; Sheidai, 2007).

The pomegranate fruit is one of the most important horticultural products in Iran with an area under cultivation of 90,605 hectare and production of 917,529 ton in 2018 (Ministry of Agriculture Jihad in Iran and FAOstat, http://www.fao.org/faostat/ en/#data).

In total, 760 cultivars of *P. granatum* have been cultivated in the germplasm of Iran Pomegranate Research Center, located in Yazd province (Madadi et al., 2017). These cultivars differ in their agronomic, morphological, physiological and biochemical characteristics such as tree type, crown form, growth power, pH, phenolic contents, peel color, etc. The chemical compounds may change based on the type of each cultivar, environmental conditions, ripening time, and storage.

The different parts of the pomegranate plant (flower, fruit, aril, peel fruit, seed, leaf and root) have different chemical composition (Saeed et al., 2019). The different parts of *P.granatum* fruit are the rapeutically beneficial and exhibit a wide variety of medicinal value. Pomegranate fruit is a rich source of secondary and primary metabolites such as flavonoids, anthocyanins and hydrolyzable tannins, fatty acids and lipids that have many therapeutic effects (Wu & Tian, 2017).

Pomegranate peel is the source of biological compounds with medical effects such as phenolic acids (hydroxycinnamic acid), flavonoids (anthocyanins, catechins, etc.) and hydrolyzable tannins (alginic and gallic acids and punicalaginine) (Smaoui et al., 2019; Alexandre et al., 2019). Many studies have been done to evaluate the properties of pomegranate peel and its properties as antioxidant, anti-inflammatory and anti-tumor (Singh et al., 2018; Kaderides et al., 2019; Pirzadeh et al., 2020). On the other hand, the seed extract of the plant was found to have anti-diabetic effect (Syed et al., 2018) and the flower extract of pomegranate prevents nephrotoxicity (El-Daly, 2016).

It is suggested that morphometric and chemical compound measurements of different pomegranate cultivars will allow us to gain basic but needed knowledge on the agronomic characteristics of pomegranate germplasm collection which have been grown under homogeneous conditions (Martinez et al., 2016). If the phenotypic variability is found to be high, then the assumption is made that they are also genotypic different. These results could lead us to further characterize the cultivars collection through genetic analysis (Martinez et al., 2016). Therefore, the aims of present study were to illustrate morphological and chemical contents diversity of pomegranate cultivars grown in the research center and agricultural education and natural resources of Yazd province. We also tried to determine if morphological and chemical variations are related to their original geographical region of cultivation.

## MATERIALS AND METHODS

In this study, we selected pomegranate cultivars that were of commercial and therapeutic importance. Pomegranate fresh fruits (Rabab, Vahshi Poostghermez, Goojagh Shahpar, Makhmal shahreza, Marmar Ramhormoz, Ardestanitorsh, Black skin Abrand Abad; 3-5 trees of each cultivars and 3-5 fruits of each tree) and petals of Golnar (*Punica granatum* var. *pleniflora*- ornamental - no fruit) were collected during September 2019. Name of cultivars are provided in Table 1.

**Morphological characteristics**

The minimum age of the selected trees was three years. The crown shape and growth strength dominant type were considered for growth type. The number of off-shooting, smooth or ragged wood surface, presence or absence of thistle on annual and mature wood after harvesting and leaf fall time were measured. The presence and amount of anthocyanin were recorded in spring as well as the appearance time of leaves.

For leaf characters, twenty mature leaves were harvested from the middle part of perennial branches and the middle leaf incision, leaf shape, leaf tip shape and leaf edge color were recorded. Finally, fruit measurements were only done on fully ripened fruits, which included peel thickness, fruit shape and cross section (Table 2).

**Extraction and Isolation of *Punica granatum* of chemicals**

For chemical analyses, we determined the quantity of phytochemicals compounds such as three phenolic acids (hydroxycinnamicacids): 1- Caffeic acid, 2-Chlorogenic acid, and 3-P-Coumaric acid, and one

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**Table 1.** Cultivar names, accession number and their regions.

| No. | Accession number | Cultivar names | Region |
|---|---|---|---|
| 1 | 68-119-1 | Rabab Poostghermez | Fars |
| 2 | 67-210-2 | Vahshi Poost ghermez Roodbar (Wild) | Gilan |
| 3 | 69-181-1 | Goojagh Shahpar | Vramin |
| 4 | 69-143-1 | Makhmal Shareza | Esfahan |
| 5 | 69-161-2 | Marmar Ramhormoz | Khoozestan |
| 6 | 69-179-4 | Ardestani Torsh | Semnan |
| 7 | 68-541-3 | Golnar Farsi Shahdad | Kerman |
| 8 | 67-233-1 | Black Skin Abrand Abad | Yazd |

flavonoid (flavonols): Quercetin in freshly collected fruit juice and peel extract by high performance liquid chromatography (HPLC) method.

All the fruits were checked for its morphological features. The sun-burned, cracked and pest fruits were excluded to achieve acceptable uniformity.

**Preparation and Extraction of Raw Pomegranate Juice**

First, the fruits were washed, following peeling out, then, the skins which are covering seeds were removed, and the juices obtained by pressing transfer. The pomegranate juices were centrifuged at 14000g for 5 min at room temperature(25°C), then the supernatant was separated and passed through a 0.2 µm RC-lter filter (Phenomenex, Torrance, CA, USA). Raw juices were kept at 4 °C and were analyzed within one week.

**Extracted from peel**

First, the fruits were peeled, then the skins were dried for ten days, and finally the dried skins were ground 3 times for 10 seconds. A total of 50 g peel powder was soaked into polar solvents including distil water and ethanol (100%) 40:60 ratios for 24hr. Then, all extracts were centrifuged at 10000g for 3 min at room temperature (25°C) then filtered by using Whatmann® No. 41 filter paper (pore size 20-25 µm). Their concentrated under reduced pressure at 40°C. The extracts were stored in brown glass containers and kept at -80°C until further use.

**HPLC condition**

In this study, Agilent Technologies Model 1260 chromatography was used. It includes components degasser, a binary pump delivery system, a manual injector and UV-Vis Detector (Agilent Technologies, Palo Alto, CA, USA). The column used in the device was column C18 (5 µm, 4.6 mm X 250 mm) Agilent Technologies.

The mobile phase in HPLC test was consisted of two solvents: Solvent A, water/acetic acid (99.5: 0.5; v/v) and Solvent B, methanol. The phenolic compounds were eluted under the following conditions: column flow of 0.8 ml/min and the temperature was set at 30°Cand 20 ul injection volume for Chlorgenic acid, Caffeic acid, P-Coumaric acid and temperature was set at 28° for Quercitin. The ultra-violet-visible spectra (scanning from 200 nm to 600 nm) were recorded for all peaks chemical compounds and identified by valid standards (phenolic standards were provided by Sigma-Aldrich (St. Louis, MO, USA): Chlorgenic acid, Caffeic acid, P-Coumaric acid and Quercitin). The detection was performed at 330 nm and 352 nm wavelengths (Karakaplan et al., 2017).

**Chemical components**

The chemical components are listed in Table 5. They are included: titrable acidity, total soluble solid, total phenolic contents, antioxidant activity, peel color and pH.

**Titrable Acidity**

Juice was extracted by hand and the juice obtained was analyzed for titratable acidity (TA). dilute 5 ml of fruit juice with 20 ml of distilled water, then add 2 to 3 drops of Phenolphthalein to the solution, until purple color was formed.

The percentage of sample acid (according to citric acid) was calculated by using 0.2 normal NaOH titration until formation of pink color (Mirdehghan & Rahimi, 2007).

$$TA\ (\%) = \frac{\text{NaOH consumed} \times \text{NaOH Normality} \times \text{Acetic acid valance gram}}{\text{Sample weight} \times ۱۰۰۰} \times 100$$

**Table 2.** Morphological features of *Punica* cultivars studied.

| cultivar | tree type | Crown form | Growth power | rained condition | Wood surface | existence Thistle on mature wood | The presence of anthocyanin dye on the branch | Middle leaf incision | Flat leaf form | Leaf tip form | Leaf edge | Fruit shape | peel thickness (mm) | Fruit: shape in cross section |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Rabab | A few trunks | Extensive | Strong | Medium | Ragged | Have not | Low | Low | Elliptical stretched | Globular | Green | Elliptic | Thin | circular |
| Roodbar | A few trunks | Extensive | Strong | Low | Smooth | Have not | Low | Low | Elliptical stretched | Sharp | etc. | Globular | Thin | circular |
| Goojagh | A few trunks | Extensive | Strong | Medium | Smooth | Low | Medium | Low | Elliptical stretched | Sharp | etc. | Globular | Thin | angular |
| Makhmal shareza | A few trunks | Extensive | Strong | Medium | ragged | Have not | Low | Low | Elliptical stretched | Globular | etc. | Globular | Thin | circular |
| Marmar Ramhormoz | A few trunks | Extensive | Weak | High | ragged | Have not | Low | Low | Elliptical stretched | Sharp | etc. | Elliptic | Thick | circular |
| Ardestani | A few trunks | Extensive | Strong | very much | ragged | Have not | Low | Low | Elliptical stretched | Globular | Green | Elliptic | Thin | circular |
| Golnar | A few trunks | Extensive | Strong | Low | Smooth | Have not | Low | Low | Elliptical stretched | Globular | Yellow | Have not | Have not | Have not |
| Black skinAbrand Abad | A few trunks | Extensive | Medium | High | ragged | Have not | Low | Low | Elliptical stretched | Sharp | Yellow | Egg shaped | Thin | circular |

**Total Soluble Solid**

Pomegranate juice was obtained by pressing manually and then used for total soluble solids (TSS) analysis.

Soluble solids were measured at room temperature using a Refractometer Model (MG- 55320).

For this purpose, a few drops of pomegranate juice were poured onto the prisms of the refractometer, and the resulting number was read as soluble solids based on the Brix grade (Mirdehghan & Rahimi, 2005).

**Total phenolic contents**

Five mg of pomegranate aryl was extracted with 10 ml of phosphate buffer solution and centrifuged for 4 min at 4 ° C. the supernatant was used to measure the amount of phenolic compounds (Serrano et al., 2005). to the volume of 200 µl of supernatant, added 300 µl of phosphate buffer, 2.5 ml 0.2 normal folin and 2 ml sodium bicarbonate then incubated at 50 ° C for 5 min. The absorption was performed by spectrophotometer (UNICO 2150 model) at 760 nm. Finally, phenolic compounds were expressed using 1 mM gallic acid standard at 100 g fresh weight.

**Antioxidant activity**

Five mg of pomegranate aril was extracted with 10 ml of phosphate buffer and centrifuged at 4°C for 20 minutes and the supernatant was separated. Then 100 µl of peroxidase was added. Optical density (OD) was measured at 730nm. The 25 µL of supernatant was added and OD was measured. After one minute reading of the absorbance was measured again. The difference amounts between two absorption reads were used for the rate of antioxidant activity. Finally, amount of antioxidant activity was expressed on the basis of mg ascorbic acid at 100 g fresh weight (Serrano et al., 2005).

**Peel color**

We used a colorimeter (Minolta cr400) to calculate surface and interior color of pomegranate peel. The color measurement method was performed by using 5 replicates for each fruit cultivars and each of them was read 4 segment of peel with a colorimeter (Mirdehghan et al., 2006).Chroma index (color intensity) can be calculated using the following formula.

$$C = \sqrt{(a)^2 + (b)^2}$$

**pH**

pH measurements were performed using a Metrohm model 744 pH meter. 50 ml of filtered fruit



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juice was calculated by pH meter (Varasteh et al., 2006).

**Statistical analyses**

For Mantel test between morphological distance and geographical distance of *Punica* cultivars obtained and to group cultivars, traits and determine their similarity used PAST ver. 2.17 (Hammer et al. 2001). for cluster analysis and draw the UPGMA dendrogram used Euclidean distance by PAST, furthermore, PCA (Principal component analysis) and PCoA (Principal Coordinate Analysis) analyses used SPSS software version 21.

## RESULTS

**Morphological analyses**

Details of the morphological characteristics studied in *Punica* cultivars are presented in Table 2. Morphological grouping of the studied *Punica* cultivars by UPGMA cluster analysis (Fig. 1), placed them in three major clusters or groups. The cultivars Makhmal Shahr reza, Ardestani, and Rabab, were grouped together in a single cluster. Among these three cultivars, Rabab and Ardestani showed closer morphological similarities.

The cultivars Mamar and Black-Skin, formed the second cluster, while three cultivars Roodbar, Golnar, and Goojagh, comprised the third cluster. The first two cultivars showed a higher degree of morphological similarities.

The Mantel test between morphological distance and geographical distance of *Punica* cultivars did not produce significant correlation (r = 0.04. P = 0.50). Therefore, it is suggested the morphological features are not related to the longitude and latitude of the cultivation area of these cultivars.

The PCA analyses of morphological characters revealed that the first two PCA component comprised about 72% of the total variation. In the first axis with about 44% of total variance, characters like the wood surface and the leaf tip had the highest positive correlation (r >0.70, data not shown), while rained conditionhad the highest negative correlation (r = -0.90). In the second axis with about 28% of total variance, characters like the leaf incision and leaf tip had the highest positive correlation (r >0.700, while the growth power had the highest negative correlation (r = -0.80). Therefore, these are the most variable morphological characteristics among *Punica* cultivars studied.

PCoA biplot of morphological characters (Fig. 2), revealed that Golnar and Rabab cultivars differs from the other cultivars mainly in growth power feature. Morphological features like the leaf lip, the leaf incision, and leaf edge, separated the cultivars Goojagh and Black-skin from the others.

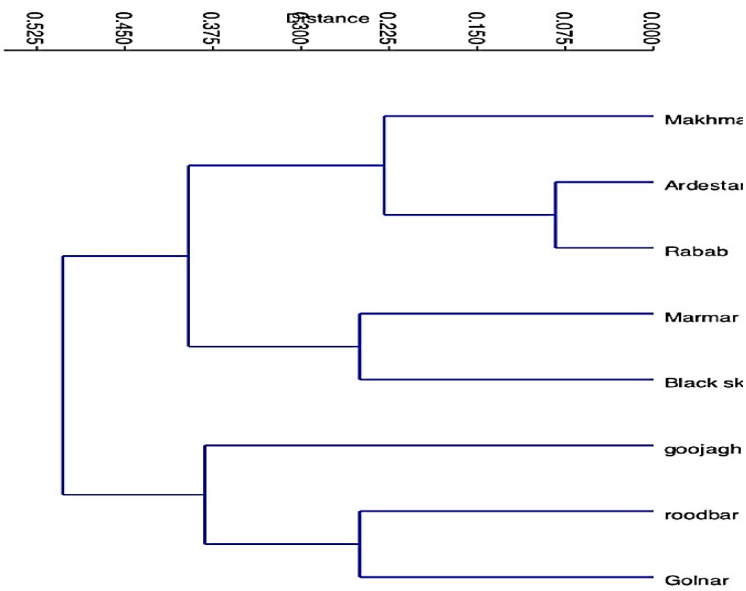


**Figure 1.** UPGMA dendrogram of the *Punica* cultivars based on morphological features.

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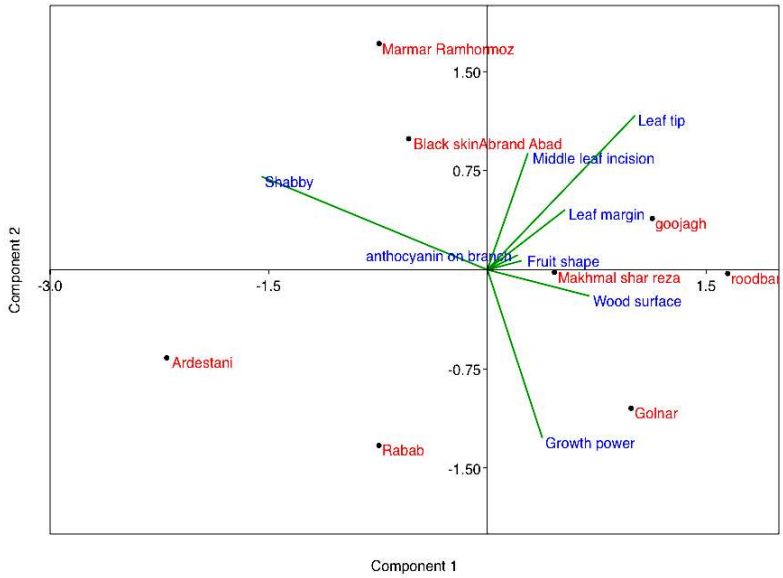


**Figure 2.** PCA ordination based on morphologicalcharacters of cultivars studied.

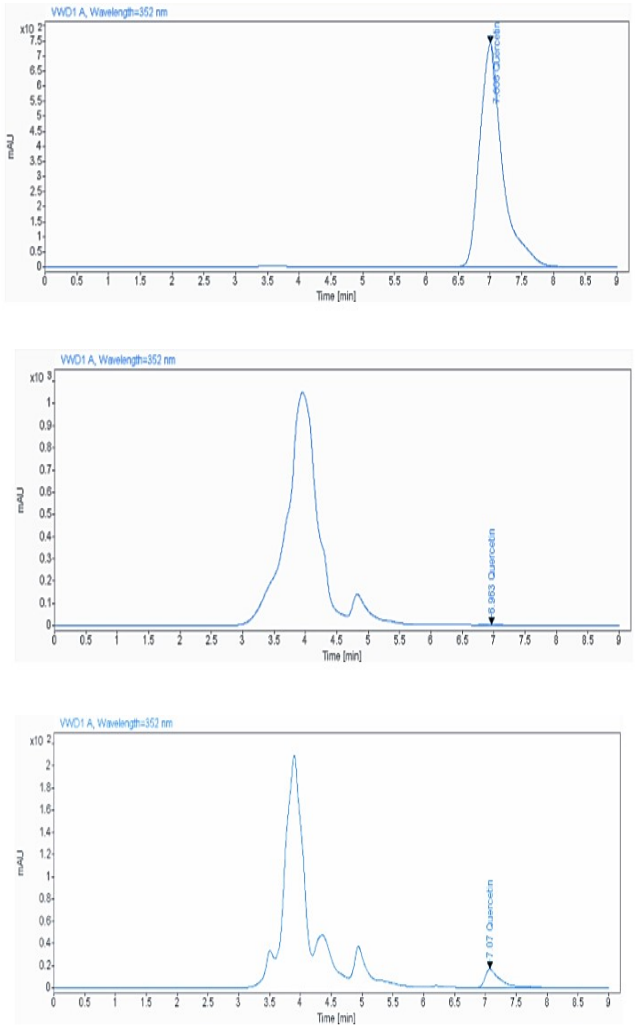


**Figure 3.** Chromatograms of HPLC analyses of Ardestanitorsh cultivar in juice and peel at 325 nm. **A.** Quercetin standard peak. **B.** pomegranate juice and C: pomegranate peel extract.



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**Table 3.** Phenolic contents and Total compounds of pomegranate juice of cultivars studied.

| Cultivar | Chlorgenic acid (mg/ml) | Caffeic acid (mg/ml) | P-Coumaric acid (mg/ml) | Quercitin (mg/ml) | TA% | TSS(°Brix) | Total phenolic contents (Gallic *acid*/100g fresh fruit) | Antioxidant activity (Ascorbic acid/100g fresh fruit) | TSS/TA | pH |
|---|---|---|---|---|---|---|---|---|---|---|
| Rabab Poostghermez | 0.007 | 0.008 | 0.14 | 0.0007 | 1.099 | 20.7 | 238 | 31 | 18.82 | 3.11 |
| Vahshi Poost Ghermez Roodbar (Wild) | 0.015 | 0.027 | 0.0003 | 0.0006 | 1.057 | 12.9 | 222 | 19 | 12.2 | 2.8 |
| Goojagh Shahpar | 0.009 | 0.02 | 0.005 | 0.0003 | 1.069 | 20 | 354 | 29 | 18.7 | 3.5 |
| Makhmal Shareza | 0.011 | 0.012 | 0.025 | 0.001 | 1.23 | 19.9 | 225 | 33 | 16.14 | 3.05 |
| Marmar Ramhormoz | 0.01 | 0.03 | 0.001 | 0.0007 | 1.27 | 20.01 | 285 | 20 | 15.72 | 3.17 |
| Ardestani Torsh | 0.014 | 0.32 | 0.011 | 0.0007 | 0.98 | 18.9 | 274 | 12 | 19.2 | 2.95 |
| Golnar Farsi Shahdad | Have not | Have not | Have not | Have not | Have not | Have not | Have not | Have not | Have not | Have not |
| Black Skin Abrand Abad | 0.007 | 0.013 | 0.016 | 0.001 | 0.97 | 18.3 | 227 | 29 | 18.74 | 3.01 |

**Table 4.** Phenolic contents and peel color of peels pomegranate cultivars studied. * Golnar flower was used.

| Cultivar | Peel color (Hue angle) | Chlorgenic acid(mg/ml) | Caffeic acid(mg/ml) | P- Coumaric acid(mg/ml) | Quercitin(mg/ml) |
|---|---|---|---|---|---|
| Rabab Poost Ghermez | 4.55 | 0.00004 | 0.0002 | 0.02 | 0.001 |
| Vahshi Poost Ghermez Roodbar (Wild) | 2.9 | 0.00007 | 0.0009 | 0.001 | 0.0015 |
| Goojagh Shahpar | 4.1 | 0.00003 | 0.00007 | 0.02 | 0.004 |
| Makhmal Sharreza | 4.4 | 0.00001 | 0.0002 | 0.002 | 0.0018 |
| Marmar Ramhormoz | 3.8 | 0.00004 | 0.0006 | 0.01 | 0.005 |
| Ardestani Torsh | 3.6 | 0.0004 | 0.0006 | 0.009 | 0.012 |
| Golnar Farsi Shahdad* | Have not | 0.0007 | 0.0002 | 0.03 | 0004 |
| Black Skin Abrand Abad | 4.35 | 0.0002 | 0.001 | 0.0001 | 0.011 |

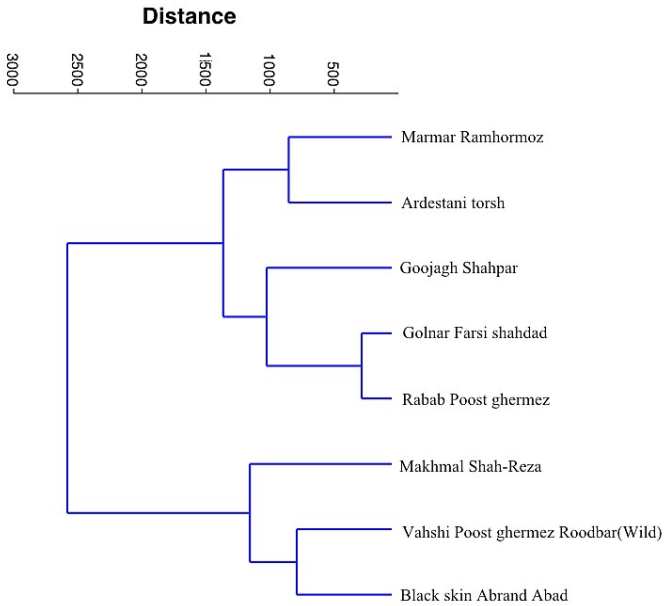


**Figure 4.** UPGMA dendrogram of *Punica* cultivars studied based on chemical data.

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**Chemical analyses**

Details of chemical components for juice and peel of *Punica* cultivars as well as HPLC chromatogram of quercetin in Ardestani cultivar are provided in Tables 3 & 4 and Figure 3.

In this study, some phenolic compounds were identified by HPLC. The results showed that the highest amount of phenolic compounds in pomegranate juice were included: chlorogenic acid in Roodbar (0.015 mg / ml) and Ardestani (0.014 mg/ml) cultivars, caffeic acid: Ardestani (0.32 mg/ml), P-Coumaricacid: Makhmal Shahr reza (0.025 mg/ml) and quercetin: Makhmal Shahreza (0.001 mg/ml) and black skin (0.001 mg/ml).

In pomegranate juice, the highest pH (3.17) and titratable acidity (1.27٪) belong to Marmar cultivar. The Rabab cultivar has the highest total soluble solid content of 20.7°Brix, the highest total phenolic content was obtained in Goojagh cultivar (354 Gallic acid/100g fresh fruit) and the highest level of antioxidant activity (33 Ascorbic acid/100g fresh fruit) was observed in Makhmal cultivar. In peels, the highest level of peel color (4.55) was measured in Rabab cultivar.

The highest levels of phenolic compounds in pomegranate peel extract was including: chlorogenic acid in Golnar cultivar (0.0007 mg/ml), caffeic acid: black skin (0.001 mg/ml), P-Coumaricacid: Golnar (0.03 mg/ml) and quercetin: Ardestani (0.012 mg/ml).

The clustring of pomegranate cultivars by UPGMA method based on chemical data is shown in Figure 4. Theses cultivars here placed in two major clusters. Five cultivars comprised the first major cluster, while they were scattered in two sub-clusters. The cultivars Marmar and Ardestani formed the first sub-cluster, while three cultivars including Goojagh, Golnar and Rabab comprised the second sub-cluster. The last two showed a higher degree of chemical similarity and were placed close to each other.

The second major cluster contains three cultivars including, Makhmal, Vahsi Poost ghermeaz, and Black skin. Members of this major cluster were placed far from those of the first major cluster, due to difference in their chemical contents.

The Mantel test performed between chemical difference and geographical distance of the studied *Punica* cultivars produced no significant association (r = -0.21, P= 0.85). Therefore, chemical differences in *Punica* cultivars are not related to the locality of cultivar cultivation.

PCA analysis of chemical data revealed that the first two PCA axes comrade about 90% of total variance. Chemical features like fruit chlorgenic acid and fruit P-coumaric acid have the highest positive correlation (r >0.70), with the PCA axes, while fruit caffein acid had the highest negative correlation (r = -0.80). Therefore, fruit chemical contents differentiate the studied *Punica* cultivars.

PCA biplot of these cultivars based on chemical data (Fig. 5), revealed that fruit chlorgenic acid and P-coumaric acid, separates Makhmal and Ardestani cultivars from the others. It is worth to mention that these two cultivars also differ from each other in these chemicals.

The fruit caffeic acid separates Goojagh cultivars from the others, while the peel caffeic acid separates Rabab and Marmar *Punica* cultivars.

Comparing the two dendrograms of *Punica* cultivars based on morphological data and chemical contents indicate certain disagreement between the two. This is well supported by the Mantel test performed between the two dendrograms. No significant correlation between these dendrograms was observed by using Mantel test (r = -0.03, P = 0.54). Moreover, reticulation analysis also produced Robinson and Foulds distance (RF) = 12 and Bipartition dissimilarity = 12.0, which indicate a major difference between the two dendrograms.

The combined dendrogram from both morphological and chemical data are depicted (Fig. 6). Three groups of *Punica* cultivars, which can be used in further hybridization and breeding programs. Cultivars Marmar and Ardestani show affinity to each other and form the first cluster. Cultivars Goojagh, Golnar, and Rabab, comprise the second cluster, while, three cultivars, Makhmal, Vahsi Poost ghermez and Black skin, showed affinity and formed the third cluster.

These findings indicate that *Punica* cultivars cultivated in different regions of Iran, differ in their genetic content and may be used in future applied and research tasks.

## DISCUSSION

Despite the importance of pomegranate, only a few number of studies have been investigated the biochemical or physiological properties of pomegaranate the establishment of its genomic and genetic infrastructures was more or less neglected (Ophir et al., 2017).

The results of the current study indicate that the differences in various *Punica* cultivars according to their morphological features were not correlated with their chemical properties. Probably, different genes control these two sets of features in *Punica* cultivars.

Martinez et al. (2016) studied the morphological and chemical traits (arils, seeds, leaves and flowers and pH, titratable acidity, total soluble solids) of pomegarante and showed that the characteristics related to fruit and grain size as well as the acidity and pH of water have the highest discriminative power of the studied

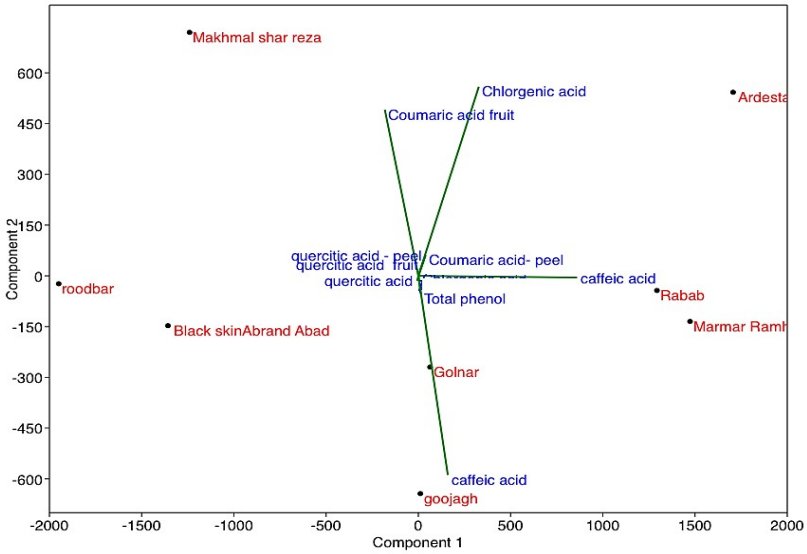


**Figure 5**. PCA biplot of pomegranate cultivars based on chemical data

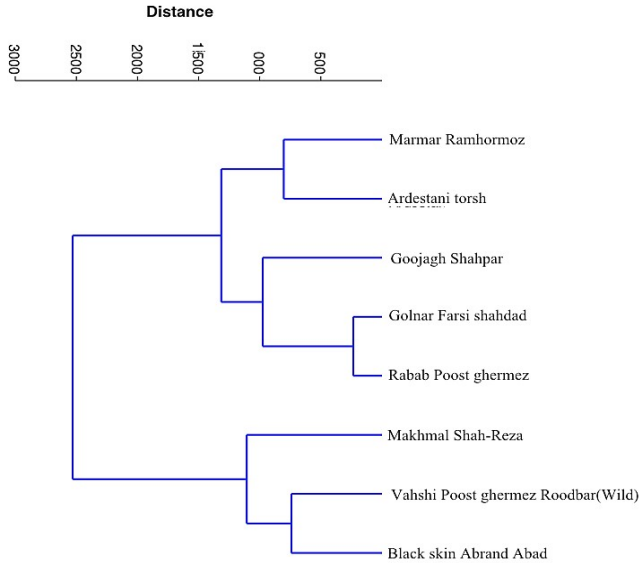


**Figure 6.** Dendrogrambased on both morphological and chemical data of *Punica* cultivars studied.

pomegranate cultivars (Martinez et al., 2016). Our findings showed extensive morphological and chemical diversity among the studied *Punica* cultivars in Iran. these results are in agreement with the report of Martinez et al., 2016.

Mirjalili et al. (2018) studied the biochemical diversity, phenolic compounds and anthocyanins in the juice of *Punica granatum* L. among 25 genotypes of Malas cultivars. The results showed that the acidity of genotypes varies between 0.57% to 2.06%, while the range of changes in our results is between 0.97% to 1.099% (Mirjalili et al., 2018). In another investigation, 21 pomegranate cultivars fromfour regions of Lorestan the highest titratable acidity was 26.4% and the lowest was 12.9% were recorded (Spahvand et al., 2017). This data could be due to the influence of climatic and geographical factors. Feyzi et al. (2015) believed that cultivars and climatic conditions have a significant effect on vitamin C, acidity and TSS (Feyzi et al., 2015). Although the range of soluble solids was 14-18.5° Brix (Mirjalili et al., 2018) and the range of soluble solids was between 13.5-17.8° Brix (Spahvand et al., 2017), our study showed a wider range between 12.9 and 20.7° Brix. This difference may stem from differences in the cultivars studied. Most of the cultivars studied here had soluble solids higher than 17, which makes them suitable for export, better quality and the start of a breeding program.



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The results showed that the range of changes in antioxidant activity among the studied genotypes was from 12 to 33, while in other studies these changes were between 56 to 93 (Mirjalili et al., 2018) and 69 to 93 (Tehranifar et al., 2014). The difference in the results can be due to differences in the measurement method.

The percentage of polyphenolic compounds in natural pomegranate juice have been recorded by HPLC method. They were 0.1, 1544 mg/L and 58.3% for amount of quercetin, total phenolic content and antioxidant activity % in black skin cultivar respectively (Hoseani et al. 2018) while in the present study, these amonts were 0.001 mg/mL, 27 gallic acid/100g, and 29 ascorbic acid/100g fresh fruit.

The caffeic acid, P-coumaric acid and chlorogenic acid were reported 0.01, 0.48 and 0.8 respectively by Karakaplan et al. (2017), while these amounts were different in present study (Table 3).The chemical composition of the fruits differs depending on the cultivar, growing region, maturity, cultivation practice, climate, and storage circumstances (Fadavi et al., 2005).

Extracts of all parts of the pomegranate fruit exhibit therapeutic properties and target a range of diseases including cancer, cardiovascular disorders, diabetes, male infertility, Alzheimer's disease, aging, and AIDS (Sreekumar et al., 2014). Therefore, to produce data on pomegranate cultivars chemical properties and genetic structure may benefit the mankind in future health care.

## Conclusion

Iran, with multiple genotypes of pomegranate, it is necessary to conduct a wide screening by researchers in order to find the most suitable cultivars and their practical use. according to the results, the cultivar is the most important factor in determining the physical properties, phenolic compounds and antioxidant activity in pomegranate, so identifying cultivars with the highest amount of polyphenols or antioxidants can be of great importance in medicine. In this article, we tried to examine some cultivars that have valuable therapeutic properties, but more research is needed to obtain important cultivars to promote human health.

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