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Evaluation of bioactive potential and phytochemical variation among *Punica granatum* var *pleniflora* genotypes

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Pomegranate (*Punica granatum* var. *pleniflora*) flowers are distinguished by their high antioxidant activity due to their high content of phenolic compounds. Iranian pomegranate flowers have been traditionally used in medicine for centuries. The present study evaluated the growth traits and bioactive characteristics of Iranian pomegranate**.** The cultivars were selected from the same site at the Natural Resources Research Centre in Yazd, Iran, to ensure consistency in assessing the specified traits. The results showed high phenotypic diversity among the cultivars, with leaf dry weight and flower dry weight exhibiting the highest coefficients of variation. Furthermore, significant differences were observed among the cultivars regarding total phenolic compound content. The methanolic extract obtained from the flowers of Zinati-e-Saveh cultivar had the highest level of total phenols (119.33 mg GAE g-1 DW), total flavonoids (72.21 mg QE g-1 DW), anthocyanins (33.36 mg g-1 DW), hydrolyzable tannins (95.94 mg TA g-1DW), and IC50 activity (25.29 µgmL-1). Principal component analysis (PCA) confirmed significant differences among the cultivars studied based on their morphological and phytochemical characteristics. These findings suggest that the studied cultivars represent a valuable genetic resource for future breeding programs.

Keywords: Antioxidant potential, Pomegranate flowers, Polyphenols, Principal component analysis

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INTRODUCTION

Pomegranate (*Punica granatum* var. *pleniflora*), belonging to the Punicaceae family, is one of the major products of tropical and subtropical regions which is extensively cultivated in the Middle East, North Africa, and many parts of the Mediterranean. Due to its attractive blooms, *P. granatum* var. *pleniflora* is valued as a drought-resistant plant, and its flowers are used in both traditional and modern medicine for treating some diseases and cosmetics. In Iran, non-fruit-bearing pomegranates with many-petaled flowers are known as 'Golnar,' and cultivars vary in flower color. Pomegranate cultivation is widespread in Fars province and Kerman province, both major centers for pomegranate production. Notable cultivars include 'Golnar-e Farsi Sarvestan' from Fars province, which features double red flowers that bloom in early May. This cultivar offers several advantages for cultivation, including climate adaptability, low water requirements, pest and disease resistance, quick yield, long shelf life, and economic viability (Sarkhosh et al., 2020). This species has been valued for centuries for its economic value in flower production and its symbolic significance in various cultures. The flower and its extracts are rich in bioactive compounds, contributing to their extensive use in health and wellness applications (Kandylis et al., 2022). Beyond their visual appeal, pomegranate flowers possess unique medicinal and therapeutic properties that distinguish them from their fruit-bearing counterparts. Research indicates that pomegranate flowers have significant levels of phenolic compounds, anthocyanins, flavonoids, and tannins, all of which contribute to their remarkable antioxidant and antimicrobial properties **(**Abdolahi et al., 2018; Gosciniak et al., 2022; Tekin & Kucukbay, 2024). Studying these specific varieties can reveal untapped potential for applications in natural medicine, cosmetic formulations, and dietary supplements.

Additionally, understanding pomegranate genotypes' morphological and genetic diversity aids their conservation and sustainable use (Ge et al., 2021)**.** Different phytochemical compounds with high antioxidant and health benefits have been extracted from pomegranate, which has been used to treat multiple ailments and disorders (Hmid et al., 2017; Tekin & Kucukbay, 2024). Numerous studies have confirmed that different parts of pomegranate, mainly the fruit and flowers, are naturally rich sources of secondary metabolites with preventative or inhibitory effects on oxidative stress and the formation of free radicals (Kandylis & Kokkinomagoulos, 2020)**.** Several bioactive compounds with diverse pharmacological activities, such as anti-tumor, antifungal, antiviral, antibacterial, and antioxidant properties**,** have been identified in extracts from dried pomegranate flowers (Abdolahi et al., 2018; Gavanji et al., 2015). In herbal medicine**,** pomegranate flowers treat diarrhea, bronchitis, dysentery, ulcers, astringent, and diabetes due to their astringent properties (Wafa et al., 2017; Ge et

al., 2021). Pomegranate flowers are rich in phenolic compounds like gallic and ellagic acids and flavonoids like punica flavone and granatum flavanyl xyloside (Maphetu et al., 2022). Moreover, triterpenes such as ursolic, oleanolic, maslinic, and Asiatic acids, along with the sterol daucosterol, have been characterized in pomegranate flowers (Sharifiyan et al., 2019; Wong et al., 2021).

Geographical variations have been observed in the active substances of different plant species**.** Parashuram et al. (2022) and Wong et al. (2021) reported that the phytochemical quantity and quality of pomegranate flowers largely depend on environmental and soil conditions, water quality, solar radiation, and cultivar type, factors that are beyond human control. Occasionally, plants within the same species show differences in morphological and biochemical traits due to variations in genotype and geographic conditions (Aziz et al., 2020; Parashuram et al., 2022). Iran is the center of pomegranate diversity, and the variations among native ornamental pomegranate genotypes are linked to differences in the morphological traits of shoots, leaves, and flowers, as well as the biological activity of flower extract (Sharifiyan et al., 2019; Abdolahi et al., 2018; Fellah et al., 2018; Elfalleh et al., 2012). The commercialization of crops has resulted in a loss of genetic diversity in many plant species, highlighting the importance of preserving native genetic resources rich in phytochemicals. Morphological assessment is an efficient and robust method for protecting gene pools and providing valuable information on phenotypic diversity (Khadivi & Arab, 2021). In this context, it is essential to evaluate the current knowledge of leave traits and floral biology in local pomegranate cultivars, as a comprehensive understanding of flower characteristics is a prerequisite for any crop breeding and development programs (Shahsavari et al., 2022).

The antioxidant capacity of flowering pomegranate varieties can vary due to genetic differences and environmental factors, highlighting the importance of detailed biochemical profiling. Such analyses are valuable for identifying promising varieties for medicinal and nutritional purposes and understanding how morphological traits correlate with biochemical composition (Parashuram et al., 2022). Research has shown that the pomegranate plant's flowers and other non-fruit parts are significant sources of bioactive compounds, presenting new opportunities for natural product development and pharmaceutical applications**.** Furthermore, understanding these biochemical variations can inform breeding strategies to enhance desirable traits, such as higher bioactive content or improved tolerance to environmental stressors. By integrating morphological observations with biochemical data, researchers can achieve a comprehensive view of how these flowering pomegranate varieties contribute to biodiversity and functional plant attributes (Elfalleh et al., 2012; Mahboubi et al., 2015; Huo et al., 2023; Ferrara et al., 2023).

Morphological and biochemical analyses are essential for studying plant diversity, as they provide comprehensive insights into various plant parts' structure, function, and potential applications. In flowering pomegranates, these analyses highlight their aesthetic and structural traits and the complex array of bioactive components that contribute to their health benefits. Research has shown that pomegranate flowers are particularly rich in phenolic acids, anthocyanins, flavonoids, and tannins, all of which play critical roles in their potent antioxidants and antimicrobial activities (Hmid et al., 2017; Fellah et al., 2018; Arlotta et al., 2022). These phytochemicals are known for their ability to neutralize free radicals, thereby reducing oxidative stress and potentially lowering the risk of chronic diseases in humans (Maphetu et al., 2022). In addition, anthocyanins contribute to the vivid colors of pomegranate flowers and are associated with various health-promoting properties, including anti-inflammatory and anti-cancer effects (Zhang et al., 2011).

While significant research has been conducted on fruit-bearing pomegranates, there remains a gap in understanding the full extent of morphological and biochemical diversity in flowering varieties. Few studies have compared the various bioactive compounds in flowering pomegranates or explored how these compounds correlate with their morphological characteristics. Addressing these gaps could enhance the appreciation of these plants' potential uses in modern production and medicine and inform strategies for their preservation and cultivation. This study aims to investigate the morphological and biochemical diversity among pomegranate varieties (*Punica granatum var. pleniflora*). By examining key morphological traits and profiling bioactive compounds such as phenolic compounds, flavonoids, and tannins, this study seeks to provide insight into these medicinal plants' unique characteristics and potential health benefits.

Understanding these attributes contributes to scientific knowledge of pomegranate biodiversity and highlights their potential applications in horticulture, traditional medicine, and as sources of natural antioxidants.

MATERIALS AND METHODS

Plant materials

This experiment was conducted in 2022 at the Natural Resources Research Centre, Yazd, Iran (latitude 31°55'N, longitude 54°16'E, altitude 1216 m). According to the Koppen climate classification system, the area's climate is classified as a hot desert climate (BWh). This site has an average annual minimum temperature of 4° C and a maximum temperature of 20.5° C, respectively, with an annual rainfall of 96 mm. The soil characteristics were determined using standard methods. The soil texture was silty sand. The organic carbon content was 0.19%, with natural materials at 24.1%, a bulk density of 1.52 g/cm3, electrical conductivity of 1.8 ds/m, pH of 7.86, absorbable phosphorus content of 0.8 ppm, and absorbable potassium content of 107.3 ppm.

In the present study, the morphological traits of five Iranian pomegranate (*Punica granatum* var. *pleniflora*) cultivars and the phenolic compounds and antioxidant potential of their flower extracts were evaluated. The studied cultivars included Khoshe Nar Baharestan Sari (KNB-S), Sarvestan-e- Fars (S-F), Zinati-e- Saveh (Z-S), Rijab-e- Kermanshah (R-K), and Kenar Takht-e- Fars (KT-F), which were selected from 12-year-old cultivars. Plant materials were harvested at the complete bloom stage, and twelve individuals from each cultivar were selected for morphological and phytochemical analysis.

Morphological characterization

At the full flowering stage, the morphological characteristics of the cultivars studied were recorded (Figure 1). The assayed morphological traits included tree height, stem diameter, petiole length, leaf length and width, leaf dry weight, number of thorns on annual and perennial shoots, and number of suckers per tree. The recorded flower morphological traits included flower length, corolla diameter, number of petals, petal length and width, stamen length, style length, and flower dry weight. The harvested samples were first rinsed with deionized water and blotted with filter paper to determine the dry weight of leaves and flowers**.** Finally, the dry weights of leaves and flowers were measured after oven-drying at 37.5˚C for 24 h.

Phytochemical assays

Flowers of the studied cultivars were harvested in May and desiccated in the shade at room temperature. The dried flowers were then powdered and extracted by maceration in 100 ml of methanol at 30˚C. The solution was covered with parafilm to prevent the solvent from evaporating and kept under continuous agitation overnight. After centrifuging at 2,000 g for 15 min, the supernatant was used to analyze phenolic compounds and antioxidant activity.

Total phenol content measurement

The total phenol content of pomegranate flowers was estimated using the Folin-Ciocalteu method, as described by Aryal et al. (2019). Briefly, 0.5 mL Folin-Ciocalteu reagent was added to the methanolic solution, followed by 1 M sodium carbonate solution. The mixture was incubated for 35 min in a water bath at 45°C. After cooling, the absorbance of the solution was read at 765 nm using a Shimadzu 1600-UV spectrophotometer. The total phenol content of each sample was expressed in mg gallic acid equivalent per g dry weight of the flower.

Total flavonoids content measurement

The total flavonoid content of the flower samples was determined based on the formation of the flavonoid aluminum complex as described by Zhao et al. (2018). One milliliter of the methanolic extract was mixed with 1 mL of 2% aluminum chloride solution. After incubation at room temperature for 15 min, the absorbance of the reaction mixture was measured at 430 nm. Using the standard curves of quercetin ($y = 0.0022x - 0.0021$; $R^2 = 0.9898$), the total flavonoid content was expressed as mg quercetin equivalent per g dry weight of the flower.

Total anthocyanin content measurement

The total anthocyanin content of the flower samples was measured using the pH-differential method, as described by Giusti and Wrolstad (2001). The methanolic extract was mixed separately with potassium chloride buffer (0.025 M, pH 1.0) and sodium acetate buffer (0.4 M, pH 4.5). After incubation at room temperature for 15 min, the absorbance of the solutions was measured at 510 and 700 nm. The anthocyanin content of each sample was expressed in mg per g flower dry weight by calculating the absorbance for each sample using the following formula:

$$A = (A510 - A700)\ pH\ 1.0 - (A510 - A700)\ pH\ 4.5$$

Figure 1. Some vegetative and reproductive characteristics of *P. granatum* var. *pleniflora* cultivars.

Hydrolysable tannins content measurement

The content of hydrolyzable tannins in pomegranate flowers was determined using the method described by Çam and Hişil (2010). One milliliter of the methanolic extract was diluted with 10 mL of distilled water and mixed with 5 mL of a 2.5% solution. The mixture was vortexed for 10 seconds, and the absorbance of the resulting red solution was measured at 550 nm against a water blank. Using the standard curves of tannic acid ($y = 0.02x - 0.0066$; $R^2 = 0.9625$), tannin content was expressed as mg of tannic acid equivalent per g dry weight.

DPPH radical scavenging assay

The scavenging activity of the samples studied was measured to assess their ability to inhibit the free radicals of 2,2- Diphenylhydrazyl (DPPH) described by Stojichevich et al. (2008). Half a milliliter of a methanolic DPPH solution (98%) was added to 1 mL of the methanolic extract of the flowers. After incubation in darkness at room temperature for 30 min, the absorbance of the samples was read at 517 nm. The radical-scavenging activity of the samples calculated as: [(A Control – A Sample) / A Control] × 100

The antioxidant activity was expressed as IC50, representing the sample concentration required to achieve 50% inhibition of free radicals.

Statistical analysis

Descriptive statistics of the quantitative and qualitative traits were estimated by calculating the mean, standard deviation, minimum, maximum, and coefficient of phenotypic variation (CV). Before data analysis, Bartlett's test was conducted to verify the homogeneity of variance among the cultivars, and the Kolmogorov-Smirnov test was used to assess data normality. Analysis of variance (ANOVA) was performed using the Generalized Linear Model (GLM) based on randomized complete block design (RCBD) with three replications. Mean comparisons for each trait were conducted using Duncan's multi-range test. Pearson correlation analysis among the studied traits was conducted using SAS software version 9.4 (https://support.sas.com/software/94/), and a heat map was generated using GraphPad software version

7.0 (https://www.graphpad.com/). Principal component analysis (PCA) was conducted using XLSTAT software version 2020 (https://www.xlstat.com/en/).

RESULTS AND DISCUSSION

The present study investigated five cultivars of *P. granatum* var*. pleniflora* for their morphological traits and phytochemical compounds of collected flowers. The studied pomegranate cultivars were compared using 23 morphological and phytochemical characteristics. Analysis of variance revealed significant variations among the cultivars for the measured traits, especially the morphological characters. These variations were further confirmed by calculating the attributes' coefficient of variation (CV). Parashuram et al. (2022) state that the CV value is independent of the measurement unit, making it a more effective index for comparing the assayed traits. Descriptive values for the morphological and phytochemical characteristics of the studied cultivars are reported in Table 1. This study observed the highest CV value for leaf dry weight (106.63%), followed by flower dry weight (64.46%). However, the CV values for main stem diameter, tannin content, leaf length, corolla diameter, and petal characteristics were all less than 10%. Traits with lower CV values were considered stable characteristics across the cultivars studied. Among the phytochemical traits, only flavonoid content exhibited a CV value greater than 10%, indicating a high phenotypic variation among the cultivars.

Morphological traits

Assessment of morphological traits in native cultivars is crucial for genetic variability evaluation in breeding programs and crop production improvement (Khadivi et al., 2020). In this study, the cultivar's heights varied from 2.23 to 3.98 m, with the R-K cultivar showing the highest height and the Z-S and KNB-S cultivars displaying the lowest height. R-K cultivar showed the highest main stem diameter, followed by KNB-S and KT-F cultivars. The main stem diameter of the S-F cultivar was significantly lower than that of the other cultivars. Moreover, the thickest sub-stems were observed in cultivars R-K and Z-S. *P. granatum* var. *pleniflora* cultivars did not show significant differences regarding leaves' petiole length, which ranged from 4.25 to 5.60 mm. The most oversized leaves were obtained in the Z-S cultivar, and the lowest leaf length and width values were related to KNB-S and KT-F cultivars, respectively. Leaf dry weight varied between 0.180 and 0.378 g. Z-S cultivar trees showed the highest leaf dry weight, 2 times higher than the lowest reported value of this trait in KT-F. The number of thorns was consistent across annual and perennial shoots; the S-F cultivar had the highest number of thorns, with 5 thorns per shoot, while the KNB-S, Z-S, and R-K cultivars had only one. The lowest sucker number (1 sucker) was observed in the R-K cultivar, while the other cultivars had 5 suckers per tree (Table 2). Differences in morphological traits among cultivars could be attributed to genetic and environmental factors, including temperature, sunlight, rainfall, and soil conditions (Parashuram et al., 2022; Khadivi & Arab, 2021). In this research, the cultivars studied were from the same location, suggesting that the observed variations were related to genetic factors (Khadivi et al., 2020).

The morphological analysis was complemented by assessing flower characteristics, revealing significant variations among the studied cultivars. The longest flowers were observed in Z-S (37.30 mm) and KNB-S (36.70 mm), while R-K and KT-F cultivars had the shortest flower length. Significant differences were also found, with Z-S having the largest (46.30 mm) and KNB-S the smallest (39.03 mm). Petal numbers varied widely, from 332.5 to 81.50 petals per tree. Cultivars R-K and KT-F had the highest petal number, whereas KNB-S and Z-S cultivars had the lowest. KT-F flowers had the largest petals, while S-F and R-K cultivars showed the shortest petal length and width, respectively. Notably, the flowers of S-F, R-K, and KT-F cultivars were sterile, lacking pistils and stamens.

The lengths of stamen and styles in the flowers of the KNB-S cultivar measured 11.80 and 11.00 mm, respectively, while in the Z-S cultivar, these measurements were 8.70 and 10.80 mm, respectively. Flowers play an important role in ornamental pomegranates as they contain different phytochemical compounds with significant antioxidant capacity (Karimi et al., 2017). Therefore, assessing flower biomass across different genotypes holds economic value (Ge et al., 2021). In this study, flower dry weight ranged from 0.50 to 0.98 g, with the highest dry weights observed in R-K and KT-F cultivars and the lowest in KNB-S cultivar (Table 3). The significant variation in flower morphology among different pomegranate genotypes was also reported by Ikram et al. (2022), who reported that floral characterization is essential for pomegranate breeding programs. A recent study by Ferrara et al. (2023) also reported a difference in flower weight among pomegranate cultivars. According to their findings, the phenotypic differences observed among

Table 1. Descriptive statistics for evaluated quantitative characteristics of *P. granatum* var. *pleniflora* cultivars.

Trait	Abbr.	Unit	Min	Max	Average	SD	CV (%)
Tree height	HT	m	2.00	4.10	2.81	0.66	28.96
Main stem diameter	MSD	mm	82.00	180.00	130.65	30.65	4.24
Stem diameter	SD	mm	4.00	9.00	6.24	1.36	18.69
Petiole length	PeL	mm	3.00	10.00	5.16	1.74	25.57
Leaf length	LL	mm	48.00	84.00	64.95	9.75	4.81
Leaf width	LW	mm	14.00	24.50	18.07	3.34	10.12
Leaf dry weight	LDW	gr	0.16	0.41	0.26	0.08	106.63
No. thorn on an annual shoot	NTA	Code	1.00	5.00	2.20	1.64	58.24
No. thorn on a perennial shoot	NTP	Code	1.00	5.00	2.20	1.64	58.24
No. sucker	NS	Code	1.00	5.00	4.20	1.64	30.51
Flower length	FL	mm	9.00	43.00	25.68	11.51	13.21
Flower corolla diameter	CFD	mm	29.50	55.00	43.22	6.92	6.09
Petal number	PN	No.	55.00	360.00	206.60	107.21	5.01
Petal length	PL	mm	19.00	31.00	23.75	4.12	8.54
Petal width	PW	mm	12.10	24.00	19.19	2.92	8.90
Stamen length	StL	mm	0.00	13.00	4.09	5.29	56.24
Style length	SL	mm	0.00	13.00	4.36	5.63	54.42
Flower dry weight	FDW	gr	0.42	1.44	0.82	0.28	64.46
Polyphenol content	PC	mg GAE g^{-1} DW	66.32	126.25	89.77	19.67	4.94
Flavonoids content	FC	mg QE g^{-1} DW	25.24	79.24	44.18	20.05	10.13
Tannin content	TC	mg TA g^{-1} DW	54.65	102.32	79.26	13.57	4.65
Anthocyanin content	AC	mg g^{-1} DW	15.11	43.20	31.56	7.44	8.64
DPPH	DPPH	μg^{-1} mL	21.30	47.05	35.50	7.76	7.85

SD: Standard deviation; CV: Coefficient of variation.

Table 2. The variations of morphological traits among the studied *P. granatum* var. *pleniflora* cultivars

Cultivar	Height (m)	Main stem diameter (mm)	Stem diameter (mm)	Petiole length (mm)	Leaf length (mm)	Leaf width (mm)	Leaf dry weight (g)	No. Thorn on an annual shoot	No. Thorn on a perennial shoot	No. Sucker per tree
KNB-S	2.33±0.13^{c}	153.00±8.33^{ab}	5.18±0.79^{c}	4.25±0.50^{a}	54.00±5.89^{b}	19.50±4.12^{ab}	0.298±0.06^{b}	1.00±0.00^{c}	1.00±0.00^{c}	5.00±0.00^{a}
S-F	2.83±0.24^{b}	87.80±5.44^{d}	5.38±1.25^{c}	6.25±3.30^{a}	68.88±10.30^{a}	15.73±1.78^{ab}	0.215±0.06^{c}	5.00±0.00^{a}	5.00±0.00^{a}	5.00±0.00^{a}
Z-S	2.23±0.22^{c}	112.50±22.17^{c}	7.13±0.85^{a}	5.08±0.87^{a}	73.88±4.52^{a}	20.18±3.55^{a}	0.378±0.07^{a}	1.00±0.00^{c}	1.00±0.00^{c}	5.00±0.00^{a}
R-K	3.98±0.13^{a}	165.00±12.91^{a}	7.25±1.50^{a}	4.60±0.42^{a}	64.33±6.55^{ab}	19.63±2.83^{ab}	0.235±0.06^{c}	1.00±0.00^{c}	1.00±0.00^{c}	1.00±0.00^{b}
KT-F	2.70±0.22^{b}	135.00±5.77^{ab}	6.25±1.26^{b}	5.60±1.91^{a}	63.65±10.42^{ab}	15.33±0.90^{b}	0.180±0.05^{c}	3.00±0.00^{b}	3.00±0.00^{b}	5.00±0.00^{a}

Values followed by the same letter within a column indicate they are not significantly different ($p < 0.05$).

Table 3. The variations of flower traits among the studied *P. granatum* var. *pleniflora* cultivars

Cultivar	Flower length (mm)	Corolla diameter (mm)	No. Petal per tree	Petal length (mm)	Petal width (mm)	Stamen length (mm)	Style length (mm)	Flower dry weight (g)
KNB-S	36.70±5.44 ^{a}	39.03±2.42 ^{d}	97.75±19.19 ^{c}	21.25±2.06 ^{c}	20.00±1.41 ^{a}	11.80±0.96 ^{a}	11.00±1.41 ^{a}	0.50±0.10 ^{b}
S-F	27.65±3.30 ^{b}	44.13±7.15 ^{b}	215.00±14.72 ^{b}	20.75±0.96 ^{c}	19.50±3.70 ^{a}	0.00±0.00 ^{c}	0.00±0.00 ^{c}	0.88±0.07 ^{a}
Z-S	37.30±5.64 ^{a}	46.30±5.72 ^{a}	81.50±21.38 ^{c}	25.00±1.58 ^{b}	19.43±1.34 ^{a}	8.70±1.70 ^{b}	10.80±2.93 ^{b}	0.76±0.07 ^{ab}
R-K	13.75±2.63 ^{c}	44.75±5.85 ^{b}	332.50±22.17 ^{a}	21.50±3.70 ^{c}	15.50±2.41 ^{b}	0.00±0.00 ^{c}	0.00±0.00 ^{c}	0.97±0.40 ^{a}
KT-F	13.00±3.03 ^{c}	41.88±11.69 ^{c}	306.25±11.09 ^{a}	30.25±0.65 ^{a}	21.50±2.08 ^{a}	0.00±0.00 ^{c}	0.00±0.00 ^{c}	0.98±0.32 ^{a}

Values followed by the same letter within a column indicate they are not significantly different ($p < 0.05$).

flowers of various cultivars are mainly due to the petalization of stamens and the development of petal transitional forms. In addition, Huo et al. (2023) analyzed genetic variation related to flowering and proposed that transcription factors play a crucial role in regulating flower organ morphogenesis in pomegranates. These studies support the present research results, attributing the observed phenotypic variations among the studied cultivars to genetic factors.

Phytochemical traits

P. granatum contains several phytochemicals, including phenolic compounds, anthocyanins, flavonoids, and tannins (Ge et al., 2021). Phenylpropanoid metabolism is a primary, secondary metabolic pathway in plant cells that produces different compounds with high antioxidant capacities. Phenylalanine ammonia-lyase (PAL) is the

key enzyme involved in phenylpropanoid metabolism, regulating the biosynthesis of phenolic compounds, mainly phenolic acids and flavonoids (Cesarino et al., 2022).

Variance analysis revealed that the cultivar type significantly influenced the phytochemical traits of the flower extract. Significant differences were observed among the studied cultivars *P. granatum var. pleniflora* concerning the content of total phenol, flavonoids, hydrolyzable tannins, and antioxidant capacity. According to Abdolahi et al. (2018), polyphenols constitute a large proportion of the antioxidants in pomegranate flowers. The study found a considerable difference between the cultivars' highest and lowest total phenol content. The highest phenol content (119.33 mg GAE g-1 DW) was recorded in the Z-S cultivar, 70% higher than the lowest total phenol content observed in KT-F flowers. The total phenol content in the studied cultivars exceeded the values reported by Abdolahi et al. (2018). The Z-S cultivar also exhibited the highest flavonoid content at 72.21 mg QE g-1 DW, while KNB-S, KT-F, and S-F cultivars had the lowest flavonoid content at 26.93, 28.78, and 30.5 mg g-1 QE DW, respectively. A similar range of phenolic compound content in Iranian pomegranate flowers was reported by Tekin and Kucukbay (2024).

Anthocyanins, derivates of flavonoids, are water-soluble pigments with significant pharmacological properties due to their high antioxidant capacity (Gosciniak et al., 2022). The antioxidant activity of anthocyanins is correlated with the number of phenolic hydroxyl groups on the B-ring of their structure (Zhang et al., 2011). In this study, no significant differences were found among the cultivars regarding anthocyanin content in pomegranate flowers, which ranged from 27.26 mg g-1 DW in KT-F to 38.03 mg g-1 DW in KNB-S. The tannin content in *P. granatum* var. *pleniflora* flowers varied from 65.69 to 95.94 mg TA g-1DW, averaging 79.26 mg TA g-1 DW. The highest content of hydrolyzable tannins was observed in the Z-S and KT-F cultivars, while the lowest levels were found in the S-F and R-K cultivars. These differences in phenolic compound content among cultivars are attributed to variations in photo-assimilation, environmental stress responses, and plant defense mechanisms (Hmid et al., 2017). In addition, the expression of genes and enzymes involved in the phenylpropanoid pathway can vary between species and within a single species (Fellah et al., 2018).

In this study, the antioxidant activity of flower extracts was assessed using IC50, where lower IC50 values indicated higher DPPH scavenging ability. The DPPH antioxidant capacity of the cultivars ranged from 25.29 to 44.87 µg mL-1. The Z-S cultivar, followed by the R-K cultivar, showed the highest antioxidant activity, while the KT-F cultivar had the lowest DPPH activity (Table 4). The superior antioxidant efficiency of the Z-S flower extract at low concentrations was attributed to its higher content of different phenolic compounds. Considerable variations in the phenolic compound content and antioxidant potential of pomegranate flowers have been reported in previous studies (Abdolahi et al., 2018; Elfalleh et al., 2012; Fellah et al., 2018; Zhang et al., 2011). Abdolahi et al. (2018) reported the antioxidant capacity of Iranian pomegranate flowers with an IC50 value of 27.92 µg mL-1 IC50.

An antioxidant assessment in this research indicates that pomegranate flowers from the studied cultivars are rich sources of natural antioxidant phenolic compounds, such as anthocyanin, flavonoids, and tannins. These findings highlight the potential of pomegranate flowers for greater utilization by pharmaceutical and food industries in developing health-promoting products with high nutritional value. Furthermore, exploring new pomegranate-based products such as ready-to-eat pomegranate flowers, frozen flowers, and syrup is recommended to support a healthier lifestyle.

Correlation analysis

One of the most used procedures by breeders in breeding programs is selection based on the results of correlation analysis (Arlotta et al., 2022). The Pearson correlation coefficients confirmed a significant relationship among the assayed traits (Figure 2). Leaf dry weight was positively correlated with leaf width (r= 0.805), stamen length (r= 0.893), style length (r= 0.811), and flower length (r= 0.813), and negatively correlated with petal number (r= -0.842). Flower dry weight was positively correlated with petal number (r= 0.847), and there was a negative correlation between the flower dry weight and the length of style (r= -0.937), stamen (r= -0.870) and flower (r= -0.841). A positive correlation was observed between anthocyanin content and style length (r= 0.938), flower length (r= 0.888), and stamen length (0.885). In addition, anthocyanin content was negatively correlated with petal number (r= -0.881) and flower dry weight (r= -0.991).

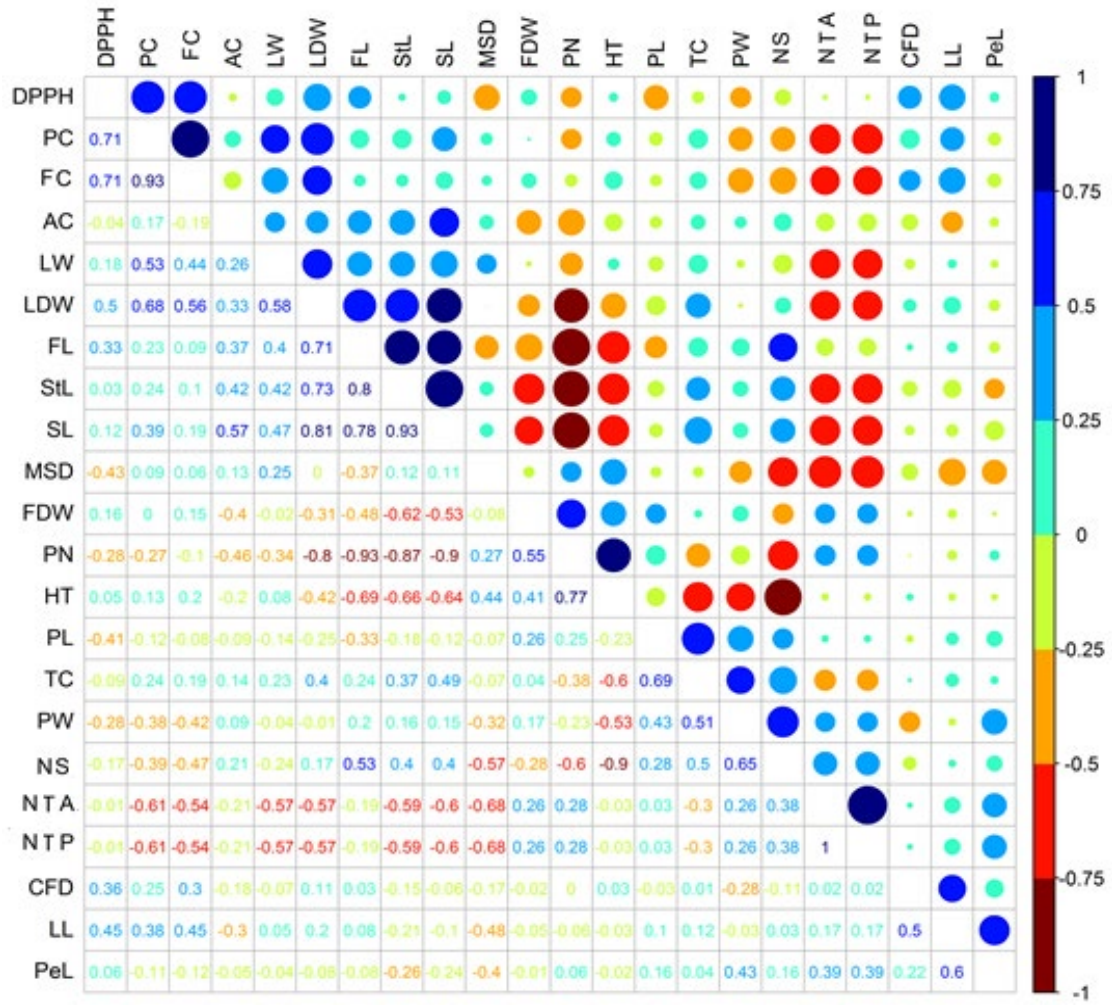

Figure 2. Pearson correlation coefficients among 23 morphological and phytochemical traits of *P. granatum* var. *pleniflora* cultivars. DPPH: Antioxidant activity; PC: Total phenol content; FC: Total flavonoids content; SD: Stem diameter; CFD; Corolla diameter; MSD: Main stem diameter; FDW: Flower dry weight; PN: Petal number; HT: Tree height; AC: Total anthocyanin content; LW: leaf width; LDW: leave dry weight; FL: Flower length; StL: Style length; SL: Stamen length; PL: Petal length; TC: Total tannin content; PW: Petal width; NS: Number of sucker; NTA: Number of thorn on an annual shoot; NTP: Number of thorn on a perennial shoot; LL: Leave length; PeL: Petiole length.

Table 4. The variations of phenolic compounds and antioxidant activity among the studied *P. granatum* var. *pleniflora* cultivars

Cultivar	Total phenol (mg GAE g^{-1} DW)	Flavonoids (mg QE g^{-1} DW)	Anthocyanin (mg g^{-1} DW)	Tannin (mg TA g^{-1} DW)	DPPH (µg mL^{-1})
KNB-S	78.54±3.08 ^{c}	26.93±1.47 ^{c}	38.03±3.91 ^{a}	77.99±2.88 ^{b}	38.01±1.95 ^{b}
S-F	76.13±3.71 ^{c}	30.59±1.51 ^{c}	30.60±4.37 ^{a}	65.69±6.32 ^{c}	40.40±1.71 ^{b}
Z-S	119.33±5.31 ^{a}	72.21±5.50 ^{a}	33.36±9.95 ^{a}	95.94±5.67 ^{a}	25.29±2.64 ^{d}
R-K	104.56±2.47 ^{b}	62.41±7.45 ^{b}	28.53±9.68 ^{a}	66.16±8.30 ^{c}	28.92±2.40 ^{c}
KT-F	70.28±3.76 ^{d}	28.78±2.36 ^{c}	27.26±4.98 ^{a}	90.53±0.80 ^{a}	44.87±2.72 ^{a}

Values followed by the same letter within a column indicate they are not significantly different ($p < 0.05$).

The correlation analysis of the assayed phytochemical traits showed a significant positive relationship between DPPH antioxidant activity and total flavonoid (r= 0.930) and phenol (r= 0.710) content. A significant correlation between different phenolic compounds and antioxidant activity has been previously reported by Montefusco et al. (2021) and Haldhar et al. (2022). According to Gosciniak et al. (2022), antioxidant potential is closely linked to reductones, which neutralize free radicals. The antioxidant activity of phenolic compounds is attributed to their redox properties, hydrogen-donating abilities, and singlet oxygen scavenging.

Principal component analysis

Several traits with high-dimensional data are the main constricts of interpreting results in biology experiments. Principal component analysis (PCA) is commonly used to facilitate the interpretation of complex data by reducing it to fewer dimensions and summarizing the key traits. PCA determines the main features with high variability that play a key role in distinguishing the effects of the applied treatments. Therefore, the selected traits can be considered an index for identifying cultivars or genotypes with the desired characteristics (Ashrafi et al., 2022).

This study conducted PCA on the morphological and phytochemical traits of the five pomegranate cultivars. PCA explained 30.38 and 22.35% of the total variance on the first and second components. Figure 3 shows the eigenvalue of the first 19 PCs of 23 assayed traits. Khadivi et al. (2020) explained 72.06% of the total variance in 23 PCs of Iranian wild pomegranate accessions. PC1 was dominated by stamen length, leaf dry weight, style length, flower length, leaf width, anthocyanin content, petal number, and throne number. PC2 included tree height, flavonoid content, stem diameter, phenol content, number of suckers, and petal width (Figure 4A). Biplot analysis based on the key characteristics of the two first PCs revealed a two-dimensional distribu f the studied cultivars. The positions of two or cultivars in the same part of the biplot graph indicate their genetic similarity (Ashrafi et al., 2022).

According to PCA, the KNB-S cultivar was separated from the others along PC1 due to its longest flower, style, and stamen. In addition, the R-K cultivar was associated with PC2, while the Z-S cultivar was characterized by its high levels of total phenol, flavonoids, and DPPH. The S-F and KT-F cultivars were clustered together with negative values for PC1 and PC2, characterized by a high number of suckers and throne (Figure 4B). These results suggest that both morphological and phytochemical traits are key factors in differentiating the studied cultivars of *P. granatum* var. *pleniflora*. Similar findings were reported by Ikram et al. (2022), who emphasized the importance of morphological traits in identifying and clustering pomegranate cultivars, accessions, and genotypes.

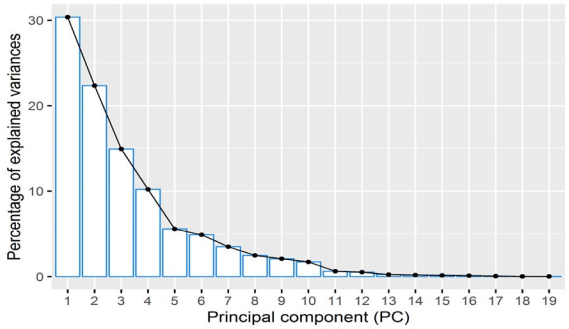

Figure 3. The eigenvalue of first 19 principal components of 23 assayed traits of *Punica granatum* var. *pleniflora* cultivars.

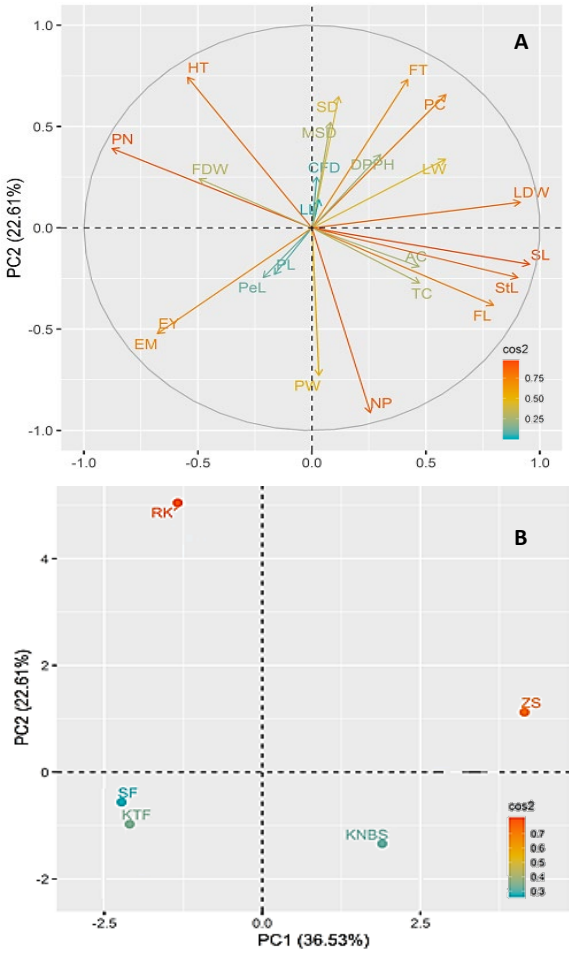

Figure 4. Principal components analysis (PCA) based on 23 assayed traits of *Punica granatum* var. *pleniflora* cultivars. DPPH: Antioxidant activity; PC: Total phenol content; FT: Total flavonoids content; SD: Stem diameter; CFD; Corolla diameter; MSD: Main stm diameter; FDW: Flower dry weight; PN: Petal number; HT: Tree height; AC: Total anthocyanin content; LW: leaf width; LDW: leave dry weight; FL: Flower length; StL: Style length; SL: Stamen length; PL: Petal length; TC: Total tannin content; PW: Petal width; NP: Number of sucker; EY: Number of thorn on an annual shoot; EM: Number of thorn on a perennial shoot; LL: Leave length; PeL: Petiole length.

CONCLUSION

The present research aimed to assay Iranian pomegranate cultivars' morphological and phytochemical diversity. The pomegranate cultivars were selected from the same site. The results demonstrated significant variations in phenotypical traits among the cultivars. Furthermore, there was considerable diversity in the content of different phenolic compounds and antioxidant activity. The methanolic extract of the Zinati-e-Saveh cultivar exhibited the highest levels of total phenols, total flavonoids, anthocyanin, hydrolyzable tannins, and antioxidant capacity. Principal Component Analysis (PCA) based on the evaluated traits further confirmed the distinct differences. Given the experimental site's similar environmental conditions, growth medium, and production operations, the observed differences are likely attributed to genetic variations. Therefore, these cultivars represent a rich genetic source for further breeding programs. Overall, considerable variation was observed in both phenotypic and phytochemical traits. This research not only provides valuable insights into each cultivar's growth, yield components, and phytochemical potential but also highlights superior traits and cultivars. Furthermore, the correlation between morphological and biochemical traits facilitates the identification of key biochemical parameters and active substances, thereby reducing time and cost.

AVAILABILITY OF DATA

The data presented in this study are available on request from the corresponding authors.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors contributed to the experiments performed. RSH contributed to the experiment design, analyzed the data, and performed statistical analyses, writing, revising and improving the manuscript. AY, MV, and MI conceptualized the idea, performed the experiments, and interpreted the results. All authors have read and approved the final manuscript.

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HERBAL ETHICS STATEMENT

The authors state that all the samples used in the research are from the collection of Yazd Natural Resources Research Centre (YNRRC) and all necessary approvals have been obtained in this regard.

REFERENCES

Abdolahi, N., Soltani, A., Mirzaali, A., Soltani, S., Balakheyli, H., Aghaei, M. (2018). Antibacterial and antioxidant activities and phytochemical properties of *Punica granatum* flowers in Iran. *Iranian Journal of Science and Technology, Transactions A: Science.* 42, 1105-1110. https://doi.org/10.1007/s40995-017-0413-8

Arlotta, C., Toscano, V., Genovese, C., Calderaro, P., Puglia, G.D., Raccuia, S.A. (2022). Nutraceutical content and genetic diversity share a common pattern in new pomegranate genotypes. *Molecules*, 27(2), 389. https://doi.org/10.3390/molecules27020389

Aryal, S., Baniya, M.K., Danekhu, K., Kunwar, P., Gurung, R., Koirala, N. (2019). Total phenolic content, flavonoid content and antioxidant potential of wild vegetables from western Nepal. *Plants*, 8(4), 96. https://doi.org/10.3390/plants8040096

Ashrafi, S.H., Bodaghi, H., Rezaei. M., 2022. Morphological diversity of indigenous wild pomegranate (*Punica granatum* L. var. *spinosa*) accessions from northeast of Iran. *Food Science & Nutrition*, 11(2), 1001-1012. https://doi.org/10.1002/fsn3.3135

Aziz, S., Firdous, S., Rahman, H., Awan, S.I., Michael, V., Meru, G. (2020). Genetic diversity among wild pomegranate (*Punica granatum*) in Azad Jammu and Kashmir region of Pakistan. *Electronic Journal of Biotechnology,* 46, 50-54. https://doi.org/10.1016/j.ejbt.2020.06.002

Çam, M., Hışıl, Y. (2010). Pressurised water extraction of polyphenols from pomegranate peels. *Food Chemistry*, 123(3), 878-885. https://doi.org/10.1016/j.foodchem.2010.05.011

Cesarino, I., Eudes A., Urbanowicz, B., Xie, M. (2022). Editorial: phenylpropanoid systems biology and biotechnology. *Frontiers in Plant Science*, 13, 866164. https://doi.org/10.3389/fpls.2022.866164

Elfalleh, W., Hannachi, H., Tlili, N., Yahia, Y., Nasri, N., Ferchichi, A. (2012). Total phenolic contents and antioxidant activities of pomegranate peel, seed, leaf and flower. *Journal of Medicinal Plants Research*, 6(32), 4724-4730. https://doi.org/10.5897/JMPR11.995

Fellah, B., Bannour, M., Rocchetti, G., Lucini, L., Ferchichi, A. (2018). Phenolic profiling and antioxidant capacity in flowers, leaves and peels of Tunisian cultivars of *Punica granatum* L. J*ournal of Food Science and Technology,* 55, 3606-3615. https://doi.org/10.1007/s13197-018-3286-8

Ferrara, G., Porfido, C., Terzano, R., Sarkhosh, A., Mazzeo, A.A. (2023). Study on the characteristics of buds and flowers in pomegranate: differences among cultivars.

Horticulturae, 9, 117. https://doi.org/10.3390/horticulturae9010117

Gavanji, S., Larki, B., Bakhtari, A. (2015). Comparative analysis of extract of *Punica granatum* var. *pleniflora* (Golnar-e-farsi) to some antibiotics on pathogens. *Journal of Essential Oil Bearing Plants*, 18(1), 168-178. https://doi.org/10.1080/0972060X.2014.960272

Ge, S., Duo, L., Wang, J., Yang, J., Li, Z., Tu, Y. (2021). A unique understanding of traditional medicine of pomegranate, *Punica granatum* L. and its current research status. *Journal of ethnopharmacology*, 271, 113877. https://doi.org/10.1016/j.jep.2021.113877

Gosciniak, A., Bazan-Wozniak, A., Pietrzak, R., Cielecka-Piontek, J. (2022). Pomegranate flower extract—the health promoting properties optimized by application of the Box–Behnken design. *Molecules*, 27(19), 6616. https://doi.org/10.3390/ molecules27196616

Giusti, M.M., Wrolstad, R.E., (2001). Characterization and measurement of anthocyanins by UV-Visible spectroscopy. *Current Protocols in Food Analytical Chemistry*, (1), F1-2. https://doi.org/10.1002/0471142913.faf0102s00

Haldhar, S.M., Kumar, R., Corrado, G., Berwal, M.K., Gora, J.S., Thaochan, N., Basile, B. (2022). A field screening of a pomegranate (*Punica granatum*) Ex-situ germplasm collection for resistance against the false spider mite (*Tenuipalpus punicae*). *Agriculture*, 12(10), 1686. https://doi.org/ 10.3390/agriculture12101686

Hmid, I., Elothmani, D., Hanine, H., Oukabli, A., Mehinagic, E. (2017). Comparative study of phenolic compounds and their antioxidant attributes of eighteen pomegranate (*Punica granatum* L.) cultivars grown in Morocco. *Arabian Journal of Chemistry*, 10, S2675-S2684. https://doi.org/10.1016/j.arabjc.2013.10.011

Huo, Y., Yang, H., Ding, W., Yuan, Z., Zhu, Z. (2023). Exploring the relationship between genomic variation and phenotype in ornamental pomegranate: A study of single and double-petal varieties. *Horticulturae*, 9, 361. https://doi.org/10.3390/horticulturae9030361

Ikram, S., Shafqat, W., Rehman, S.U., Qureshi, M.A., Din, S.U., Ikram, S., Jaskani, M.J. (2022). Floral characterization of pomegranate genotypes to improve hybridization efficiency. *Plants*, 12(1), 165. https://doi.org/10.3390/plants12010165

Kandylis, P., Kokkinomagoulos, E. (2020). Food applications and potential health benefits of pomegranate and its derivatives. *Foods*, 9(2), 122. https://doi.org/10.3390/foods9020122

Karimi, M., Sadeghi, R., Kokini, J. (2017). Pomegranate as a promising opportunity in medicine and nanotechnology, *Trends in Food Science & Technology*, 69, 59-73. https://doi.org/10.1016/j.tifs.2017.08.019

Khadivi, A., Arab, M. (2021). Identification of the superior genotypes of pomegranate (*Punica granatum* L.) using morphological and fruit characters. *Food Science & Nutrition*, 9(8), 4578-4588. https://doi.org/10.1002/fsn3.2450

Khadivi, A., Mirheidari, F., Moradi, Y., Paryan, S. (2020). Morphological variability of wild pomegranate (*Punica granatum* L.) accessions from natural habitats in the Northern parts of Iran. *Scientia Horticulturae*, 264, 109165. https://doi.org/10.1016/j.scienta.2019.109165

Maphetu, N., Oshiomame Unuofin, J., Prenate Masuku, N., Olisah, C., Lebelo, S.L. (2022). Medicinal uses, pharmacological activities, phytochemistry, and the molecular mechanisms of *Punica granatum* L. (pomegranate) plant extracts: A review. *Biomedicine & Pharmacotherapy,* 153, 113256. https://doi.org/10.1016/j.biopha.2022.113256

Montefusco, A., Durante, M., Migoni, D., De Caroli, M., Ilahy, R., Pek, Z., Lenucci, M.S. (2021). Analysis of the phytochemical composition of pomegranate fruit juices, peels and kernels: A comparative study on four cultivars grown in Southern Italy. Plants, 10(11), 2521. https://doi.org/10.3390/plants10112521

Parashuram, S., Singh, N.V., Gaikwad, N.N., Corrado, G., Roopa Sowjanya, P., Basile, B., Marathe, R.A. (2022). Morphological, biochemical, and molecular diversity of an Indian ex situ collection of pomegranate (*Punica granatum* L.). *Plants*, 11(24), 3518. https:// doi.org/10.3390/plants11243518

Sarkhosh, A., Yavari, A.M., Zamani, Z. (2020). The pomegranate: Botany, production and uses; CABI: Wallingford, UK.

Shahsavari, S., Noormohammadi, Z., Sheidai, M., Farahani, F., Vazifeshenas, M.R. (2022). A bioinformatic insight into the genetic diversity within pomegranate cultivars: From nuclear to chloroplast genes. *Genetic Resources and Crop Evolution,* 69, 1207–1217. https://10.1007/s10722-021-01297-z

Sharifiyan, F., Mirjalili, S.A., Fazilati, M., Poorazizi, E., Habibollahi, S. (2019). Variation of ursolic acid content in flowers of ten Iranian pomegranate (*Punica granatum* L.) cultivars. *BMC Chemistry*, 13, 1-7. https://doi.org/10.1186/s13065-019-0598-3

Stojichevich, S.S., Stanisavljevich, I.V., Velichkovich, D.T., Lazic, M.L. (2008). Comparative of the antioxidant and antimicrobial activities of *Sempervium marmoreum* L. extracts obtained by various extraction techniques. *Journal of the Serbian Chemical Society,* 73(6), 597–607. https://doi.org/10.2298/JSC0806597S

Tekin, Z., Kuçukbay, F. (2024). Pomegranate leaves, buds, and flowers: phytochemical, antioxidant, and comparative solvent analyzes. *Foods and Raw Materials*, 13(1), 155-171. http://doi.org/10.21603/2308-4057-2025-1-630

Wafa, B.A., Makni, M., Ammar, SKhannous, L., Hassana, A. B., Bouaziz, M., Gdoura, R. (2017). Antimicrobial effect of the Tunisian Nana variety *Punica granatum* L. extracts against *Salmonella enterica* (serovars Kentucky and Enteritidis) isolated from chicken meat and phenolic composition of its peel extract. *International Journal of Food Microbiology*, 241, 123-

131. https://doi.org/10.1016/j.ijfoodmicro.2016.10.007

Wong, T.L., Strandberg, K.R., Croley, C.R., Fraser, S.E., Venkata, K.N., Fimognari, C., Bishayee, A. (2021). Pomegranate bioactive constituents target multiple oncogenic and oncosuppressive signaling for cancer prevention and intervention. *Seminars in Cancer Biology*, 73, 265-293. https://doi.org/10.1016/j.semcancer.2021.01.006

Zhang, L., Fu, Q., Zhang, Y. (2011). Composition of anthocyanins in pomegranate flowers and their antioxidant activity. *Food chemistry*, 127(4), 1444-1449. https://doi.org/10.1016/j.foodchem.2011.01.077

Zhao, L., Liu, W., Xiong, S., Tang, J., Lou, Z.H., Xie, M.X., Liao, D.F. (2018). Determination of total flavonoids contents and antioxidant activity of *Ginkgo biloba* leaf by near-infrared reflectance method. *International Journal of Analytical Chemistry*, 2018(1), 8195784. https://doi.org/10.1155/2018/8195784.

Supplementary Figures

Sarvestan-e- Fars

Zinati-e- Saveh

Kenar Takht-e- Fars

Khoshe Nar Baharestan Sari

Rijab-e- Kermanshah