

Phenotypic and Phytochemical Diversity among Different Accessions of Gijavash (*Froriepia subpinnata* (Ledeb.) Baill.: An Endemic Medicinal Plant Grown in Iran

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ABSTRACT

Froriepia subpinnata is an endemic endangered medicinal plant growing indigenous to Caspian coast forest in the north of Iran. This research was done to obtain primary information on morphological and phytochemical variation for the next step of domesticating and breeding of this plant. Twenty-three morphological traits measured indicated high variation among 52 accessions. There was a significant positive correlation between leaf dimension, plant height, and attributes related to biomass data. The range of phytochemical attributes analyzed was for total phenols (355.6-941.3 mg GAE 100 g⁻¹ FW), total flavonoids (204.33-540.74 mg CAT 100 g⁻¹ FW), total carotenoids (1.52 to 3.15 µg 100g⁻¹ FW) and antioxidant capacity (31.36-81.82 DPPH%). The HPLC analysis results showed that chlorogenic acid was the dominant phenolic compound generally found in this plant. Total carotenoids had no significant correlation with other biochemical traits, while the rest exhibited a linear relationship with each other. UPGMA cluster based on combined data of morphological and biochemical traits showed 3 distinct groups with high inter and intra-regional variations. Also, these information were combined with molecular data to separate environment and genetic effects. The high level of variation of phytochemical and morphological traits among and within populations could be a useful tool for future breeding and selection programs.

Keywords: Breeding program, HPLC analysis, Phenolic compound, Threatened plant.

INTRODUCTION

Gijavash (*Froriepia subpinnata* (Ledeb.) Baill. Syn: *Bupleurum subinatum*, *Froriepia nuda*) is a biennial medicinal and aromatic plant belonging to Apiaceae. It is a self-pollinated plant, up to 220 cm height with white or purple flowers and small achene fruits (Mozaffarian, 2015). Gijavash leaves are used in people's diet and it is the only endemic threatened species of *Froriepia* genus in north of Iran (Mozaffarian, 2015).

Bupleurum L. including 200 species are extensively distributed in the north hemisphere, and usually used for their medicinal attributes (Mabberley, 2008). Previous studies have stated many physiological activities such as antiviral (Ashour *et al.*, 2014), immunomodulatory (Guinea *et al.*, 1994), anti-inflammatory (Bremner *et al.*, 2009), antiproliferative (Hsu *et al.*, 2004), and antioxidant activity (Liu *et al.*, 2006) for various species of *Bupleurum*. Also, recent researches have confirmed the existence of various compounds in *Bupleurum* plants such

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as saikosaponins (Liang *et al.*, 2014), flavonoids (Gevrenova *et al.*, 2015), total phenolic content (Nabavi *et al.*, 2008), and coumarins (Pistelli, 2006). Nowadays, collecting plants from their wild habitats is the main way to supply materials of the medicinal plant industries. Therefore, it exposes the ecotypes to annihilation (Bogers *et al.*, 2006). Also, we know that morphological traits, chemical composition, and biological activities of plants are affected by the genetic and environmental aspects (Heywood, 2002). Thus, the first step to prevent annihilation of endemic plants and supply plant raw materials is analyzing distribution zones, domestication, and cultivation of wild medicinal plant (Iezzoni and Pritts, 1991; Bogers *et al.*, 2006).

Due to lack of earlier literature about morphological and phytochemical attributes, this study was done to reveal the morphological and biochemical variation of *Froriepia subpinnata* collected from different natural habitats in Iran.

MATERIALS AND METHODS

Plant Materials

Fifty-two accessions of *F. subpinnata* were gathered from different regions of Guilan province, Iran (Table 1). Fresh leaves were immediately frozen in liquid nitrogen

and kept at -80°C for future studies.

Morphological Analysis

Voucher specimens were deposited in herbarium of the University of Guilan. Morphological studies were conducted on fully flowered fresh plants and herbarium materials. Twenty-three morphological traits, from at least 10 plants, were measured. These included plant height (by ruler), leaf and leaflet number, leaf and leaflet length (by ruler and caliper), leaf and leaflet width (by ruler and caliper), fresh weight of leaves, stem and root, terminal leaflet length and width (by caliper), 1,000 seeds weight, internode length, peduncle length of umbels and umbellete (by caliper), number of flowers and umbels per plant, secondary stem number and stem diameter (by caliper). To measure the dry weight, leaves, stem, and roots were put in the oven at 70°C for 48 hours, then, the dry weight was measured by scale.

Determination of Total Phenolic Content

Total Phenolic Content (TPC) was measured by using Folin-Ciocalteu reagent (Dewanto *et al.*, 2002) with some

Table 1. List of 52 germplasm accessions of *F. subpinnata* gathered from Guilan Province of Iran.

Accessions number	Origin	Latitude (N)	Longitude (E)	Average of altitude (m)	Voucher number
G1 to G6	Astaneh-ye Ashrafiyeh	$37^{\circ} 15'$	$49^{\circ} 56'$	-11	APF53098
G7 to G11	Rasht	$37^{\circ} 16'$	$49^{\circ} 35'$	83	APF54005
G12 to G15	Kiashahr	$37^{\circ} 25'$	$49^{\circ} 56'$	-23	APF54014
G16 to G19	Lahijan	$37^{\circ} 12'$	$50^{\circ} 0'$	60	APF54018
G20 to G23	Langrud	$37^{\circ} 11'$	$50^{\circ} 9'$	16	APF54024
G24 to G28	Rudsar	$37^{\circ} 8'$	$50^{\circ} 16'$	-17	APF54028
G29 to G32	Sangar	$37^{\circ} 10'$	$49^{\circ} 41'$	109	APF54033
G33 to G37	Someehsara	$37^{\circ} 18'$	$49^{\circ} 18'$	34	APF54038
G38 to G42	Fuman	$37^{\circ} 13'$	$49^{\circ} 19'$	45	APF54041
G43 to G47	Shaft	$37^{\circ} 10'$	$49^{\circ} 24'$	76	APF54045
G48 to G52	Siyahkal	$37^{\circ} 9'$	$49^{\circ} 52'$	42	APF54052

modification as follows: a 190 μL of distilled water added to 10 μL of the methanolic extract (methanol and acetic acid 85:15 v/v) of each sample followed by addition of 1 mL of 10% Folin-Ciocalteu reagent. After 5 minutes, 800 μL of 7.5% sodium carbonate solution was added and the samples were put in darkness for 90 minutes before measuring the absorbance at 760 nm using an UV/Vis spectrophotometer (model PG Instrument +80, Leicester, United Kingdom). TPC was calculated as mg Gallic Acid (GA) equivalents per 100 g Fresh Weight (FW) basis using a standard curve developed with gallic acid.

Determination of Total Carotenoid Content

Total Carotenoid (TCC) was measured *via* spectrophotometer (Mínguez-Mosquera and Pérez-Gálvez, 1998). A 100-mg powdered tissue was added to 1,200 μL acetone 80% (v/v) and kept at room temperature for 30 minutes. Afterward, the mixture was centrifuged at 5,000 rpm for 10 minutes, and the supernatant was separated for analysis. The absorbance of the solution was determined at 470 nm. The pigment concentration was calculated and expressed as $\mu\text{g } 100 \text{ g}^{-1} \text{ FW}$.

Determination of Total Flavonoid Content

One gram of tissue powdered by liquid nitrogen was added to 10 mL of extraction solvent (methanol/ acetic acid 85:15 v/v) and kept overnight at room temperature (Cordenunsi *et al.*, 2003). The mixture was centrifuged at 10,000 rpm for 10 minutes at 4°C, and the supernatant was separated for future measurement. Total Flavonoid Contents (TFC) were analyzed by a colorimetric method described by Shin *et al.* (2007) with some modifications. Thirty μL of each sample was added to 1.8 mL of methanol. Afterward, 75 μL of 5% NaNO_2

was added and allowed to stand at room temperature for 5 minutes, then, 75 μL of 3% $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ was added. After 5 minutes, 0.5 mL of 1M NaOH was added to the mixture and put in darkness for 15 minutes. The absorbance was measured at 506 nm and results were expressed as catechin equivalents using a standard curve developed with catechin.

Antioxidant Capacity

DPPH free radical scavenging assay was applied to estimate Antioxidant Capacity (AC) according to method described by Sánchez-Moreno *et al.* (1998) with some modifications. A sample of 10 μL of extract was added to 990 μL of DPPH and allowed to stand in darkness at room temperature for 20 minutes. Then, the Absorbance (A) was read at 517 nm versus the blank. Three replicates were carried out for each sample. The radical scavenging capacity was calculated using the following equation:

$$\text{DPPH}\% = \left(\frac{A_{\text{blank}} - A_{\text{sample}}}{A_{\text{blank}}} \right) \times 100 \quad (1)$$

HPLC Analysis

Phenolic profiles were determined described by Bakhshi and Arakawa (2006). Twelve accessions based on genetic clustering (Jorkesh *et al.*, 2019) were selected for High Performance Liquid Chromatography (HPLC) analysis. The methanolic extracts (Methanol: Acetic acid 85:15 v/v) were filtered through a 0.45 μm single-use syringe filter. Afterward, 20 microliters of filtered specimen were injected in HPLC (Waters, 1525, Milford, USA) equipped with a UV-visible detector (Waters Dual λ Absorbance 2487), C18 column: Waters Symmetry C18, 4.6 mm \times 150 mm (Waters, Dublin, Ireland), at 274 and 320 nm. Phenolic compounds were identified by comparing their retention time and UV-vis spectral data to those of injected standards. Cumarin, ferulic acid and



chlorogenic acid standards were bought from Sigma-Aldrich (Canada Ltd).

Data analysis

Variance analysis was performed for all morphological and biochemical traits by SAS software ver 9.1. Coefficients of Variation (CV%) were determined as indicators of variability. Correlation (Pearson correlation coefficient) analysis among different attributes was subjected in SPSS ver. 22. The phytochemical and morphological similarity coefficients according to Euclidean distance were calculated. It was used for the cluster analysis and making of dendrogram through the Unweighted Pair-Group Method using Arithmetic average (UPGMA), performed by NTSYS ver. 2.10 (Rohlf, 2000). Finally, to check the fitness of the cluster, cophenetic correlation coefficient was calculated.

RESULTS

Morphological Characterization

Means and coefficient of variation for 23 morphological traits are presented in Table 2. The highest variation was observed in leaflet width and leaves number by 51.6 and 48.7% of CV, respectively (Table 2). The three lowest CVs among all traits belonged to the 1000 seeds weight (CV= 21%), leaflet number (CV= 28.6%), and flowers number per plant (CV= 30.9%) (Table 2). The plant height varied between 67.2 cm (G8) and 189 cm (G41) and 1000 seeds weight varied between 4.32 g (G7) and 7.31 g (G29). Differences in leaf length and leaflet length were high and varied from 6.42 cm (G49) to 29.16 cm (G38) and 10.2 mm (G36) to 30.7 mm (G38), respectively. The maximum number of flowers and umbels per plant was observed in P34 (149 and 124, respectively). The minimum number of flowers and umbels per plant belonged to G8 and G51 (49 and 28, respectively).

The analysis of correlation among morphological traits showed that plant height had a significant positive correlation with the number of leaves and leaflets and all traits concerning weight such as leaves, stem, and roots. Also, there were significant positive correlations in the leaf and leaflet dimensions with 1,000 seeds weight, flowers and umbels number per plant (data not presented). These results are in good agreement with Hadian *et al.* (2010) and Khadivi-Khub *et al.* (2014). To provide a reduced dimension model and understand the relationships between traits as well as between accessions (Khadivi-Khub *et al.*, 2012), factor analysis was used based on Principal Component (PCA). The results showed the first five-component account for 90.05% of total variations. The first and second components accounted for 41.82 and 31.80 % of the total variation, respectively.

Phytochemical Characterizations

Among all biochemical characterization, the lowest and highest variations were observed in antioxidant activity and total carotenoid by 27.48 and 34.3%, respectively (Table 3). TPC ranged from 355.6 to 941.3 mg GAE 100 g⁻¹ FW. The lowest amount of TPC belonged to the P43 accessions from Shaft, whereas the highest TPC was observed in P20 accessions from Langrud. The TFC range was between 204.33-540.74 mg CAT 100 g⁻¹ FW. The highest and lowest amounts of TFC were belonged to the G28 accessions from Rudsar and G31 accessions from Sangar, respectively. The variations in terms of TCC ranged from 1.52 to 3.15 µg 100 g⁻¹ FW. Among all accessions, the G1 accessions from Astaneh-ye Ashrafiyeh and G30 from Sangar had the lowest and highest of TCC, respectively. Also, AC's range was between 31.36-81.82%. The G20 accessions had the highest AC, whereas the lowest AC was measured in G31 accessions from Someh-Sara site. The results indicated significant positive relationship between TPC, TFC and AC, but there was no

Table 2. Descriptive statistics for morphological traits among studied *F. subpinnata* accessions.

No.	Character	Unit	Populations ^a										Mean	CV (%)
			As	Ra	Ki	Lh	Ln	Ru	Sa	So	Fu	Sh	Si	
1	Plant height	cm	146.9	81.6	111.9	165.6	159.6	108.6	134.2	91.8	180.3	106.1	171	132
2	Leaves number	n	44	19.6	22.5	56.7	49.9	23.2	37.2	21.3	60	24.5	54.4	37.2
3	Leaf length	cm	13.8	18.2	17.3	14.5	15.9	24	18.8	17.4	21.6	17.8	16.2	17.59
4	Leaflet number	n	21.2	13.7	16.6	23	23	17.1	19	13.7	25.2	15.9	21.9	19.08
5	Leaflet length	mm	13.8	15.8	18.2	17.1	17.7	21.3	20.2	19.9	23.7	18.3	18.7	18.57
6	Leaf fresh weight	g	245	111	165	292	289	169	232	143	328	165	318	222.6
7	Leaf dry weight	g	28.2	22.6	38.2	57.2	55.8	38.5	51.2	32.8	67.2	35.5	64.9	46.32
8	Stem fresh weight	g	91.9	35	70.2	111.9	106.4	72.1	85.1	48	136	60.8	119.9	84.7
9	Stem dry weight	g	28.9	12.1	25.2	37	34.2	26.5	31.6	17	40.7	21.1	39.9	28.36
10	Root fresh weight	g	87.8	31.8	56.2	105.1	100.8	53.1	81.7	40	117.7	54.3	111.2	75.8
11	Root dry weight	g	37.3	13.5	26	45.6	44.2	25.3	37.5	18.2	53.7	24.8	51	34
12	Leaf width	cm	8.85	9.87	8.98	7.29	8.34	10.75	9.84	8.21	12.37	9.06	8.96	9.36
13	Leaflet width	mm	8	8.4	10.1	9.3	9.7	15.3	13.6	11.6	14.6	11	9.8	11
14	Terminal leaflet length	mm	16	17.3	19.6	18.6	20.5	25.9	23.8	24.6	26.5	22.2	22.3	21.55
15	Terminal leaflet width	mm	10	9.74	11.2	11.4	11.9	18	14.7	12.9	16	12	11.2	12.64
16	1000 Seeds weight	g	5.5	5.37	5.71	6.41	5.56	6.51	6.49	6.14	6.27	5.92	6.25	6
17	Internode length	cm	6.42	9.12	7.61	6.28	8.65	9.47	9.51	9.39	9.22	7.47	7.95	8.26
18	Umbellete peduncle length	mm	4.73	5.50	4.64	5.53	6.71	6.86	6.64	5.14	6.93	6.54	6.60	5.97
19	Umbles peduncle length	cm	19.52	23.07	18.98	23.59	27.80	27.61	27.58	21.86	29.25	26.84	27.73	24.8
20	Secondary stem number	n	7.16	7.4	7.5	7.5	8.5	9.2	8	9.4	8.2	8.6	8.4	8.17
21	Flowers number per plant	n	70.3	67.6	77.5	74.5	81.25	101.6	106.5	100.8	101.4	87	74.6	85.5
22	Umbeles number per plant	n	55	49.2	60.25	56.72	66	85.8	90.25	84.4	84.2	67.8	56.4	68.5
23	Stem diameter	mm	4.99	6.41	6.77	6.01	7.34	7.89	8.56	8.69	9.24	6.91	6.76	7.23

^a As= Astaneh-ye Ashrafiyeh, Ra= Rasht, Ki= Kiashahr, Lh= Lahijan, Ln= Langrud, Ru= Rudsar, Sa= Sangar, So= Somehsara, Fu= Fuman, Sh= Shaft, Si= Siyahkal.

Table 3. Descriptive statistics for biochemical traits among studied *F. subpinnata* accessions.

Character	Unit	Populations ^a											CV (%)
		As	Ra	Ki	Lh	Ln	Ru	Sa	So	Fu	Sh	Si	
Total phenolic content	mg GAE 100 g ⁻¹ FW	685.6	575.7	724.8	754.4	842.6	620.6	599.8	524.2	625.7	522.6	659.0	28
Total flavenoid content	mg CAT 100 g ⁻¹ FW	393.8	330.7	416.3	433.4	484.0	356.5	344.5	301.8	359.4	311.6	378.5	29.65
Antioxidant activity	DPPH %	59.7	50.3	63.1	65.7	73.3	54.1	52.3	45.8	54.6	46.8	56.1	27.48
Total carotenoid	Mg 100 g ⁻¹ FW	2.20	2.33	2.49	2.61	2.44	2.62	2.72	2.46	2.7	2.54	2.63	34.3

^a As= Astaneh-ye Ashrafiyeh, Ra= Rasht, Ki= Kiashahr, Lh= Lahijan, Ln= Langrud, Ru= Rudsar, Sa= Sangar, So= Somehsara, Fu= Fuman, Sh= Shaft, Si= Siyahkal.

statistically significant correlation between TCC and these parameters (data not presented).

Cumarine, ferulic acid, and chlorogenic acid were identified and measured. Variations of phenolic compound are presented in Table 4. The coumarin content ranged between 102.40 $\mu\text{g g}^{-1}$ FW (P8) and 211.28 $\mu\text{g g}^{-1}$ FW (G20). The highest ferulic acid content (148.92 $\mu\text{g g}^{-1}$ FW) was observed at G37 accession and the lowest (40.34 $\mu\text{g g}^{-1}$ FW) belonged to G43 accession. Also, the G38 had the highest level of chlorogenic acid by 669.47 $\mu\text{g g}^{-1}$ FW, whereas the lowest content was measured in G12 accession by 147.3 $\mu\text{g g}^{-1}$ FW of (Table 4).

Combined Data

UPGMA cluster based on combined data of morphological and biochemical traits showed three distinct groups (Figure 1), such that accessions originating from the same region could not take the same place together completely, indicating high intra-regional diversity. For instance, accessions from Astaneh-ye Ashrafiyeh and Shaft were in different groups. The first distinct cluster included 22 accessions and the second and third main clusters contained 24 and 6 accessions, respectively. In some cases, high similarities were observed between inter and intra-regional accessions. For example, all accessions from Rasht and Lahijan were in the same cluster. Also, some accessions from Astaneh-ye Ashrafiyeh and Langrud had high similarities with each other. The similarities between accessions from different region shows the possibility of gene flow between the populations (Sefc *et al.*, 2000).

By combining morphological, biochemical, and molecular data (Jorkesh *et al.*, 2019) of these populations, it is possible to separate the effects of environment and genetics. For example, the accessions from Rasht were in the same group in both molecular and morpho-biochemical clusters.

Table 4. Phenolic compounds ($\mu\text{g g}^{-1}$ FW) in accessions of *F. subpinnata*.^a

Accessions	Coumarin	Ferulic acid	Cholorogenic acid
G4	142ef ^a	95e	212f
G8	179c	45g	611b
G12	102h	113c	148g
G16	136f	82f	589b
G20	211a	42g	590b
G27	155de	87ef	522c
G35	204ab	108cd	306e
G37	189bc	149a	481d
G38	161d	47g	669a
G41	122g	103d	299e
G43	195b	40g	503cd
G49	164d	131b	481d

^a a-g values in columns followed by the same letter are not significantly different, $P < 0.01$. Means separated by Tukey's HSD test in CRD design.

Table 5. Morphological and biochemical traits based on clustering accessions of *F. subpinnata*.

Character	First group	Second group	Third group
Total phenolic content	708.27	560.87	701.43
Total flavenoid content	406.8	329.25	402.92
Antioxidant activity	61.58	49.95	61.03
Total carotenoid	2.47	2.50	2.71
Plant height	165.2	95.91	154.7
Leaves number	52.97	20.93	44.66
Leaf length	15.51	18.13	23.02
Leaflet number	23.02	15.02	20.86
Leaflet length	1.70	1.78	2.73
Leaf fresh weight	295.08	143.00	275.92
Leaf dry weight	58.76	32.15	57.39
Stem fresh weight	114.38	51.44	109.45
Stem dry weight	37.27	18.16	36.44
Root fresh weight	105.39	43.98	95.08
Root dry weight	46.64	20.16	43.44
Leaf width	8.17	9.49	13.24
Leaflet width	9.40	10.79	17.86
Terminal leaflet length	19.12	20.98	32.76
Terminal leaflet width	11.2	12.12	20.03
1000 Seeds weight	5.87	5.93	6.72
Internode length	7.19	8.41	11.60
Umbellete peduncle length	5.83	5.52	8.25
Umbels peduncle length	23.82	22.76	36.74
Secondary stem number	7.54	8.12	10.66
Flowers number per plant	76.31	85.54	119
Umbels number per plant	59.54	68.62	100.83
Stem diameter	6.30	7.04	11.06

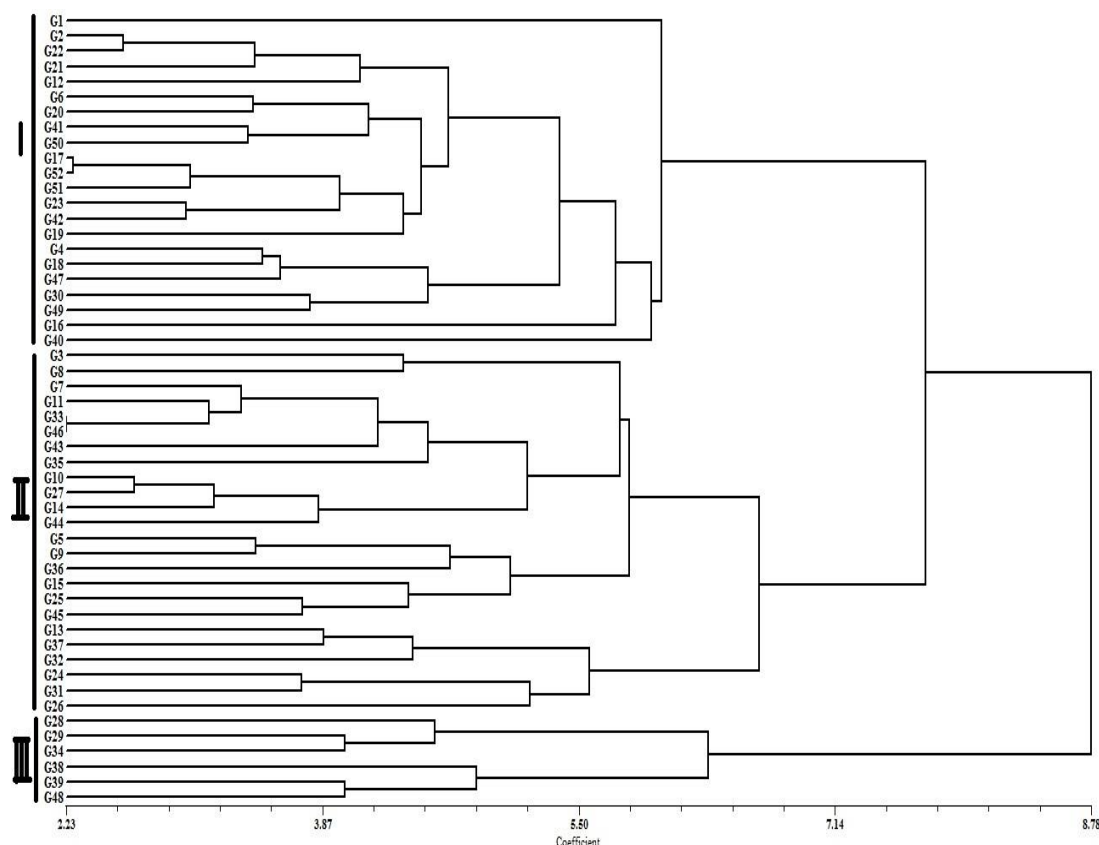


Figure 1. UPGMA cluster analysis for accessions of Gijavash based on the combined data of morphological and biochemical traits. Cluster numbers are Roman numerals on the left vertical axis.

Therefore, it was found that environmental conditions could not significantly affect morpho-biochemical traits of Rasht accessions. Also, all accessions from Astaneh-ye Ashrafiyeh were put in the same molecular cluster, while they were put into different clusters based on morpho-biochemical traits. Thus, it can be concluded that environmental conditions of Astaneh-ye Ashrafiyeh region had significant effect on morpho-biochemical traits. Morpho-biochemical traits were reported for each group (Table 4). Generally, the first group had the highest performance in terms of biochemical and biomass data followed by the third group, while the accessions placed in second group had the lowest biochemical and morphological traits (Table 5).

DISCUSSION

All measured parameters had significant differences among different accessions. The present research was the first investigation of morphological and biochemical traits of Gijavash accessions through comprehensive collection from natural habitat. The highest morphological variation was observed in leaflet width and leaves number. Based on our knowledge, there is not any literature on Gijavash morphological traits. Because of photosynthesis and production of secondary metabolites, leaf dimension and parameters concerning biomass are the most important characters that can be considered for cultivation and breeding programs (Khadivi-

Khub *et al.*, 2014). Thus, accessions G38 and G41 from Fuman, which have the higher value of leaf and biomass, are promising cultivars for future breeding programs. Another tool that could be considered for breeding and selection program is the existence of a correlation between various attributes (Pank *et al.*, 2004). In this study, there were significant positive correlations between biochemical traits and some morphological traits. For instance, TPC, TFC, and AC generally showed the same relationship with morphological traits: they had significant positive correlation with plant height, leaves number, leaflet number, and traits concerning biomass data, but there was no relationship between these traits and length of leaf and leaflet. However, these results were not observed for TCC. The TCC showed a significant correlation with traits related to leaf and leaflet dimensions, flower, and umbel number per plant (data not presented). Also, there were positive or negative correlations between leaf dimension, plant height, and biomass data.

In recent years, antioxidant and phenolic compounds of plants have been noticed in food industry and pharmacology (Ghahremani-majd *et al.*, 2012). The antioxidant activity was in remarkable range in P40 from Fuman, whereas it was negligible in P31 from Someh Sara. Previously, $0.42 \pm 0.03 \text{ mg mL}^{-1}$ and $12.8 \text{ g } 100 \text{ g}^{-1} \text{ DW}$ were reported for the IC_{50} and DPPH radical scavenging activity in *Froriepia subpinnata* (Gijavash), respectively (Nabavi *et al.*, 2008; Salmanian and Sadeghi, 2012). Phenols and polyphenolic compounds, such as flavonoids, are found in food products originating from plant sources, and they have shown significant antioxidant activities (Van Acker *et al.*, 1996). In pervious researches, total phenols and flavonoids have been reported to range between $68.83\text{--}75.7 \text{ mg galic acid equivalent g}^{-1}$ and $35.2\text{--}43.5 \text{ mg quercetin equivalent g}^{-1}$ (Nabavi *et al.* 2008; Salmanian and Sadeghi, 2012). In our study, total phenolic and flavonoids results were higher than those reported

elsewhere. It has been accepted that antioxidant activity of plants might be attributed to their phenolic compounds (Cook and Samman, 1996). The results indicated that among phenolic compound, Chlorogenic acid followed by coumarin were mostly detectable in *F. subpinnata*. These results are in agreement with finding in other plants (Tokalov *et al.*, 2004; Noroozisharaf *et al.*, 2015). In the present study, a high positive correlation was observed between total phenolic, antioxidant activity, and flavonoids content, while these characters did not exhibit significant correlation with carotenoid content. Similar findings to this study have been reported by other researchers (Orak, 2007; Tulipani *et al.*, 2008; Noroozisharaf *et al.*, 2015).

The result showed high variation among accessions due to the morphological and biochemical characteristics and they seem to have a high potential for breeding programs. UPGMA based on combined data of morphological and biochemical traits shows three distinct groups with high inter and intra-regional diversity. With respect to molecular data (Jorkesh *et al.*, 2019) and these finding, it was realized that the genetic and environmental conditions affect morpho-biochemical traits. In some cases, the environmental conditions could significantly affect morpho-biochemical traits of *F. subpinnata*.

CONCLUSIONS

Based on our knowledge, there were insufficient information about Gijavash plant, so, this study could provide primary knowledge of morphological and phytochemical variations in *F. subpinnata* accessions from different natural habitats, for the first time. The results from this research can be used in geographical location investigating of this plant for further programs such as domestication. Based on the results, the great variability in the morphological and biochemical traits and existence of positive/negative



relationship between morphological and biochemical traits increased the hopes for domestication and improvement of this plant.

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REFERENCES

1. Ashour, M. L., El-Readi, M. Z., Hamoud, R., Eid, S. Y., El Ahmady, S. H., Nibret, E., Herrmann, F., Youns, M., Tahrani, A. and Kaufmann, D. 2014. Anti-Infective and Cytotoxic Properties of *Bupleurum marginatum*. *Chin. Med.*, **9**(1): 4.
2. Bakhshi, D. and Arakawa, O. 2006. Induction of Phenolic Compounds Biosynthesis with Light Irradiation in the Flesh of Red and Yellow Apples. *J. Appl. Hortic.*, **8**(2): 101-104.
3. Bogers, R. J., Craker, L. E. and Lange, D. 2006. Medicinal and Aromatic Plants: Agricultural, Commercial, Ecological, Legal, Pharmacological and Social Aspects. *Wageningen UR Frontis Series*. 17.
4. Bremner, P., Rivera, D., Calzado, M., Obón, C., Inocencio, C., Beckwith, C., Fiebich, B., Muñoz, E. and Heinrich, M. 2009. Assessing Medicinal Plants from South-Eastern Spain for Potential Anti-Inflammatory Effects Targeting Nuclear Factor-Kappa B and Other Pro-Inflammatory Mediators. *J. Ethnopharmacol.*, **124**(2): 295-305.
5. Cook, N. and Samman, S. 1996. Flavonoids—Chemistry, Metabolism, Cardioprotective Effects, and Dietary Sources. *J. Nutr. Biochem.*, **7**(2): 66-76.
6. Cordenunsi, B., Nascimento, J. D. and Lajolo, F. 2003. Physico-Chemical Changes Related to Quality of Five Strawberry Fruit Cultivars during Cool-Storage. *Food Chem.*, **83**(2): 167-173.
7. Dewanto, V., Wu, X., Adom, K. K. and Liu, R. H. 2002. Thermal Processing Enhances the Nutritional Value of Tomatoes by Increasing Total Antioxidant Activity. *J. Agric. Food Chem.*, **50**(10): 3010-3014.
8. Gevrenova, R., Kondeva-Burdina, M., Denkov, N. and Zheleva-Dimitrova, D. 2015. Flavonoid Profiles of Three Bupleurum Species and *in Vitro* Hepatoprotective of Activity Bupleurum flavum Forsk. *Pharmacogn. Mag.*, **11**(41): 14.
9. Ghahremani-majd, H., Dashti, F., Dastan, D., Mumivand, H., Hadian, J. and Esna-Ashari, M. 2012. Antioxidant and Antimicrobial Activities of Iranian Mooseer (*Allium hirtifolium* Boiss) Populations. *Hortic. Environ. Biotechnol.*, **53**(2): 116-122.
10. Guinea, M., Parellada, J., Lacaille-Dubois, M. and Wagner, H. 1994. Biologically Active Triterpene Saponins from *Bupleurum fruticosum*. *Planta Med.*, **60**(02): 163-167.
11. Hadian, J., Ebrahimi, S. N. and Salehi, P. 2010. Variability of Morphological and Phytochemical Characteristics among *Satureja hortensis* L. Accessions of Iran. *Ind. Crop Prod.*, **32**(1): 62-69.
12. Heywood, V. H. 2002. The Conservation of Genetic and Chemical Diversity in Medicinal and Aromatic Plants. *Biodivers.*, Springer, PP. 13-22.
13. Hsu, Y-L., Kuo, P-L., Weng, T-C., Yen, M-H., Chiang, L-C. and Lin, C-C. 2004. The Antiproliferative Activity of Saponin-Enriched Fraction from *Bupleurum Kaoi* Is through Fas-Dependent Apoptotic Pathway in Human Non-Small Cell Lung Cancer A549 Cells. *Biol. Pharm. Bull.*, **27**(7): 1112-1115.
14. Iezzoni, A. F. and Pritts, M. P. 1991. Applications of Principal Component Analysis to Horticultural Research. *HortScience*, **26**(4): 334-338.
15. Jorkesh, A., Hamidoghli, Y., Olfati, J., Samizadeh Lahiji, H., Bakhshi, D. and Pala Paul, J. 2019. Genetic Diversity of *Froriepia subpinnata* Ledeb. Bail (Gijavash) an Endangered Medicinal Plant from Iran Revealed by ISSR and IRAP Markers. *J. Hort. Research.*, **22**(2): 38-45
16. Khadivi-Khub, A., Salehi-Arjmand, H. and Hadian, J. 2014. Morphological and

- Phytochemical Variation of *Satureja bachtiarica* Populations from Iran. *Ind. Crop. Prod.*, **54**: 257-265.
17. Khadivi-Khub, A., Zamani, Z. and Fatahi, M. R. 2012. Multivariate Analysis of *Prunus subgen. Cerasus* Germplasm in Iran Using Morphological Variables. *Genet. Resour. Crop Evol.*, **59(5)**: 909-926.
 18. Liang, Z., Oh, K., Wang, Y., Yi, T., Chen, H. and Zhao, Z. 2014. Cell Type-Specific Qualitative and Quantitative Analysis of Saikosaponins in Three *Bupleurum* Species Using Laser Microdissection and Liquid Chromatography–Quadrupole/Time of Flight–Mass Spectrometry. *J. Pharmaceut Biomed.*, **97**: 157-165.
 19. Liu, C. T., Chuang, P. T., Wu, C. Y., Weng, Y. M., Chen, W. and Tseng, C. Y. 2006. Antioxidative and *in Vitro* Hepatoprotective Activity of *Bupleurum kaoi* Leaf Infusion. *Phytother. Res.: An International Journal Devoted to Pharmacological and Toxicological Evaluation of Natural Product Derivatives*, **20(11)**: 1003-1008.
 20. Mabberley, D. J. 2008. Mabberley's Plant-Book: A Portable Dictionary of Plants, Their Classifications and Uses. Edition 3, Cambridge University Press, UK. 1040 pp., USD 90.00, GBP 50.00,
 21. Mínguez-Mosquera, M. I. and Pérez-Gálvez, A. 1998. Color Quality in *paprika oleoresins*. *J. Agric. Food Chem.*, **46(12)**: 5124-5127.
 22. Mozaffarian, V. 2015. *Recognition of Medicinal and Aromatic Herbs of Iran*. Farhange Moaser Press, Tehran, Iran.
 23. Nabavi, S., Ebrahimzadeh, M., Nabavi, S. and Jafari, M. 2008. Free Radical Scavenging Activity and Antioxidant Capacity of *Eryngium caucasicum* Trautv and *Froripia subpinnata*. *Pharmacology Online*, **3**: 19-25.
 24. Noroozisharaf, A., Lahiji, H. S., Hatamzadeh, A. and Bakhshi, D. 2015. Phytochemical Attributes of Endemic Endangered Primrose (*Primula heterochroma* Stapf.) Accessions Grown in Iran. *Physiol. Mol. Biol. Pla.*, **21(4)**: 573-581.
 25. Orak, H. H. 2007. Total Antioxidant Activities, Phenolics, Anthocyanins, Polyphenoloxidase Activities of Selected Red Grape Cultivars and Their Correlations. *Scientia Horticulturae*, **111(3)**: 235-241.
 26. Pank, F., Pfefferkorn, A. and Kruger, H. 2004. Evaluation of a Summer Savory Collection (*Satureja hortensis* L.) with Regard to Morphology, Precocity, Yield Components and Essential Oil and Carvacrol Content. *Z. Arzn Gew Pfl.* 72-78. *J. Z. Arzn Gew Pfl.* 72-78.
 27. Pistelli, L. 2006. The Chemistry and Biological Activity of the Genus *Bupleurum* in Italy. *Bupleurum Species: Scientific Evaluation and Clinical Applications Boca Raton*. CRC/Taylor & Francis, FL, PP. 117-131.
 28. Rohlf, F. 2000. *NTSYS-PC, Numerical Taxonomy and Multivariate Analysis System: V. 2.1*. Exeter Publishing Setauket, New York.
 29. Salmanian, S. and Sadeghi, A. 2012. Measurement of Total Phenol, Flavonoid, Carotenoid and Antioxidant Activity of Gijavash. *3rd National Symposium of Iran Agricultural Biotechnology*, Mashahd, Iran.
 30. Sánchez-Moreno, C., Larrauri, J. A. and Saura-Calixto, F. 1998. A Procedure to Measure the Antiradical Efficiency of Polyphenols. *J. Sci. Food Agric.*, **76(2)**: 270-276.
 31. Sefc, K., Lopes, M., Lefort, F., Botta, R., Roubelakis-Angelakis, K., Ibanez, J., Pejić, I., Wagner, H., Glössl, J. and Steinkellner, H. 2000. Microsatellite Variability in Grapevine Cultivars from Different European Regions and Evaluation of Assignment Testing to Assess the geographic Origin of Cultivars. *Theor. Appl. Genet.*, **100(3-4)**: 498-505.
 32. Shin, Y., Liu, R. H., Nock, J. F., Holliday, D. and Watkins, C. B. 2007. Temperature and Relative Humidity Effects on Quality, Total Ascorbic Acid, Phenolics and Flavonoid Concentrations, and Antioxidant Activity of Strawberry. *Postharvest Biol. Tec.*, **45(3)**: 349-357.
 33. Tokalov, S. V., Kind, B., Wollenweber, E. and Gutzeit, H. O. 2004. Biological Effects of Epicuticular Flavonoids from *Primula denticulata* on Human Leukemia Cells. *J. Agric. Food Chem.*, **52(2)**: 239-245.
 34. Tulipani, S., Mezzetti, B., Capocasa, F., Bompadre, S., Beekwilder, J., De Vos, C. R., Capanoglu, E., Bovy, A. and Battino, M. 2008. Antioxidants, Phenolic Compounds, and Nutritional Quality of Different Strawberry Genotypes. *J. Agric. Food Chem.*, **56(3)**: 696-704.



35. Van Acker, S. A., Tromp, M. N., Griffioen, D. H., Van Bennekom, W. P., Van Der Vijgh, W. J. and Bast, A. 1996. Structural

Aspects of Antioxidant Activity of Flavonoids. *Free Radic. Biol. Med.*, **20(3)**: 331-342.

تنوع فنوتیپی و فیتوشیمیایی جمعیت‌های مختلف گیجاواش (*Froriepia subpinnata* (Ledeb.) Bail. - گیاه دارویی بومی رشد یافته در ایران

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چکیده

گیجاواش گیاه دارویی بومی و در معرض خطر انقراض است که در نوار ساحلی دریای خزر در شمال ایران رشد می‌کند. این تحقیق در راستای به دست آوردن اطلاعات اولیه ریخت‌شناختی و فیتوشیمیایی در روند اصلاح و اهلی کردن این گیاه صورت گرفت. بیست و سه صفت ریخت‌شناختی اندازه‌گیری شد که تنوع بالایی بین ۵۲ توده مشاهده گردید. همبستگی مثبت و معنی‌داری بین ابعاد برگ، ارتفاع گیاه و عملکرد بیوماس گیاه وجود داشت. دامنه تغییرات صفات فیتوشیمیایی اندازه‌گیری شده برای فنل کل (۹۴۱/۳ - ۳۵۵/۶ میلی گرم گالیک اسید در صد گرم وزن تر)، فلاونوئید کل (۵۴۰/۷۴ - ۲۰۴/۳۳ میلی گرم کاتچین در صد گرم بافت تر)، کارتنوئید کل (۱/۵۲ تا ۳/۱۵ میکروگرم در صد گرم وزن تر) و ظرفیت آنتی‌اکسیدانی (۸۱/۸۲ - ۳۱/۳۶ درصد) بود. نتایج حاصل از آنالیز توسط دستگاه HPLC نشان داد که کلروژنیک اسید ترکیب فنولی غالب در این گیاه است. کارتنوئید کل همبستگی معنی‌داری با سایر خصوصیات بیوشیمیایی نداشت در حالیکه بقیه صفات بیوشیمیایی همبستگی خطی با یکدیگر داشتند. گروه‌بندی نمونه‌ها براساس روش UPGMA و برپایه داده‌های ریخت‌شناختی و بیوشیمیایی، نمونه‌ها را به سه گروه مشخص تقسیم کرد که دارای تنوع درون و بین منطقه‌ای بالایی نشان دادند. علاوه بر این، این اطلاعات با داده‌های مولکولی ترکیب شد تا بتوان اثرات محیط و ژنتیکی را جدا کرد. تنوع بالای فیتوشیمیایی و ریخت‌شناختی بین و درون جمعیت‌ها می‌تواند یک ابزار سودمند برای روند اصلاح و انتخاب در این گیاه باشد.