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Pomological and phytochemical diversity in Iranian populations of Caucasian whortleberry (*Vaccinum arctostaphylos* L.)

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ABSTRACT

Caucasian whortleberry (*Vaccinium arctostaphylos* L.), locally named Qare-Qat, is a shrub native to humid Caucasus area including northwest of Iran. It is a medicinal plant which has been intensively utilized in Iranian folk medicine as an antihypertensive and antidiabetic agent for many years. In order to determine the pomological and phytochemical properties of various caucasian whortleberry populations, aerial parts samples were collected from 11 locations in native regions. Current study indicated significant differences in pomological and phytochemical characteristics of Caucasian whortleberry (*V. arctostaphylos* L.) populations collected from 11 locations in 3 different province. Our results showed that the highest berry and flesh weight, number of seeds per berry, berry length and width, number of cluster per inflorescence and flowering stem length were observed from plants collected from Saghezchi-A population in Ardabil province, while the lowest values were obtained from Zendaneh population in Gilan province. In addition, the highest content of anthocyanin, total phenolics and antioxidant in both fruits and leaves of *V. arctostaphylos* L. were mainly recorded from Saghezchi-A population followed by Khanghah population. According to studied traits, *V. arctostaphylos* L. populations were divided into three different groups. Saghezchi-A, Khanghah and Saghezchi-A populations were placed in a same group with mainly the best pomological and phytochemical properties. These locations were characterized by high solar radiation. Therefore, they can be exploited for selection of suitable genotypes for organizing the berry breeding programs and taking advantage of this plant in garden establishment and fruit production investigations.

1. Introduction

The genus Vaccinium compromises about 450 species which are widely grow around the world and exhibit a high level of morphological diversity (Song and Hancock, 2011). The species within this genus present different levels of ploidy (2x, 4x and 6x; x = 12), which results in evident morphological differences. Caucasian whortleberry (*Vaccinum arctostaphylos* L.), belonging to Ericaceae family, is a shrub native to Caucasus area and is known as Qare-qat in Iran (Ehlenfeldt and Ballington, 2012). They are adapted to hilly side and slopes of persistent cloud over canopy, and most common at shaded forests of Caspian Sea in the north and northwest of the country (Sedaghathoor and Saeidi-Mehrvarz, 2006). In recent years, berry fruits have been considered as an important medicinal and industrial plant because of their uses as colorants and antioxidants or as health boosting activity for their phenolic and anthocyanin compounds. They also used as functional food for diabetics, hyperlipidemia and some other disorders (Hasanloo et al., 2011; Soltani et al., 2014; Khalili Musavi et al., 2016). In addition, the leaves of the plant collected by inhabitants and sale as herbal tea for treatment blood pressure and some urinary disorders because of its polyphenols content (Khalili et al., 2011).

The quality of berry fruits is often related to the phenolic and anthocyanin compounds that have been raised as desirable factors in selection of suitable genotypes. Occurrence of phenolic and anthocyanin compounds that are believed to provide health benefits in decreasing the risk of diseases, particularly certain cancers and eye disease refers to their antioxidant activities. Several studies have shown that the content of anthocyanins and polyphenolic compounds in berries are influenced by the cultivar, growing season and location (Howard et al., 2003; Dragovic-Uzelac et al., 2010). For instance, Martinussen et al. (2009)

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reported that total phenolics content in bilberry was increased with decreasing temperature. In addition, temperature, water stress and light significantly affected concentration of anthocyanins in bilberries (Åkerström et al., 2010). Antioxidative activity of the fruits and leaves extracts have a key role in human health (Howard et al., 2003; Nickavar and Amin, 2004). Antioxidant activity often closely related to the concentrations of anthocyanins and total phenolics (Prior et al., 1998) and factors that will affect their content also influence antioxidant activity.

Increasing demand for fruits and leaves of this plant by food industries and customers causes rural people harvest aerial parts, and competitively pick unripe fruits up. This undesirable habit prevents plant to be propagated naturally by seeds. Consequently commercialization and garden establishment to provide adequate amounts of crop and prevent plant dying out has been necessitated. For commercial producing of berries row material, and overcoming the market demand, cultivars with satisfied amounts of active ingredients should be developed. Breeders need new genes to introduce in varieties and make variation to select high performance lines to achieve the high yield and qualitative varieties. Accessions in new origins may lead to new genes that can be used to improve in characteristics of existing cultivars (Zoratti et al., 2015). Considering the issue, an investigation was carried out to evaluate the extent of diversity of *Vaccinum arctostaphylos* L. in Iranian populations. Probably existing variation could be used in breeding programs for development of cultivars with suitable levels of metabolites, and good adaptation, which in turn may promote the cultivation of this high value medicinal bushy plant and prevent it dying out.

2. Material and methods

2.1. Plant materials

The fruits and leaves of 11 *V. arctostaphylos* L. accessions were collected from different geographical regions of Iran. Geographic coordinates including latitude, longitude and altitude of each region were recorded using Global Positioning System (GPS) (Table 1). In all the studied sites, different soil samples were taken in the root depth of the plants (rhizosphere) and analyzed to determine the main physicochemical properties of the soil including texture, pH and electrical conductivity (EC) (Table 1). Moreover, solar radiation and canopy covering were determined by different scores range from 1 to 5. Low scores indicate that the plants grow under canopy and low sunlight. As the score increases, the canopy covering is lowered and the plant exposed to direct sunlight. The samples were collected from three different bushes and were transported to a research laboratory in Mohaghegh Ardabili University, Ardabil, Iran. Different pomological characteristics including inflorescence length (mm), flowering stem length (mm), number of cluster on inflorescence, number of fruit on cluster, berry width (mm), berry length (mm) and berry weight (g) were recorded in the laboratory.

2.2. Essential oil extraction

Leaves and fruits samples were dried at room temperature and finely powdered and kept in the paper bags for further analysis. The essential oil (EO) was isolated by hydrodistillation method for 3 h, using Celevenger apparatus according to the European pharmacopoeia (European Pharmacopoeia, 2005). Then EO content (%) was measured based on mass of EO obtain (g) extracted from 40 g mass of dried aerial part at flowering stage (g) using the following formula.

$$\text{EO content \%} = [\text{mass of EO obtain (g)}/\text{mass of dry matter (g)}] \times 100 \quad (1)$$

2.3. Preparation of extraction

Freeze-dried samples (0.25 g) were milled and extracted with 50 mL of 1% HCl in methanol. Extraction was carried out by stirring for 48 h. This was repeated in triplicate. The extracts were pooled, and this mixture was used for further procedures either immediately or after deep freezing (−80 °C) for no longer than 4 days.

2.4. Determination of total phenolic content

Total phenolics (TP) content was determined according to Folin-Ciocalteu method explained by Singleton and Rossi (1965). 1 g of the leaf and/or berry powder was soaked in 40 mL methanol 80% (v/v) and was laid on a magnetic plate at room temperature for 3 h. Resultant was centrifuged at 5000 rpm for 20 min at 4 °C. The supernatant was filtered, kept at 4 °C, and protected from light until further analysis. Briefly, 0.2 mL of the methanolic extract was homogenized with 0.2 mL Folin-Ciocalteu reagent and 2 mL of distilled water, thereafter resultant solutions were maintained in a dark place at room temperature for 1 h. Absorbance was read using a UV–vis spectrophotometer (UV-2550, Shimadzu, Japan) at 725 nm. The standard curve was adapted by gallic acid and the results were expressed as mg gallic acid equivalent per 100 g of fresh weight (GAE/100 g FW).

2.5. Determination of total anthocyanin (TA)

Total anthocyanin (TA) was estimated by a pH differential method

Table 1
Geographical origins of *V. arctostaphylos* L. populations.

No.	Province name	Location name	Latitude (N)	Longitude (E)	Altitude (m)	Mean annual temp. [°C]	Rainfall [mm/year]	Solar radiation score[a]	Soil		
									texture	EC (μS/cm)	pH
1	Ardabil	Aladizgae	38° 17'.895 N	48° 37'.833 E	1353	17.05	2542	3	Loam	133.2	4.87
2	Ardabil	Sooha	38° 16'.865 N	48° 41'.530 E	1719	16.70	2636	3	Sandy loam	176.0	4.38
3	Gilan	Zendaneh	37° 32'.730 N	48° 45'.035 E	1635	17.35	2528	1	Sandy loam	521.5	5.04
4	Gilan	Sakaraoni-Brin	37° 33'.693 N	48° 47'.753 E	1683	17.39	2584	4	Sandy loam	388.1	5.52
5	Ardabil	Saghezchi-A	38° 13'.969 N	48° 41'.834 E	1651	16.78	2636	5	Sandy loam	293.4	4.88
6	Ardabil	Saghezchi-B	38° 15'.000 N	48° 41'.236 E	1729	16.72	2636	5	Sandy loam	112.1	4.84
7	Gilan	Sobatan	37° 57'.926 N	48° 45'.128 E	1735	17.13	2626	3	Sandy loam	216.8	5.25
8	Gilan	Feshe Madan	37° 38'.456 N	48° 48'.529 E	1414	18.01	2862	2	Sandy loam	154.4	5.08
9	Gilan	Matash	37° 38'.211 N	48° 45'.716 E	1795	17.45	2584	1	Sandy loam	196.7	5.37
10	Ardabil	Khanghah	38° 27'.235 N	48° 34'.432 E	1635	16.83	2454	5	Loam	226.1	5.66
11	Mazandaran	Kelardasht	36° 32'.754 N	51° 07'.349 E	1704	17.52	1814	1	Loam	273.3	6.04

[a] Low scores indicate the placement of the plant under canopy and low sunlight. As the score increases, the canopy cover is lowered and the plant exposed to direct

(Giusti and Wrolstad, 2005). The absorbance was measured at two different wavelengths 700 nm and 510 nm, using buffer with pH 1.0 and pH 4.5, respectively. The molecular weight of 449.2 for cyanidin 3-glucoside was used in equation to calculate total monomeric anthocyanin pigments, and results expressed as mg cyanidin 3-glucoside per 100 g fresh weight.

2.6. Ferric reducing/antioxidant power (FRAP) assay

The antioxidant activities of the leaves and/or fruits extracts were determined using ferric reducing/antioxidant power (FRAP) method developed by Benzie and Strain (1996). The FRAP reagent contains 2.5 mL of a 10 mmol/L tripyridyltriazine (TPTZ: $C_3N_3[C_{15}H_{12}N_3]$) solution in 40 mmol/L HCl plus 2.5 mL of 20 mmol/L $FeCl_3$, and 25 mL of 0.3 mol/L acetate buffer, pH 3.6. Then 80 µL of sample extract was mixed with 3.6 mL of FRAP reagent and 0.4 mL of distilled water, and the absorbance of the samples was measured at 593 nm. Different concentrations of iron (II) sulfate ($FeSO_4$) were used as the standard. The amounts are expressed as mmol of antioxidants as FeSO4 per 100 g of fresh weight of plant.

2.7. Data analysis

Analysis of variance appropriate to the experimental design was done by SAS software (ver. 9.1). Mean comparison of the traits were done using Duncan multiple range tests at $p \leq 0.05$ significant level. The simple correlation coefficients were calculated to determine the relationships between the studied traits as Pearson correlation coefficient. Hierarchical cluster analysis of studied populations was based on the Euclidean distances using Wards method. In order to determine the most variable characteristics among the populations, factor analysis based on principal component analysis (PCA) was performed.

3. Results and discussion

3.1. Pomological traits

The results of analysis of variance for different traits are presented in Table 2. Our results indicated that there were significant differences among *V. arctostaphylos* L. populations collected from various locations for berry fresh weight and flesh weight (Table 2). The highest value of berry fresh weight (4.13 ± 0.009 g) and Flesh fresh weight (0.396 ± 0.009 g) were related to the plants collected from the Saghezchi-A population while their lowest values were found in the samples collected from Zendaneh (Table 3). Furthermore, flesh/berry weight ratio was also differed in various populations. As shown in Table 3, the highest ratio was observed in the samples collected from Saghezchi-A (0.961 ± 0.001) followed by Matash (0.947 ± 0.0003), while the lowest value was displayed from Brin-Sakaraoni (0.920 ± 0.002).

According to Table 3, number of seeds per berry ranged from a low (36.54 ± 2.74) in Aladizgae to a high (68.98 ± 2.86) in Saghezchi-A. In addition, number of berry per cluster and cluster per inflorescence were significantly influenced by the geographical origin of the plant (Table 2). As shown in Table 3, the highest number of berry per cluster was obtained from plants in the Sooha (5.56 ± 0.11) followed by Khanghah (5.54 ± 0.12) location, while the lowest number (2.55 ± 0.48) was recorded from the Zendaneh location. The samples collected from Saghezchi-A showed the highest number of cluster per inflorescence (3.89 ± 0.11), and the lowest values were observed in the plants from Kelardasht (1.89 ± 0.11).

In the current experiment, inflorescence length ranged from 37.11 mm from the Matash population to 58 mm from the Kelardasht population (Table 3). Moreover, the maximum and the minimum length of flowering stem were observed in the plants from the Saghezchi-A (166.67 ± 12.3 mm) and Kelardasht populations (42.44 ± 2.45 mm), respectively. Berry length, width and shape index were significantly influenced by the geographical origin of the plant (Table 2). According to Table 3, the plants grown in Saghezchi-A location had the highest berry length (10.68 ± 0.36 mm) and width (10.41 ± 0.31 mm), while the lowest values of these traits were observed in the Zendaneh and Brin-Sakaraoni locations. The shape of Feshe Madan berries were longer than other populations as the shape index value was 1.361 ± 0.059. The most of the populations displayed berries round in shape. In general, the highest values of pomological traits were mainly observed from plants collected from Saghezchi-A population which was characterized by high solar radiation. However, the lowest values were obtained from Zendaneh population in which the plants were mainly grown under shade condition. Variation in pomological characteristics of *V. arctostaphylos* L. has been reported previously. For instance, Ayaz et al. (2001) showed that the average fruit size of V. arctostaphylos L. ranged from 4.8 ± 0.03, 7.2 ± 0.1 to 9.2 ± 0.3 mm. In addition, Özgen et al. (2014) observed that berry weight, width and length ranged from 0.52 to 1.19 g, 9.48–12.24 mm and 10.52–13.43 mm, respectively, so, our findings were lower than them. This means that *V. arctostaphylos* L. has a very wide range and diversity.

3.2. Phytochemicals

In the current study, fruit essential oil content, fruit and leaf

Table 2
Analysis of variance for some pomological and phytochemical traits of *V. arctostaphylos* populations.

Source of Variance	df	Mean of square										
		Berry fresh weight	Flesh fresh weight	Flesh/Berry weight ratio	No. seed/ fruit	Berry length	Berry width	Berry shape index	No. fruit /cluster	No. cluster /inflorescence	Inflorescence length	Flowering stem length
Population	10	0.013**	0.012**	0.00038**	316.1**	8.71**	11.99**	0.045**	3.77**	0.95*	369.1**	6141.3**
Error	22	0.0017	0.0018	0.00007	49.11	0.36	0.36	0.0023	0.68	0.41	84.3	155.4
CV (%)		16.9	17.9	0.94	13.9	7.78	8.41	4.38	20.7	23.8	18.05	11.4

Source of Variance	df	Mean of square						
		Essential oil	Fruit anthocyanin	Leaf anthocyanin	Fruit total phenol	Leaf total phenol	Fruit antioxidant	Leaf antioxidant
Population	10	0.00038**	1322.4**	782.8**	21,799.7**	15,492.4**	108.4**	86.5**
Error	22	0.0001	111.4	23.2	1422.2	863.7	3.36	2.26
CV (%)		32.6	8.44	5.91	7.37	7.86	10.0	8.62

*,** Significant at $P \leq 0.05$ and $P \leq 0.01$, respectively. NS: Not Significant.

Table 3
Pomological traits of *V. arctostaphylos* from various Populations.

	Population				
	Aladizgae	Sooha	Zendaneh	Brin-Sakaraoni	Saghezchi-A
Berry fresh weight (g)	0.241 ± 0.057bcd	0.219 ± 0.003bcd	0.171 ± 0.006d	0.178 ± 0.002 cd	0.412 ± 0.009a
Flesh fresh weight (g)	0.228 ± 0.057bc	0.205 ± 0.003bc	0.157 ± 0.006c	0.164 ± 0.002c	0.396 ± 0.009a
Flesh/Berry weight ratio	0.937 ± 0.012bc	0.933 ± 0.001bcd	0.923 ± 0.003 cd	0.920 ± 0.002d	0.961 ± 0.001a
No. seed/ fruit	36.54 ± 2.74e	53.44 ± 5.25bcd	44 ± 1.5de	50.55 ± 4.3 cd	68.98 ± 2.86a
Berry length (mm)	5.923 ± 0.07e	7.623 ± 0.29d	5.82 ± 0.35e	5.686 ± 0.029e	10.68 ± 0.36a
Berry width (mm)	5.643 ± 0.08de	7.456 ± 0.24c	4.48 ± 0.11f	5.42 ± 0.049def	10.41 ± 0.31a
Berry shape index	1.052 ± 0.009c	1.021 ± 0.007c	1.298 ± 0.06ab	1.049 ± 0.014c	1.026 ± 0.004c
No. fruit /cluster	2.79 ± 0.105 cd	5.566 ± 0.11a	2.55 ± 0.48d	3.22 ± 0.48bcd	4.55 ± 0.29ab
No. cluster /inflorescence	2.12 ± 0.21bc	3.11 ± 0.11abc	2.55 ± 0.11bc	2.22 ± 0.29bc	3.89 ± 0.11a
Inflorescence length (mm)	48.66 ± 5.41bc	47.33 ± 5.87bc	78.11 ± 8.38a	41.0 ± 1.33bc	56.66 ± 1.83b
Flowering stem length (mm)	125.5 ± 2.06 cd	143.5 ± 9.3bc	45 ± 1.01f	51.2 ± 4.32f	166.6 ± 12.3a

	Population					
	Saghezchi-B	Sobatan	Feshe Madan	Matash	Khanghah	Kelardasht
Berry fresh weight (g)	0.285 ± 0.051b	0.251 ± 0.0004bc	0.203 ± 0.0005 cd	0.253 ± 0.003bc	0.286 ± 0.002b	0.242 ± 0.019b-cd
Flesh fresh weight (g)	0.268 ± 0.051b	0.235 ± 0.0008bc	0.189 ± 0.0005bc	0.239 ± 0.003bc	0.270 ± 0.002b	0.228 ± 0.019b-c
Flesh/Berry weight ratio	0.939 ± 0.008bc	0.937 ± 0.001bc	0.931 ± 0.0004bcd	0.947 ± 0.0003ab	0.942 ± 0.0004b	0.941 ± 0.006b
No. seed/ fruit	64.443 ± 5.94ab	46.78 ± 1.49de	45.89 ± 5.35de	39.99 ± 4.04de	59.766 ± 5.48abc	44.1 ± 1.73de
Berry length (mm)	8.076 ± 0.70	9.267 ± 0.15bc	6.39 ± 0.049e	8.466 ± 0.16 cd	9.833 ± 0.53ab	7.81 ± 0.39d
Berry width (mm)	7.78 ± 0.74c	8.98 ± 0.09b	4.709 ± 0.179ef	7.676 ± 0.117c	9.66 ± 0.58ab	6.433 ± 0.43d
Berry shape index	1.039 ± 0.009c	1.03 ± 0.01c	1.361 ± 0.059a	1.102 ± 0.007c	1.017 ± 0.009c	1.216 ± 0.027b
No. fruit /cluster	5 ± 0.38a	3.33 ± 0.507bcd	2.78 ± 0.58 cd	4.21 ± 0.45abc	5.54 ± 0.12a	4.556 ± 0.98ab
No. cluster /inflorescence	3.22 ± 0.86ab	2.77 ± 0.29abc	2.89 ± 0.11abc	2.53 ± 0.39bc	2.55 ± 0.58bc	1.89 ± 0.11c
Inflorescence length (mm)	50.44 ± 1.72bc	49.66 ± 11.9bc	41 ± 1.70bc	37.09 ± 3.42c	51.55 ± 2.99bc	58.003 ± 0.88b
Flowering stem length (mm)	154.8 ± 13.7ab	125.6 ± 3.78 cd	105.6 ± 5.66de	93.7 ± 6.91e	143.4 ± 4.28bc	42.4 ± 2.45f

The means with the same letters in each row indicates no significant difference between populations at the 5% level of probability.

Table 4
Phytochemical traits of *V. arctostaphylos* from various populations.

	Population									
	Aladizgae	Sooha	Zendaneh	Brin-Sakaraoni	Saghezchi-A	Saghezchi-B	Sobatan	Feshe Madan	Matash	Khanghah	Kelardasht
Essential oil (%)	0.0249 ± 0.005b	0.025 ± 0.005b	0.023 ± 0.009b	0.027 ± 0.007b	0.0255 ± 0.008b	0.0255 ± 0.014b	0.0403 ± 0.06b	0.031 ± 0.01b	0.027 ± 0.008b	0.026 ± 0.006b	0.0622 ± 0.17a
Fruit anthocyanin (mg/100 g. FW. Cyd-3-gl. Eq.)	112.31 ± 6.9c	140.63 ± 1.54b	131.51 ± 6.67b	106.3 ± 6.81 cd	162.43 ± 0.88a	132.04 ± 10.4b	107.72 ± 4.3 cd	91.24 ± 4.5d	112.03 ± 5.8c	147.9 ± 7.59ab	130.84 ± 5.03b
Leaf anthocyanin (mg/100 g. FW. Cyd-3-gl. Eq.)	67.70 ± 3.42d	82.01 ± 0.74c	81.35 ± 4.09c	62.30 ± 3.20de	105.39 ± 0.51a	88.20 ± 4.57bc	91.10 ± 0.97b	57.74 ± 1.91e	66.85 ± 1.61d	101.97 ± 3.47a	93.52 ± 2.38b
Fruit total phenolics (mg/100 g. FW. GA. Eq.)	420.61 ± 12.6d	536.19 ± 14.3b	413.08 ± 29.1d	506.1 ± 19.5bc	652.52 ± 35.7a	607.28 ± 21.1a	477.9 ± 35.1bcd	460 ± 12.9 cd	472 ± 12.1bcd	634.2 ± 8.37a	442.7 ± 16.4 cd
Leaf total phenolics (mg/100 g. FW. GA. Eq.)	303.59 ± 8.9de	380.5 ± 1.28bc	306.62 ± 16de	340.11 ± 5.5 cd	481.19 ± 16.8a	450.15 ± 35.8a	335.23 ± 27.8 cd	281.1 ± 13.7e	350. ± 15.3bcd	485.92 ± 3.57a	395.83 ± 4.37b
Fruit antioxidant (mmol FeSO4 / 100 g Fw)	14.07 ± 0.31d	19.42 ± 0.49c	11.62 ± 0.70d	14.19 ± 0.98d	29.46 ± 1.12a	23.30 ± 0.72b	20.79 ± 1.13bc	13.59 ± 1.81d	13.80 ± 1.95d	26.72 ± 0.30a	14.82 ± 0.46d
Leaf antioxidant (mmol FeSO4 / 100 g Fw)	14.34 ± 0.12e	18.93 ± 0.31d	11.57 ± 0.53e	13.86 ± 0.80e	27.28 ± 0.87a	22.09 ± 0.72bc	19.79 ± 0.98 cd	13.22 ± 1.68e	12.99 ± 1.37e	24.59 ± 0.34b	13.36 ± 0.36e

The means with the same letters in each row indicates no significant difference between populations at the 5% level of probability.

anthocyanin, total phenolics and antioxidant contents were significantly influenced by various geographical origin of the plant (Table 2). Although essential oil content in the fruits of *V. arctostaphylos* L. were very low, it ranged a low 0.0238 ± 0.009% in Zendaneh to a high 0.062 ± 0.17% in Kelardasht. As shown in Table 4, the highest content of anthocyanin in fruits (162.43 ± 0.88 mg/100 g. FW. Cyd-3-gl. Eq.) and leaves (105.39 ± 0.51 mg/100 g. FW. Cyd-3-gl. Eq.) of *V. arctostaphylos* L. were obtained from Saghezchi-A population followed by Khanghah, while the lowest contents were observed in the samples collected from Feshe Madan populations (Table 4). In addition, the highest amount of total phenolics in the fruits (652.52 ± 35.7 mg/100 g. FW. GA. Eq.) was recorded from Saghezchi-A population followed by Khanghah (634.26 ± 8.37 mg/100 g. FW. GA. Eq.), while the lowest value was obtained from Zendaneh population (413.08 ± 29.1 mg/100 g. FW. GA. Eq.). The maximum (485.92 ± 3.57 mg/100 g. FW. GA. Eq.) and the minimum (281.14 ± 13.7 mg/100 g. FW. GA. Eq.) amount of total phenolics in the leaves were obtained from plants collected from Khanghah and Feshe Madan locations, respectively (Table 4).

The FRAP assay measures the ability of an antioxidant to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ and form an intense blue Fe^{2+}-TPTZ complex with maximum absorption at 593 nm. In the current study the results of ferric ion- reducing activities of the extract of *V. arctostaphylos* L. are shown in Table 4. Antioxidant content of fruit extract ranged from a low of 11.62 ± 0.71 mmol FeSO4/100 g Fw in Zendaneh to a high of 29.46 ± 1.12 mmol FeSO4/100 g Fw in Saghezchi-A (Table 4). Moreover, the highest and the lowest amount of leaf antioxidants were observed in the samples collected from Saghezchi-A and Zendaneh locations, respectively (Table 4). These variations may occur because of both genetic and environmental effects.

According to the results, the highest amount of anthocyanin and total phenolics were mainly observed in the samples collected from Khanghah and Saghezchi-A where plants were growing under direct radiation of sun light (solar radiation score was around 5). The lowest contents of mentioned phytochemicals were obtained from Zendaneh and Feshe Madan populations that were growing comparatively under shad conditions. Therefore, increasing amounts of anthocyanin and phenolic compounds, subsequently antioxidant content of extracts may be influenced by sun radiation. Zupan et al. (2014) found that excessive sun irradiation increased levels of total phenolics in light-exposed apples compared with the shaded part of the same fruit or fruit growing in full shade. Jakopic et al. (2010) reported that anthocyanin content in apple skin was strongly light-dependent and that the use of reflective cover increased their synthesis.

In current study, higher solar radiation in Khanghah and Saghezchi-A provides a better growing condition resulting in a higher accumulation of phytochemicals in the leaves and fruits of *V. arctostaphylos* L. plants. Synthesis and accumulation of secondary metabolites (such as phenolics) is a common plant mechanism to cope with environmental stresses, and their function is to help the plant overcome unfavorable conditions (Veberic, 2016). Under environmental stresses, plants may close their stomata lead to reduction of photosynthesis and production of reactive oxygen species (ROS). It has been recognized that polyphenolics show antioxidant activity, so they could play a key role in ROS scavenging process (Akula and Ravishankar, 2011). Previous studies have reported wide variation in pomological and phytochemical properties of this plant from Turkey (Özgen et al., 2014; Celik and Ilkay, 2013). Rieger et al. (2008) revealed that anthocyanin content was decreased with increasing the altitude, although solar radiation increases (Blumthaler et al., 1997) and temperatures generally decrease with rising altitude. In accordance with our results, in a study, Hasanloo et al. (2011) reported variation in antioxidant activity, total phenolic and anthocyanin content in *V. arctostaphylos* L. fruit in comparison with four different wild Caucasian whortleberry genotypes in Iran. In another study on two samples from natural habitats of Gilan province of Iran, researchers showed that total phenolic content ranged from

Table 5
Correlation between pomological and phytochemical characteristics in *V. arctostaphylos* L. populations.

		(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
(1)	Berry fresh weight	1															
(2)	Flesh fresh weight	1**	1														
(3)	No. seed/ fruit	0.679*	0.671*	1													
(4)	Berry length	0.845**	0.842**	0.619*	1												
(5)	Berry width	0.838**	0.833**	0.658*	0.965**	1											
(6)	No. fruit /cluster	0.495	0.49	0.622*	0.649*	0.682*	1										
(7)	No. cluster /inflorescence	0.648*	0.645*	0.758**	0.553	0.552	0.334	1									
(8)	Inflorescence length	−0.001	0.001	0.087	−0.038	−0.106	−0.135	0.028	1								
(9)	Flowering stem length	0.699*	0.694*	0.613*	0.621*	0.706*	0.503	0.728*	−0.248	1							
(10)	Essential oil	−0.067	−0.066	−0.264	0.09	−0.044	0.038	−0.445	0.079	−0.433	1						
(11)	Fruit anthocyanin	0.656*	0.655*	0.696*	0.608*	0.625*	0.685*	0.475	0.488	0.409	−0.127	1					
(12)	Leaf anthocyanin	0.688*	0.683*	0.645*	0.788**	0.766**	0.609*	0.387	0.488	0.405	0.211	0.839**	1				
(13)	Fruit total phenol	0.741**	0.733*	0.945*	0.723*	0.784**	0.728*	0.685*	−0.113	0.713*	−0.321	0.679*	0.62*	1			
(14)	Leaf total phenol	0.767**	0.761**	0.839**	0.778**	0.803**	0.836**	0.459	0.092	0.536	−0.027	0.831**	0.813**	0.897**	1		
(15)	Fruit antioxidant	0.846**	0.838**	0.877**	0.855**	0.903**	0.669*	0.689*	0.02	0.791**	−0.174	0.716*	0.794**	0.926**	0.875**	1	
(16)	Leaf antioxidant	0.83**	0.823**	0.874**	0.823**	0.887**	0.645*	0.709*	0.013	0.824**	−0.23	0.702*	0.766**	0.921**	0.847**	0.996**	1

** Correlation is significant at the 0.01 level (2-tailed).
* Correlation is significant at the 0.05 level (2-tailed).

Table 6
Eigenvectors of the first five principal component axes from PCA analysis of variables in studied *V. arctostaphylos* L. populations.

Traits	Components		
	1	2	3
Berry fresh weight (g)	0.891	−0.013	−0.11
Flesh fresh weight (g)	0.885	−0.011	−0.11
No. seed/ fruit	0.873	−0.089	0.232
Berry length (mm)	0.883	0.117	−0.301
Berry width (mm)	0.913	0.011	−0.27
No. fruit /cluster	0.735	0.126	−0.246
No. cluster /inflorescence	0.715	−0.387	0.327
Inflorescence length (mm)	0.048	0.688	0.69
Flowering stem length (mm)	0.768	−0.485	−0.013
Essential oil	−0.166	0.69	−0.587
Fruit anthocyanin	0.793	0.386	0.331
Leaf anthocyanin	0.811	0.548	0.062
Fruit total phenol	0.923	−0.19	0.045
Leaf total phenol	0.916	0.208	−0.053
Fruit antioxidant	0.979	−0.047	−0.006
Leaf antioxidant	0.969	−0.099	0.033
% of Variance	65.599	11.95	8.44
Cumulative %	65.599	77.552	85.991

263.17 to 471.26 mg GAEg-1 and the amount of total anthosyanin ranged from 74.3 to 145.29 mg Cyd. Eg^{-1} (Khalili Musavi et al., 2016). It was found that in other berry fruits such as bilberry the same variation was being existent (DahlØ, 2011; Dragovic-Uzelac et al., 2010; Ytterdal, 2011).

3.3. Correlation results

The correlations among the different variables were calculated using Pearson'scorrelation coefficients. As shown in Table 5, berry fresh weight (As the most important trait) was positively and significantly correlated with flesh weight, Number of seed per fruit, berry length, berry width, Number of cluster per inflorescence, flowering stem length and anthocyanin, total phenolics and antioxidant contents in both leaf and fruit. Similar to berry fresh weight, the values for flesh weight also exhibited a positive and significant correlation with most of the pomological and phytochemical traits. Furthermore, there was no significant coefficient between the essential oil content and studied traits. The anthocyanin, total phenolics and antioxidant contents in both leaves and fruits of *V. arctostaphylos* L. had the higher positive correlations with all the pomological traits, except inflorescence length, but they were negatively correlated with essential oil content. In addition, these traits exhibited a positive and significant correlation with one another, indicating that their production was closely linked (Table 5).

3.4. Pomological and phytochemical variability

3.4.1. Cluster and factor analysis

To determine the pomological and phytochemical variability, a principal component analysis (PCA) was done using a correlation matrix of all measured traits. The first three components of the PCA explained 85.99% of the total variation (Table 6). The first component contributed to 65.59% followed by 11.95 and 8.44%, respectively. The first component included the berry and flesh weight, number of seeds per fruit, berry length and width, number of fruits per cluster, number of cluster per inflorescence, flowering stem length and leaf and fruit anthocyanin, total phenolics and antioxidant contents (Table 6). The second component was formed of the essential oil content, while the final component was the inflorescence length.

Cluster and PCA biplot analysis based on the pomological and/or phytochemical variables were conducted to classify various *V. arctostaphylos* L. populations. According to Fig. 1, based on pomological traits, *V. arctostaphylos* L. populations were divided into three major

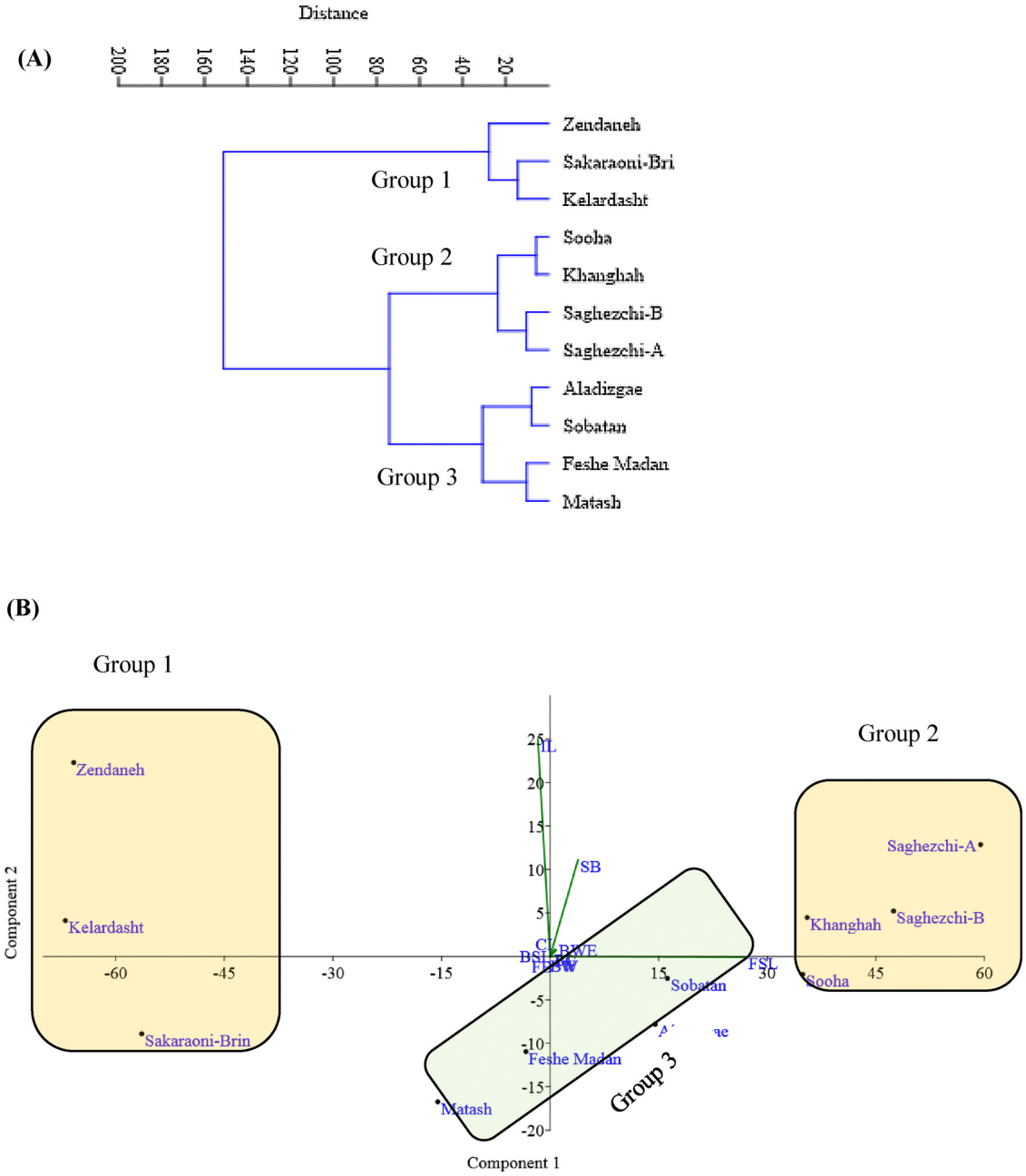

Fig. 1. Cluster (A) and PCA biplot (B) analysis of *V. arctostaphylos* L. populations based on pomological traits.

groups. Group 1 was comprised of the Zendaneh, Brin-Sakaraoni and Kelardasht populations which showed mainly high inflorescence length and berry shape index. The second cluster is formed by populations including, Sooha, Khanghah, Saghezchi-A and Saghezchi-B populations. These populations were characterized by high flowering stem length, number of cluster per inflorescence, seed per fruit, fruit per cluster, flesh fresh weight, berry fresh weight, berry length and Berry width. In addition, group 3 comprised of four populations (Aladizgae, Fesh Madan, Sobatan and Matash) which showed lower values for pomological traits.

In relation to phytochemical traits, the cluster and PCA analysis divided the 11 *V. arctostaphylos L.*populations into three major groups. According to Fig. 2, group 1 was made-up of three populations (including Khanghah, Saghezchi-A and Saghezchi-B) with high amount of leaf anthocyanin, fruit anthocyanin, leaf total phenolics, fruit total phenolics, fruit antioxidant and leaf antioxidant. Group 2 and comprised of three (including Aladizgae, Fesh Madan and Zendaneh) and five (Kelardasht, Sooha, Sakaraoni-Bin, Sobatan, Matash) populations which showed low amounts of the phytochemicals. The pomological and phytochemical diversity found in the populations may be attributed to genetic diversity as well as environmental factors (Hadian et al., 2011; Djabou et al., 2012). Our results indicated the most of the variation in wild populations of *V. arctostaphylos* L. is relatively distributed among the populations rather than within them, indicating a relative notable population differentiation caused by the low level of gene flow among them (Hadj Ali et al., 2012). It has been reported that the pomological and phytochemical traits of Vaccinium species has been strongly influenced by the geographical coordinates like altitude and climatic parameters such as temperature and mean annual rainfall (Dahlø, 2011; Hasanloo et al., 2011; Zoratti et al., 2015).

4. Conclusion

Current study indicated significant differences in pomological and

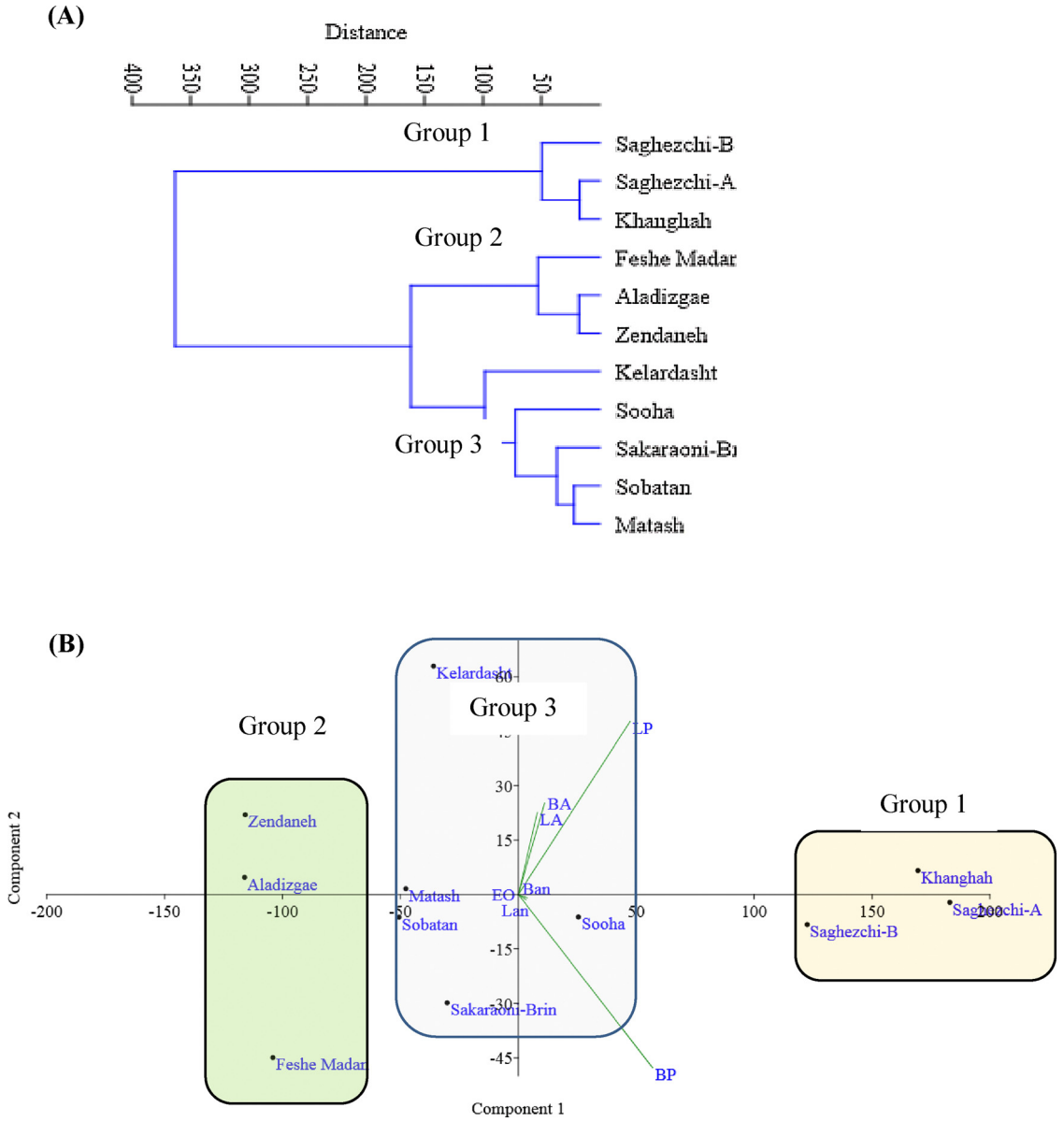

Fig. 2. Cluster (A) and PCA biplot (B) analysis of *V. arctostaphylos* L. populations based on phytochemical traits.

phytochemical characteristics of Caucasian whortleberry (*V. arctostaphylos* L.) populations collected from 11 locations in 3 different provinces. In the case of pomological traits, the highest berry and flesh weight, number of seeds per berry, berry length and width, number of cluster per inflorescence and flowering stem length were observed from plants collected from Saghezchi-A population in Ardabil province which was characterized by high solar radiation. However, the lowest values were obtained from Zendaneh population in Gilan province, in which the plants were mainly under shade condition. In addition, the highest content of anthocyanin, total phenolics and antioxidant in both fruits and leaves of *V. arctostaphylos* L. were mainly recorded from Saghezchi-A population followed by Khanghah population. According to studied traits, *V. arctostaphylos* L. populations were divided into three different groups. Saghezchi-A, Khanghah and Saghezchi-A populations were placed in a same group with mainly the best pomological and phytochemical properties. These locations were characterized by high solar radiation. Therefore, they can be exploited for selection of suitable genotypes for organizing the berry breeding programs and taking advantage of this plant in garden establishment and fruit production investigations.

Declaration of interest

The authors declare that there is no conflict of interest. The authors alone are responsible for the content of the paper.

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