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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