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Original article

Chemical constituents of sea buckthorn (*Hippophae rhamnoides* L.) fruit in populations of central Alborz Mountains in Iran

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Abstract

Background and objectives: *Hippophae rhamnoides* L. known as sea buckthorn is a deciduous medicinal shrub belonging to Elaeagnaceae family**.** In this study, the most important chemical constituents of sea buckthorn were evaluated in wild populations of central Alborz Mountains in Iran during the growth season of 2014 and 2015. **Methods:** Phytochemical analysis of fruit pulp and seed oil traits was performed using different methods of chromatography such as spectrophotometry, HPLC and GC. **Results:** Based on the results of combined analysis of variance, significant ($p \leq 0.01$) difference ranges between populations were found in respect to fruit dry weight (21.32 to 32.03%), total phenolic compounds (20.78 to 34.60 mg/g), extractable tannin (1.99 to 5.74 mg/g), glucose (38.14 to 110.70 mg/g), total carotenoids (0.80 to 1.17 mg/g), lycopene (0.13 to 0.20 mg/g), β-carotene (0.18 to 0.26 mg/g), total flavonoids (0.98 to 2.80 mg/g), total soluble solids (TSS) (11.85 to 31.50%), vitamin C (1.47 to 8.96 mg/g), seed oil content (4.51 to 7.91%), and two major unsaturated fatty acids including linoleic acid (28.71 to 37.44%) and linolenic acid (21.52 to 28.28%). Factor analysis based on principal component analysis (PCA) revealed most important traits with the highest correlation factor such as vitamin C, carbohydrates, TSS, fruit dry weight (FDW), and tannin for the first component. **Conclusion**: content of vitamin C was the main variable in chemical constituents for effective detection of original wild populations of central Alborz Mountains. Accordingly, sea buckthorn populations were divided into four main clusters and groups with high diversity based on their chemical compositions.

Keywords: chemotypes, GC, *Hippophae rhamnoides* L., HPLC, vitamin C

Introduction

Sea buckthorn (*Hippophae rhamnoides* L.) is a valuable multipurpose medicinal plant belonging to Elaeagnaceae family and native in temperate zone of Asia, Europe, and North America. It also grows in a distinct area from the Alborz Mountains in Persia to Caucasia and eastern Turkey [1]. Sea buckthorn is a thorny nitrogen-fixing shrub with high nutraceutical and therapeutical properties. The active constituents of this plant are reputed to have considerable medicinal effects and are frequently used for curing cough, skin wounds, cardiovascular diseases, improving blood circulation, they also have antioxidant activity [2,3]. Sea buckthorn

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leaves, seeds and fruits possess an exclusive composition of natural compounds but important therapeutic uses of this shrub are related to its yellowish-orange fruits. The fruits contain phenolic compounds including flavonoids, flavones, phenolic acids, and tannins [2,4]. These compounds have shown antioxidant, cytoprotective, cardioprotective and wound healing effects [5]. However, ascorbic acid (vitamin C) is the most important medicinal factor in the juice of sea buckthorn fruits [6] and acts as an antioxidant and sustains cell membrane integrity [7]. Fruits also contain carbohydrates (such as glucose, fructose and xylose) in the form of sugars [6]. Various carotenoids (such as lycopene and *β*-carotene) are the major substances existing in a large amount in sea buckthorn fruits pulp [7,8] and act as antioxidant and help in collagen synthesis and epithelialization [9]. Total flavonoids from the leaves and fruits of *Hippophae* genus are a group of compounds containing seven kinds of flavonoids while isorhamnetin and quercetin are the main constituents. These flavonoids have a wide range of curative effects on the cardiovascular diseases [10]. There are two sources of oil in sea buckthorn fruits, the seed oil and the oil held in the pulpy fruit parts surrounding the seed. Seed oil contains high amounts of unsaturated fatty acids and has important therapeutic effects such as preventing heart disease and arthritis and immunomodulatory, neuroprotective and anti-tumor effects [3]. According to the fact that fruits of sea buckthorn contain many kinds of vitamins, trace elements and other biologically active substances, it has been prepared as a natural pill for the prevention and treatment of various diseases.

The sea buckthorn shrubs grow widely in central and northern provinces of Iran and have been used in folk medicine. In order to adaptation to the environment, plant populations in different regions show genetic diversity which may influence the phytochemical composition and biological activity of plants active substances and chemical constituents [11]. Furthermore, previous studies have demonstrated that medicinal plants produce various contents of secondary metabolites in different environments, resulting in differences in their medicinal qualities [12]. According to these facts, the phytochemical and nutritional composition of sea buckthorn berries vary considerably because of genetic variation, parts analyzed, climate and growing conditions, variation between years, the degree of ripening, storage conditions, time of harvesting, and method of processing and analysis [7,8,13-15].

Nevertheless, no such studies have been conducted to evaluate the variation pattern in natural wild populations of sea buckthorn of any regions of Iran. The present study was carried out to determine the variations in phytochemical traits of natural populations of sea buckthorn growing in central Alborz Mountains in Iran. Such studies can provide a systematic mapping of the chemical composition of sea buckthorn berries of different origins. The results of this study are useful to identify suitable sea buckthorn populations when organizing the berry breeding programs and also provide important information for food and pharmaceutical industry.

Experimental

Plant material

Ten sea buckthorn populations were collected and evaluated from their different natural habitat in central Alborz Mountains of Iran in mid-October 2014 and 2015. Voucher specimens have been deposited at the Herbarium of Medicinal Plants Institute (MPI), ACECR, Karaj, Iran. Geographical origins of the 10 sea buckthorn populations and their GPS coordinates have been shown in table 1.

The areas range between longitudes 35° 45′ E and 36° 29′ E, latitudes 50° 26′ N and 51° 47′ N, and altitudes 1481 and 2380 m. Collected fruits samples were kept in a -80 °C refrigerator until phytochemical analysis. The solvents and chemicals used in the present study including, gallic acid standard, quercetin standard, Folin-

Table 1. Geographical origins of *Hippophae rhamnoides* populations

Population No.	Herbarium No.	Region originated	Latitude (N)	Longitude (E)	Altitude (m)
1	MPIH-4511	Parachan	36° 14' 45" N	50° 56' 49" E	2339
2	MPIH-4517	Khodkavand	36° 08' 35" N	50° 49' 59" E	2231
3	MPIH-4510	Dehdar	36° 11' 22" N	51° 03' 06" E	2328
4	MPIH-4512	Shahrak	36° 10' 32" N	50° 46' 47" E	1830
5	MPIH-4517	Jajrood	35° 45' 53" N	51° 41' 35" E	1481
6	MPIH-4519	Dizin	36° 06' 18" N	51° 21' 18" E	2380
7	MPIH-4525	Zarabad	36° 29' 36" N	50° 26' 14" E	1802
8	MPIH-4526	Moallemkelaye	36° 27' 13" N	50° 28' 44" E	1615
9	MPIH-4520	Baladeh	36° 11' 24" N	51° 47' 35" E	2070
10	MPIH-4522	Gachsar	36° 06' 54" N	51° 19' 32" E	2293

Ciocalteu reagent, polyvinyl-polypirrolidone (PVP), and methanol of analytical grade were purchased from Merck, Germany. One hundred fruits dry weight (g) and fruit dry weight percentage (%) were measured for all populations. All phytochemical measurements were done in the laboratory of cultivation and development Department of Medicinal Plants Institute (except seed oil GC analysis that was performed in the Animal Science Department of Tarbiat Modares University, Tehran, Iran).

Determination of total phenolics content

The amount of total phenolics in methanol extracts of dry fruits was determined with the Folin-Ciocalteu reagent. Gallic acid was used as the standard and the total phenolics were presented as mg/g gallic acid equivalents (GAE). Concentrations of 0.01, 0.02, 0.03, 0.04, and 0.05 mg/mL of gallic acid were prepared in methanol. Thus, the calibration curve of gallic acid was drawn. Concentration of 0.1 and 1 mg/mL of plant extract were also prepared in methanol and 0.5 ml of each sample were entered in test tubes and mixed with 2.5 mL of a 10 fold dilute Folin-Ciocalteu reagent and 2 mL of 7.5% sodium carbonate. The tubes were covered with parafilm and permitted to stand for 30 min at room temperature before the absorbance was read at 760 nm (UV-2601 double beam UV/VIS spectrophotometer, China) spectrometrically [16].

Determination of tannin content

Tannin content in each sample was determined using insoluble polyvinyl-polypirrolidone (PVP), which binds tannins [17]. Briefly, 1 mL of extract was dissolved in methanol (1 mg/ml), mixed with 100 mg PVP, vortexed, kept for 15 min at 4 °C and then centrifuged for 10 min at 3,000 rpm. In the clear supernatant, the non-tannin phenolic compounds were determined in the same way as the total phenolic compounds. Tannin content was calculated as a difference between total and non-tannin phenolic content.

Determination of fruit sugars

Soluble sugar content determination was done with phenol-sulphuric method [18]. Standard curves were prepared to quantify glucose, fructose, xylose and arabinose contents. Sugars concentration was determined by spectrophotometry method (UV-2601 double beam UV/VIS spectrophotometer/China) at 480 nm for xylose and arabinose, at 485 nm for glucose and at 490 nm for fructose. Sensitivity of this method ranged from 10 to 100 µg of sugars and the quantification was made from calibration curve using glucose, fructose, xylose and arabinose as standards and calculation were performed by equation of the linear regression obtained from the calibration curve. The sugars content was expressed on a dry weight basis.

Determination of total flavonoids content
For determination of total flavonoids content of each fruit extract, we used a conventional method [19]. Based on this method, each prepared sample (1 mL) was mixed with 4 mL of distilled water and subsequently with 0.3 mL of a $NaNO_2$ solution (10%). After 5 min, 0.3 mL $AlCl_3$ solution (10%) was added fallowed by 2 mL of NaOH solution (1%) to the mixture. Immediately, the mixture was thoroughly mixed and absorbance was then determined at 510 nm (UV-2601 double beam UV/VIS spectrophotometer, China) versus the blank. The S\standard curve of quercetin was prepared (0-12 mg/mL) and the results were expressed as quercetin equivalents (mg quercetin/mg dried extract).

Determination of total carotenoids, lycopene and β-carotene content
For carotenoids extraction and determination method, a mixture of hexane/ethanol/ acetone (2:1:1) containing 0.05% butylated hydroxytoluene (BHT) was used. For total carotenoid quantification, the absorbance of the hexane extract was read at 450 nm using a UV-2601 double beam UV/VIS spectrophotometer (China) [20]. Also for *β*-carotene and lycopene determination the dried methanolic extract (100 mg) was vigorously shaken with 10 mL of acetone–hexane mixture (4:6) for 1 min and filtered through Whatman No. 4 filter paper. The absorbance of the filtrate was measured at 453, 505, 645 and 663 nm [21]. Contents of *β*-carotene and lycopene were calculated according to the following equations:

$$\text{lycopene (mg/100 mL)} = -0.0458\ A_{663} + 0.372\ A_{505} - 0.0806\ A_{453}$$

$$\beta\text{-carotene (mg/100 mL)} = 0.216\ A_{663} - 0.304\ A_{505} + 0.452\ A_{453}$$

HPLC quantification of vitamin C
One g of sample (fruit flesh) was homogenized repeatedly with 0.01 mol/L metaphosphoric acid (6 × 20 mL) and centrifuged at 2200 × g for 5 min (Hanil smart R17 Centrifuge, Korea). The supernatant was filtered through a 0.45 µm filter and analyzed immediately. Juice samples were also treated with metaphosphoric acid, centrifuged and filtered. A 20 µL sample was injected into a Knauer Wellchrom HPLC (Germany) equipped with a K-1001 pump Phenomenex C-18 ODS-2 column (5 µm, 250 mm × 4.60 mm; Luna) and a K-2501 UV detector set at 246 nm, and 3.7 mmol/L phosphate buffer (pH 4) at a flow rate of 1 mL/min as the mobile phase. Authentic standards of ascorbic acid (0.5-5 µg/mL) were used for optimizing the HPLC conditions [22].

Total soluble solids content (TSS) measurement
TSS contents in the fruits of each population were expressed by the Brix of fresh juice. The measurement was taken by placing a drop of filtered juice on the prism of a digital refractometer (KRUSS Co. Germany, HR Series).

Seed oil extraction
Ten grams of the dried seeds were milled and placed in an extraction thimble and extracted with organic solvent *n*-hexane using a 250 mL capacity Soxhlet apparatus for 8 h (60 °C) in 3 replications [23]. The oil was then separated by rotary-evaporator under reduced pressure at 35 °C.

GC analysis of fatty acid methyl esters
Determination of the fatty acids was done by gas chromatographic measurement of the prepared samples. We used a Unicam 4600 GC instrument equipped with a flame ionization detector and a split/splitless injector. A fused-silica capillary column BPX70 (SGE, Melbourne, Australia) with 30 m length, 0.22 mm internal diameter and 0.25 µm thickness was used for analysis. Injector and detector temperatures were 230 and 250 °C, respectively. Oven conditions were 180 °C increased to 220 °C at a rate of 2 °C/min and maintained for 5 min. Helium was the carrier gas and nitrogen was used as the make-up gas at a

flow rate of 30 ml/min. The quantification of fatty acid methyl esters composition was realized by integration of the FID peak area and comparing their retention times with standards methyl esters to be expressed by percentage [24].

Data analysis

Analysis of variance was performed for all traits by SPSS Statistics (ver. 22) software. ANOVA analysis and mean comparison of the traits were done by using Duncan multiple range tests at $p \leq 0.05$ significant level. In order to determine the most variable characters among the populations, factor analysis based on principal component analysis (PCA) was performed. Hierarchical cluster analysis of studied populations was based on the Euclidean distances of traits using Wards method. The simple correlation coefficient was calculated to determine the relationships between the studied traits using the Pearson correlation coefficient.

Results and discussion

According to the obtained results, all studied traits except vitamin C, trans-oleic acid and linoleic acid changed significantly ($p \leq 0.01$) in the experimental years. Of course linolenic acid changed significantly at a level of 5% in experimental years. Also, the variance analyses showed that the various populations had significant differences in respect of all studied traits ($p \leq 0.01$) and their mean. Only seed oil content in 2014 changed significantly at a level of 5% among the populations.

It was found that the average of the 100 dry fruits weight in the second year (4.40 g) was more than the first year (3.94 g). Regarding to this parameter, the highest value (6.13 g) of 100 fruits dry weight was related to Zarabad population in 2015. Also, the fruit dry weight percentage showed higher average in 2015 (26.93%) in comparison with 2014 (23.91%). The maximum and minimum fruit dry weight percentages were reported from Parachan (34.52 %) in 2015 and Zarabad (19.07 %) in 2014, respectively (table 2).

Mean comparison results showed that the highest and lowest values of phenolic contents were related to Zarabad (45.08 mg/g) in 2015 and Jajrood (18.37 mg/g) in 2014, respectively (table 2). In a study, in relation to seventeen natural population of sea buckthorn from Trans-Himalaya, the fruits were found to be rich in total phenolic content ranging from 9.64 to 107.04 mg/g [25]. Another study reported significant variation in total phenolic content (21.31-55.38 mg GAE/g DW) among 10 Sea buckthorn genotypes in Turkey [26]. The result of two mentioned studies had similar ranges to our study. Also the maximum and minimum content of extractable tannin were found in Baladeh (7.98 mg/g) in 2015 and Shahrak (1.71 mg/g) in 2014, respectively (table 2).

Sugar is a major ingredient of sea buckthorn fruits, as it plays a valuable role in determining the sweetness of its juice. It was indicated that fruit measured sugars in the second year were more than the contents in the first year. The highest value of glucose (125.57 mg/g), fructose (132.06 mg/g), xylose (76.13 mg/g) and arabinose (123.62 mg/g) were related to Shahrak population in 2015. But, the lowest values of these traits were observed in Parachan population in 2014 year (table 2). A study explained that Sugar components are important ingredients of sea buckthorn juice and glucose and fructose account for around 90% of the total sugar content for Chinese and Russian origins [27].

Various colors of sea buckthorn berries are related to the occurrence of carotenoids that are thought to provide health benefits in decreasing the risk of diseases, particularly certain cancers and eye disease [28]. The higher average content of fruit total carotenoid, lycopene and β-carotene were observed in the first year (1.11, 0.18 and 0.25 mg/g) in comparison to the second year (0.95, 0.16 and 0.22 mg/g). The maximum content (1.29, 0.21 and 0.28 mg/g) of these factors occurred in Dizin in 2014; whereas, the minimum content of these traits were in Jajrood (0.71, 0.12 and 0.17 mg/g) in 2015. In a study in Sweden, sea buckthorn cultivars comprised from 0.12 to 1.42 mg/g of dry weight total carotenoids depending on cultivar, harvest time, and year [9].

Table 2. Results of mean comparisons for fruit traits among studied *Hippophae rhamnoides* populations during 2014 and 2015 harvesting season

		Fruit traits									
Populations	**Year**	**100 fruits dry weight (g)**	**Fruit dry weight (%)**	**Total phenol (mg/g)**	**Tannin (mg/g)**	**Glucose (mg/g)**	**Fructose (mg/g)**	**Xylose (mg/g)**	**Arabinose (mg/g)**	**Carotenoid (mg/g)**	**Lycopene (mg/g)**
Parachan	2014	4.83^{a}	29.53^{a}	20.50^{de}	2.03^{de}	30.78^{d}	22.91^{e}	19.26^{e}	37.16^{d}	1.15^{bc}	0.19^{ab}
	2015	4.97^{bc}	34.52^{a}	24.26^{cd}	6.85^{a}	57.07^{f}	54.26^{f}	35.36^{f}	61.25^{de}	1.19^{a}	0.20^{a}
	Mean	4.90^{ab}	32.03^{a}	22.38^{c}	4.44^{b}	43.92^{fg}	38.59^{fg}	27.84^{ef}	49.73^{fg}	1.17^{a}	0.20^{a}
Khodkavand	2014	4.00^{bc}	25.62^{bcd}	18.75^{e}	3.53^{bc}	51.47^{bc}	44.64^{d}	32.95^{d}	57.56^{c}	0.92^{d}	0.15^{c}
	2015	4.15^{cde}	25.29^{d}	36.22^{b}	4.96^{b}	80.39^{d}	81.10^{d}	48.81^{d}	81.82^{c}	0.92^{bc}	0.15^{cd}
	Mean	4.08^{cd}	25.46^{bcd}	27.48^{b}	4.25^{bc}	65.93^{d}	62.87^{d}	40.88^{c}	69.69^{d}	0.92^{e}	0.15^{d}
Dehdar	2014	3.36^{cd}	20.68^{fg}	20.40^{de}	2.03^{de}	62.22^{b}	55.96^{c}	39.89^{c}	67.62^{b}	0.84^{d}	0.14^{c}
	2015	3.88^{de}	25.70^{cd}	36.02^{b}	3.62^{b}	53.10^{fg}	51.79^{f}	33.11^{fg}	57.80^{ef}	0.77^{de}	0.13^{ef}
	Mean	3.62^{de}	23.19^{de}	28.21^{b}	2.82^{de}	57.66^{e}	53.87^{e}	35.98^{d}	62.19^{e}	0.80^{f}	0.13^{e}
Shahrak	2014	2.80^{d}	19.13^{g}	22.91^{bc}	2.26^{de}	95.84^{a}	93.44^{a}	58.56^{a}	92.74^{a}	1.17^{abc}	0.19^{ab}
	2015	4.21^{cde}	24.40^{de}	28.18^{c}	1.71^{c}	125.57^{a}	132.06^{a}	76.13^{a}	123.62^{a}	0.95^{bc}	0.16^{cd}
	Mean	3.51^{e}	21.77^{e}	25.54^{b}	1.99^{e}	110.70^{a}	112.75^{a}	67.34^{a}	108.18^{a}	1.06^{cd}	0.17^{c}
Jajrood	2014	2.83^{d}	21.57^{efg}	18.37^{e}	1.71^{e}	84.09^{a}	79.68^{b}	51.65^{b}	85.50^{a}	1.06^{c}	0.17^{b}
	2015	2.77^{f}	21.06^{e}	36.30^{b}	3.32^{bc}	97.19^{c}	101.04^{c}	59.31^{c}	97.89^{b}	0.71^{e}	0.12^{f}
	Mean	2.80^{f}	21.32^{e}	27.34^{b}	2.52^{de}	90.64^{c}	90.36^{c}	55.48^{b}	91.69^{c}	0.88^{ef}	0.15^{de}
Dizin	2014	4.19^{ab}	29.18^{ab}	22.01^{cd}	2.13^{de}	44.42^{c}	37.13^{d}	28.51^{d}	50.75^{c}	1.29^{a}	0.21^{a}
	2015	3.37^{ef}	24.46^{de}	22.93^{cd}	4.75^{b}	31.86^{i}	28.25^{h}	20.31^{h}	38.21^{g}	0.86^{cde}	0.14^{def}
	Mean	3.78^{de}	26.82^{b}	22.47^{c}	3.44^{cd}	38.14^{g}	32.69^{g}	24.41^{f}	44.48^{g}	1.07^{bcd}	0.18^{bc}
Zarabad	2014	3.52^{c}	19.07^{g}	22.27^{cd}	4.34^{ab}	47.67^{c}	40.75^{d}	30.65^{d}	54.03^{c}	1.15^{bc}	0.19^{ab}
	2015	6.13^{a}	32.73^{ab}	45.08^{a}	6.88^{a}	68.46^{e}	67.96^{e}	41.79^{e}	71.08^{d}	1.18^{a}	0.19^{ab}
	Mean	4.83^{ab}	25.90^{bc}	33.67^{a}	5.61^{a}	58.07^{e}	54.36^{e}	36.22^{d}	62.56^{e}	1.17^{ab}	0.19^{ab}
Moallemkelaye	2014	4.55^{ab}	26.93^{abc}	19.56^{e}	2.89^{cd}	49.30^{bc}	42.08^{d}	31.61^{d}	55.51^{c}	1.09^{c}	0.18^{b}
	2015	4.62^{bcd}	27.85^{cd}	22.00^{d}	3.98^{b}	45.33^{gh}	42.73^{g}	28.13^{g}	54.18^{ef}	1.06^{ab}	0.18^{bc}
	Mean	4.59^{abc}	27.39^{b}	20.78^{c}	3.43^{cd}	47.31^{f}	42.40^{f}	29.87^{e}	54.84^{f}	1.07^{bcd}	0.18^{bc}
Baladeh	2014	4.65^{ab}	24.43^{cde}	26.06^{a}	3.49^{bc}	40.83^{cd}	41.29^{d}	30.27^{d}	53.46^{c}	1.15^{bc}	0.19^{ab}
	2015	4.41^{cd}	24.01^{de}	43.15^{a}	7.98^{a}	42.98^{h}	39.47^{g}	27.06^{g}	48.54^{fg}	0.87^{cd}	0.15^{de}
	Mean	4.53^{bc}	24.22^{cd}	34.60^{a}	5.74^{a}	41.90^{fg}	40.38^{f}	28.67^{e}	51.00^{f}	1.01^{d}	0.17^{c}
Gachsar	2014	4.66^{ab}	22.91^{def}	24.99^{ab}	4.46^{a}	84.00^{a}	79.84^{b}	48.26^{b}	86.08^{a}	1.23^{ab}	0.20^{a}
	2015	5.50^{ab}	29.29^{bc}	27.49^{cd}	3.89^{b}	114.63^{b}	120.07^{b}	69.65^{b}	117.04^{a}	1.00^{bc}	0.16^{cd}
	Mean	5.08^{a}	26.10^{bc}	26.24^{b}	4.17^{bc}	99.32^{b}	99.95^{b}	58.95^{b}	101.56^{b}	1.11^{abc}	0.18^{abc}
Mean	**2014**	**3.94^{b}**	**23.91^{b}**	**21.58^{b}**	**2.89^{b}**	**59.06^{b}**	**53.77^{b}**	**37.16^{b}**	**64.04^{b}**	**1.11^{a}**	**0.18^{a}**
	2015	**4.40^{a}**	**26.93^{a}**	**32.16^{a}**	**4.79^{a}**	**71.66^{a}**	**71.87^{a}**	**43.97^{a}**	**75.14^{a}**	**0.95^{b}**	**0.16^{b}**
	Mean	**4.17**	**25.42**	**26.87**	**3.84**	**65.36**	**62.82**	**40.56**	**69.59**	**1.03**	**0.17**

*Means in each column followed by the same letter (a-g) are not significantly different according to Duncan's multiple range test at the 5% level of probability. The obtained values were expressed as mean from three replications.

In another study between six Romanian sea buckthorn varieties, total carotenoid content varied between 0.53 and 0.97 mg/g dry weight in berries [8].

Flavonoids have several biological activities and are one of the several important constituents of sea buckthorn leaves and fruits. High content of flavonoid has been raised as a desirable factor in selection of sea buckthorn suitable genotypes. The highest fruit flavonoid content in 2014 and 2015 (2.40 and 3.19 mg/g) were reported from Baladeh and the lowest content of this trait in 2014 and 2015 (1.04 and 0.92 mg/g) were in Dehdar population. In a study, the flavonoid content in fruits of sea buckthorn in different origins showed clear differences between 0.18% to 0.56% among genotypes originating from China, Russia, Finland, and Canada [13]. Soluble

solids offer a main fraction of the sea buckthorn fruit juice, as a consequence of the high amount of organic acids and sugars of its berry [14]. The result showed that the maximum content of this trait was observed in Shahrak population (35.20%) in 2015 and the minimum content in Parachan (8.60%) in 2014. In a recent research, the quantity of total soluble solids in fruit juice was determined refractometrically as °Brix, ranges from 9.3 to 22.74% which is consistent with our results [29].

The quality of sea buckthorn fruit is often based on the nutritional value, especially vitamin C (ascorbic acid). Previous studies have reported a typical variation of 2-2500 mg/100 g of vitamin C content in sea buckthorn berries [14,30,31]. Thus sometimes vitamin C amount in sea buckthorn fruit is 5 to 100 times higher than any other known fruit or vegetable [32] (such as orange (50 mg/100 g), strawberries (64 mg/100 g), tomatoes (12 mg/100 g) and kiwi fruit (100-400 mg/g) [33]. According to this high amount of ascorbic acid, its fruit juice offers the antioxidant activity as a scavenger of free radicals and inhibits the formation of potentially carcinogenic compounds [10]. In our study, the highest amount of vitamin C were observed in Shahrak (10 mg/g=1000 mg/100 g) in 2014 and the lowest amount in Parachan (1.39 mg/g=139 mg/100 g) in the same year.

Seed oil is important because of high amounts of unsaturated fatty acids (oleic acid, linoleic acid and linolenic acid) and populations with high content of these fatty acids are more valuable. Maximum content of seed oil was reported from Jajrood (8.28%) in 2015 and minimum content in Dehdar population (4.35%) in 2014. The highest content of cis-oleic acid was found in Khodkavand (21.07 mg/g) in 2014 and the lowest amount in Moallemkelaye (5.93 mg/g) in 2015 (table 3). The average content of fruit trans-oleic acid in 2014 and 2015 years were 3.99 and 3.96 %, respectively. The highest and lowest content of trans-oleic acid in average of two years were in Shahrak (4.64%) and Zarabad (2.79%). Also, the results showed that the highest and lowest linoleic acid content in two years was related to Baladeh (37.44%) and Zarabad (28.71%) populations, respectively. Both maximum and minimum content of linolenic acid existed in Dehdar (30.73%) and Khodkavand (20.02%) in 2014 (table 3).

In accordance with our results, in a study, researchers showed linoleic and linolenic acids comprised about 70% of seed oil fatty acids in sea buckthorn berries of different origins [3].

Factor analysis was used based on principal components to provide a reduced dimension model indicating differences measured among groups. Principal components analysis (PCA) allows to evaluate multicollinear data and to determine the traits most suitable for classification [34]. PCA indicated five components explaining 90.88% of the total variance. The first three components (PC1–PC3) explained 72.85% of the total variation (table 4). In the first component (PC1), some characteristics such as fruit dry weight percentage, tannin, carbohydrates, carotenoids, TSS and vitamin C showed the highest variance. Also, in PC2 100 fruits dry weight, fruit carotenoids (total, lycopene and β-carotene) and linolenic acid of seed oil showed the highest variance. While, the highest variance was observed for fruit total phenol and trans-oleic acid, linoleic acid and linolenic acid for seed oil in PC3.

Cluster analysis (CA) based on Wards method at similarity coefficient of 10, divided populations into four main groups with high diversity (figure 1).

The first main group was divided into five populations, consisted of populations from Dizin, Moallemkelaye, Parachan, Khodkavand, and Baladeh, with similar characteristics such as lower amount of TSS and vitamin C. The second group was comprised of Zarabad and Gachsar populations with similar characteristic such as higher content of β-carotene and lower content of seed oil and linoleic acid in oil.

Table 3. Results of mean comparisons for fruit traits among studied *Hippophae rhamnoides* populations during 2014 and 2015 harvesting season

		Fruit traits								
Populations	**Year**	**Fruit *β*-carotene (mg/g)**	**Fruit flavonoid (mg/g)**	**Fruit TSS brix (%)**	**Fruit vitamin C (mg/g)**	**Seed oil (%)**	**Cis-oleic acid (%)**	**Trans-oleic acid (%)**	**Linoleic acid (%)**	**Linolenic acid (%)**
Parachan	2014	0.26^{bc}	1.24^{de}	8.60^{e}	1.39^{c}	5.28^{bcd}	13.51^{cd}	4.35^{bc}	33.18^{c}	25.28^{bc}
	2015	0.26^{a}	1.39^{efg}	15.10^{e}	1.55^{e}	7.38^{ab}	13.24^{a}	4.04^{bcd}	34.24^{bc}	22.33^{e}
	Mean	0.26^{a}	1.31^{ef}	11.85^{e}	1.47^{e}	6.33^{bc}	13.37^{b}	4.20^{abc}	33.71^{cd}	23.80^{bcd}
Khodkavand	2014	0.24^{c}	1.10^{e}	13.70^{cd}	4.01^{bc}	5.27^{bcd}	21.07^{a}	5.15^{a}	37.74^{ab}	20.02^{d}
	2015	0.24^{ab}	1.96^{cde}	20.60^{cd}	2.62^{e}	5.78^{c}	11.35^{ab}	3.77^{cd}	34.89^{bc}	23.02^{de}
	Mean	0.24^{bc}	1.53^{de}	17.15^{c}	3.32^{cd}	5.52^{cde}	16.21^{a}	4.46^{ab}	36.31^{ab}	21.52^{e}
Dehdar	2014	0.19^{d}	1.04^{e}	16.60^{cd}	4.52^{b}	4.69^{cd}	14.20^{cd}	4.91^{ab}	29.62^{def}	30.73^{a}
	2015	0.17^{d}	0.92^{g}	15.70^{e}	4.53^{c}	5.52^{c}	8.62^{c}	2.54^{e}	31.52^{bcde}	25.83^{bc}
	Mean	0.18^{e}	0.98^{f}	16.15^{cd}	4.52^{c}	5.11^{de}	11.41^{cd}	3.73^{cd}	30.57^{ef}	28.28^{a}
Shahrak	2014	0.25^{c}	1.82^{bc}	27.80^{a}	10.00^{a}	6.75^{ab}	14.93^{cd}	4.87^{ab}	34.58^{bc}	26.55^{b}
	2015	0.20^{cd}	2.46^{bc}	35.20^{a}	7.92^{a}	7.01^{b}	8.59^{c}	4.42^{bc}	34.63^{bc}	23.95^{cde}
	Mean	0.23^{cd}	2.14^{bc}	31.50^{a}	8.96^{a}	6.88^{b}	11.76^{cd}	4.64^{a}	34.61^{bc}	25.25^{b}
Jajrood	2014	0.25^{c}	1.35^{de}	25.10^{ab}	8.07^{a}	7.55^{a}	18.05^{b}	3.27^{de}	39.72^{a}	22.28^{cd}
	2015	0.17^{d}	1.30^{fg}	23.60^{c}	5.36^{bc}	8.28^{a}	13.09^{a}	4.70^{ab}	30.98^{cde}	28.90^{a}
	Mean	0.21^{d}	1.32^{ef}	24.35^{b}	6.71^{b}	7.91^{a}	15.57^{a}	3.99^{bcd}	35.35^{abc}	25.59^{b}
Dizin	2014	0.28^{a}	2.11^{ab}	14.07^{cd}	1.77^{c}	5.28^{bcd}	14.09^{cd}	3.73^{cd}	31.26^{cde}	22.14^{cd}
	2015	0.19^{d}	2.75^{ab}	13.80^{e}	4.44^{c}	5.56^{c}	9.96^{bc}	3.24^{de}	32.58^{bcd}	25.35^{bcd}
	Mean	0.23^{bc}	2.43^{b}	13.93^{de}	3.11^{d}	5.42^{cde}	12.02^{bcd}	3.48^{d}	31.92^{de}	23.74^{bcd}
Zarabad	2014	0.26^{abc}	1.60^{cd}	17.30^{c}	2.18^{bc}	5.89^{abcd}	14.69^{cd}	2.67^{e}	28.86^{ef}	25.05^{bc}
	2015	0.27^{a}	2.13^{bcd}	17.50^{de}	2.55^{e}	5.66^{c}	10.87^{b}	2.91^{e}	28.57^{e}	25.79^{bc}
	Mean	0.26^{a}	1.87^{cd}	17.40^{c}	2.36^{de}	5.78^{cd}	12.78^{bc}	2.79^{e}	28.71^{f}	25.42^{b}
Moalemkelaye	2014	0.24^{c}	1.64^{cd}	15.30^{cd}	3.32^{bc}	5.30^{bcd}	15.99^{bc}	3.79^{cd}	26.90^{f}	20.53^{d}
	2015	0.24^{ab}	2.47^{bc}	14.90^{e}	3.98^{cd}	7.30^{ab}	5.93^{d}	5.39^{a}	35.33^{b}	23.31^{de}
	Mean	0.24^{bc}	2.05^{c}	15.10^{cd}	3.65^{cd}	6.30^{bc}	10.96^{d}	4.59^{a}	31.11^{e}	21.92^{de}
Baladeh	2014	0.26^{abc}	2.40^{a}	12.70^{d}	1.89^{bc}	6.47^{abc}	12.96^{d}	3.51^{d}	32.86^{cd}	22.14^{cd}
	2015	0.19^{cd}	3.19^{a}	13.40^{e}	2.74^{de}	7.31^{ab}	8.01^{c}	4.77^{ab}	42.03^{a}	27.11^{ab}
	Mean	0.23^{cd}	2.80^{a}	13.05^{de}	2.31^{de}	6.89^{b}	10.49^{d}	4.14^{abc}	37.44^{a}	24.62^{bc}
Gachsar	2014	0.28^{ab}	2.21^{ab}	22.70^{b}	8.59^{a}	4.35^{d}	14.61^{cd}	3.68^{cd}	33.58^{c}	22.93^{cd}
	2015	0.22^{bc}	1.70^{ef}	30.40^{b}	6.60^{b}	4.67^{c}	11.17^{ab}	3.85^{cd}	29.29^{de}	22.66^{e}
	Mean	0.25^{ab}	1.95^{c}	26.55^{b}	7.59^{b}	4.51^{e}	12.89^{bc}	3.76^{cd}	31.43^{de}	22.79^{de}
Mean	2014	**0.25^{a}**	**1.65^{b}**	**17.39^{b}**	**4.57**	**5.68^{b}**	**15.41^{a}**	**3.99**	**32.83**	**23.76^{b}**
	2015	**0.22^{b}**	**2.02^{a}**	**20.02^{a}**	**4.23**	**6.45^{a}**	**10.08^{b}**	**3.96**	**33.41**	**24.82^{a}**
	Mean	**0.23**	**1.84**	**18.70**	**4.40**	**6.06**	**12.75**	**3.98**	**33.12**	**24.29**

*Means in each column followed by the same letter (a-g) are not significantly different according to Duncan's multiple range test at the 5% level of probability. The obtained values were expressed as mean from three replications.

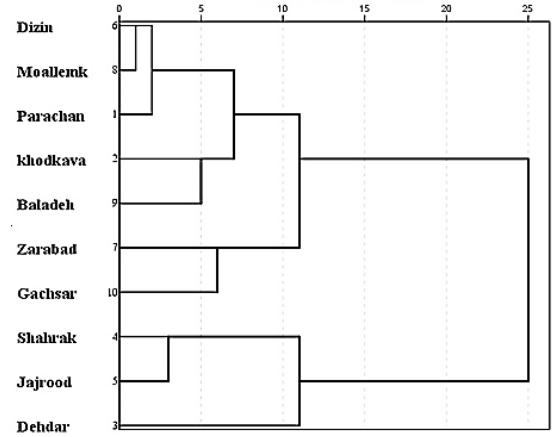

Figure 1. Wards cluster analysis of *Hippophae rhamnoides* populations based on studied chemical constituents

The third group divided to Shahrak and Jajrood populations with similarity in lower value of fruit dry weight percentage and tannin content and higher amount of carbohydrates, TSS, vitamin C and seed oil quantity. The fourth group comprised of Dehdar population. This population was recognized with a low amount of carotenoids (total lycopene and *β*-carotene) and flavonoids. Simple correlation coefficient analysis showed the existence of significant positive and negative correlations among studied traits (table 5). We mentioned some of the more important correlations between them. Altitude of the natural

Table 4. Eigenvectors of the first three principal component axes from PCA analysis of fruit variables in studied *H. rhamnoides* populations.

Character	Component		
	1	2	3
100 fruits dry weight	-0.70**	0.54**	-0.16
Fruit dry weight	-0.81**	0.30	0.23
Fruit total phenol	0.01	-0.19	-0.56**
Fruit tannin	-0.74**	0.15	-0.23
Fruit glucose	0.88**	0.46	-0.10
Fruit fructose	0.88**	0.45	-0.10
Fruit xylose	0.89**	0.44	-0.09
Fruit arabinose	0.87**	0.47	-0.10
Fruit carotenoid	-0.53**	0.79**	-0.04
Fruit lycopene	-0.54**	0.78**	-0.03
Fruit *β*-carotene	-0.53**	0.79**	0.06
Fruit flavonoid	-0.27	0.32	0.06
Fruit TSS Brix	0.87**	0.48	-0.13
Fruit vitamin C	0.91**	0.33	-0.07
Seed oil (%)	0.29	-0.17	0.42
Cis-oleic acid	0.29	0.05	0.28
Trans-oleic acid	0.31	0.03	0.83**
Linoleic acid	0.26	-0.15	0.66**
Linolenic acid	0.32	-0.53**	-0.61**
Eigenvalue	7.698	3.878	2.266
% of variance	40.514	20.409	11.928
Cumulative (%)	40.51	60.92	72.85

** Eigenvalues are significant ≥ 0.50

habitat of populations had negative correlation with seed oil content (r=-0.71, $p\leq0.05$).One hundred fruits dry weight exhibited positive correlation with fruit tannin (r=0.74, $p\leq0.05$), carotenoids (r=0.71, $p\leq0.05$), *β*-carotene (r=0.73, $p\leq0.05$) and lycopene (r=0.72, $p\leq0.05$) content. Fruit dry weight percentage had negative correlation with fruit vitamin C content (r=-0.65, $p\leq0.05$). Also, fruit tannin had a negative correlation with fruit vitamin C (r=-0.69, $p\leq0.05$). Fruit glucose had positive correlation with fruit total soluble solid (r=0.98, $p\leq0.01$) and vitamin C (r=0.93, $p\leq0.01$). It was evident that fruit *β*-carotene had negative correlation with linolenic acid of seed oil (r=-0.63, $p\leq0.05$). Fruit TSS had positive correlation with fruit vitamin C (r=0.96, $p\leq0.01$).

There was a wide variability in chemical constituents among different *H. rhamnoides* populations in central regions of Alborz

Table 5. Correlations between fruit characteristics in *Hippophae rhamnoides* populations

Variables	Altitude	100 fruits dry weight	Fruit dry weight (%)	Phenol	Tannin	Glucose	Fructose	Xylose	Arabinose	Carotenoide	Lycopen	β-caroten	Flavonoid	TSS	Vitamin C	Seed oil %	Cis-oleic acid	Trans-oleic acid	Linoleic acid	Linolenic acid
Altitude	1																			
100 fruits dry weight	0.33	1																		
Fruit dry weight	0.44	0.72*	1																	
Phenol	-0.09	0.07	-0.43	1																
Tannin	0.23	0.74*	0.46	0.56	1															
Glucose	-0.31	-0.32	-0.58	0.01	-0.52	1														
Fructose	-0.32	-0.32	-0.59	0.04	-0.50	0.99**	1													
Xylose	-0.33	-0.34	-0.60	0.04	-0.51	0.99**	1.00**	1												
Arabinose	-0.32	-0.30	-0.58	0.03	-0.49	0.99**	0.99**	0.99**	1											
Carotenoid	0.04	0.71*	0.65*	-0.17	0.44	-0.12	-0.12	-0.13	-0.12	1										
Lycopene	0.05	0.72*	0.66*	-0.17	0.46	-0.13	-0.13	-0.15	-0.13	0.99**	1									
***β*-carotene**	0.02	0.73*	0.65*	-0.08	0.57	-0.10	-0.10	-0.11	-0.09	0.90**	0.90**	1								
Flavonoid	-0.05	0.26	-0.02	0.17	0.34	-0.16	-0.13	-0.14	-0.13	0.44	0.43	0.32	1							
TSS	-0.36	-0.35	-0.61	-0.01	-0.55	0.98**	0.98**	0.98**	0.98**	-0.06	-0.08	-0.09	-0.03	1						
Vitamin C	-0.29	-0.44	-0.65*	-0.14	-0.69*	0.93**	0.93**	0.93**	0.93**	-0.21	-0.23	-0.29	-0.05	0.96**	1					
Seed oil	-0.71*	-0.49	-0.33	0.09	-0.21	0.12	0.14	0.16	0.12	-0.11	-0.11	-0.14	0.08	0.14	0.10	1				
Cis-oleic acid	-0.08	-0.28	-0.05	-0.04	-0.09	0.31	0.29	0.30	0.29	-0.23	-0.22	0.13	-0.53	0.19	0.09	0.11	1			
Trans-oleic acid	-0.15	-0.18	-0.05	-0.44	-0.41	0.21	0.22	0.23	0.22	-0.19	-0.18	-0.18	0.03	0.18	0.27	0.37	0.04	1		
Linoleic acid	-0.04	-0.31	-0.24	0.15	-0.01	0.11	0.14	0.15	0.12	-0.29	-0.28	-0.17	0.20	0.06	0.06	0.55	0.29	0.63	1	
Linolenic acid	-0.05	-0.45	-0.49	0.40	-0.25	0.10	0.10	0.11	0.08	-0.43	-0.43	-0.63*	-0.33	0.11	0.16	0.16	-0.26	-0.39	-0.21	1

Mountains. In conclusion, PCA indicated five components which the first three components (PC1–PC3) explained 72.85% of the total variation. In addition, vitamin C, carbohydrates (xylose, glucose, fructose, and arabinose), TSS, fruit dry weight, and tannin of *H. rhamnoides* fruit in PC1 were the most important efficient traits in Alborz Mountains for identifying the chemotypes and populations. Therefore, the content of vitamin C was the main variable in chemical constituents for effective detection of original wild populations of central Alborz Mountains. According to hierarchical cluster analysis, studied populations were divided into four main groups with high diversity. Since medicinal effects of this species is more related to fruit vitamin C and seed oil content (contained unsaturated fatty acids), we can say that populations of Shahrak and Jajrood which revealed higher amounts of vitamin C (8.96 mg/g) and oil content of seed (7.91%) were elite populations in mean results of two studied years, respectively. These populations may be generated by an interaction between the growth of suitable genotypes and an appropriate regional climate. The wide range of variation among the sea buckthorn populations in this study can be exploited for selection of suitable genotypes for organizing the berry breeding programs and exploitation of this plant in pharmacognostic 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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