

[Context Sensitive Links](#)[Look Up Full Text](#)[Israel ULS](#)

Save to EndNote online

[Add to Marked List](#)

1 of 1

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

By: Kuhkheil, A (Kuhkheil, A.)^[1]; Badi, HN (Badi, H. Naghdi)^[2]; Mehrafarin, A (Mehrafarin, A.)^[2]; Abdossi, V (Abdossi, V.)^[1]

RESEARCH JOURNAL OF PHARMACOGNOSY

Volume: 4 Issue: 3 Pages: 1-12

Published: SUM 2017

Document Type: Article

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), beta-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

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

KeyWords Plus: ANTIOXIDANT ACTIVITY; HARVESTING TIME; VITAMIN-C; BERRIES; LEAVES; CAROTENOIDS; ACID; HPLC; OIL

Author Information

Reprint Address: Mehrafarin, A (reprint author)

+ ACECR, Med Plants Res Ctr, Inst Med Plants, Karaj, Iran.

Addresses:

+ [1] Islamic Azad Univ, Dept Hort, Sci & Res Branch, Tehran, Iran

+ [2] ACECR, Med Plants Res Ctr, Inst Med Plants, Karaj, Iran

E-mail Addresses: A.Mehrafarin@gmail.com

Citation Network

In Web of Science Core Collection

0

Times Cited



Create Citation Alert

34

Cited References

[View Related Records](#)

Use in Web of Science

Web of Science Usage Count

1

Last 180 Days

1

Since 2013

[Learn more](#)

This record is from:

Web of Science Core Collection

- Emerging Sources Citation Index

Suggest a correction

If you would like to improve the quality of the data in this record, please [suggest a correction](#).

Publisher

IRANIAN SOC PHARMACOGNOSY, NO 8 SHAMS ALLEY, VALI-E-ASR ST, TEHRAN, 00000, IRAN

Categories / Classification

Research Areas: Pharmacology & Pharmacy

Web of Science Categories: Pharmacology & Pharmacy

[See more data fields](#)

◀ 1 of 1 ▶

Cited References: 34

Showing 30 of 34 [View All in Cited References page](#)

(from Web of Science Core Collection)

- 1. Carotenoids in Sea Buckthorn (*Hippophae rhamnoides* L.) Berries during Ripening and Use of Pheophytin a as a Maturity Marker** Times Cited: 44
By: Andersson, Staffan C.; Olsson, Marie E.; Johansson, Eva; et al.
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY Volume: 57 Issue: 1 Pages: 250-258 Published: JAN 14 2009
- 2. DETERMINATION OF OPTIMUM HARVESTING TIME FOR VITAMIN C, OIL AND MINERAL ELEMENTS IN BERRIES SEA BUCKTHORN (*HIPPOPHAE RHAMNOIDES*)** Times Cited: 4
By: Arif, Shazia; Ahmed, Syed Dilnawaz; Shah, Asad Hussain; et al.
PAKISTAN JOURNAL OF BOTANY Volume: 42 Issue: 5 Pages: 3561-3568 Published: OCT 2010
- 3. Simultaneous estimation of phenolic acids in sea buckthorn (*Hippophae rhamnoides*) using RP-HPLC with DAD** Times Cited: 41
By: Arimboor, Ranjith; Kumar, K. Sarin; Arumughan, C.
JOURNAL OF PHARMACEUTICAL AND BIOMEDICAL ANALYSIS Volume: 47 Issue: 1 Pages: 31-38 Published: MAY 12 2008
- 4. Flavonoid content and composition in leaves and berries of sea buckthorn (*Hippophae* spp.) of different origin** Times Cited: 3
By: Barl, B; Akhov, L; Dunlop, D; et al.
BERRY CROP BREEDING, PRODUCTION AND UTILIZATION FOR A NEW CENTURY Book Series: ACTA HORTICULTURAE Issue: 626 Pages: 397-405 Published: 2003
- 5. Total phenols, ascorbic acid, beta-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities** Times Cited: 220
By: Barros, Lillian; Ferreira, Maria-Joao; Queiros, Bruno; et al.
FOOD CHEMISTRY Volume: 103 Issue: 2 Pages: 413-419 Published: 2007
- 6. Sea buckthorn products: Manufacture and composition** Times Cited: 176
By: Beveridge, T; Li, TSC; Oomah, BD; et al.
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY Volume: 47 Issue: 9 Pages: 3480-3488 Published: SEP 1999
- 7. EXTRACTION, FATTY ACID PROFILE AND ANTIOXIDANT ACTIVITY OF SESAME EXTRACT (*Sesamum Indicum* L.)** Times Cited: 20
By: Carvalho, R. H. R.; Galvao, E. L.; Barros, J. A. C.; et al.
BRAZILIAN JOURNAL OF CHEMICAL ENGINEERING Volume: 29 Issue: 2 Pages: 409-420 Published: APR-JUN 2012
- 8. Effects of growing location on the contents of secondary metabolites in the leaves of four selected superior clones of *Eucommia ulmoides*** Times Cited: 24
By: Dong, Juane; Ma, Xihan; Wei, Qin; et al.

9. **COLORIMETRIC METHOD FOR DETERMINATION OF SUGARS AND RELATED SUBSTANCES** **Times Cited: 31,419**
By: DUBOIS, M; GILLES, KA; HAMILTON, JK; et al.
ANALYTICAL CHEMISTRY Volume: 28 Issue: 3 Pages: 350-356 Published: 1956
10. **Properties of some citrus seeds. Part 3. Evaluation as a new source of protein and oil** **Times Cited: 33**
By: El-Adawy, TA; Rahma, EH; El-Bedawy, AA; et al.
NAHRUNG-FOOD Volume: 43 Issue: 6 Pages: 385-391 Published: DEC 1999
11. **The genotypic effects on the chemical composition and antioxidant activity of sea buckthorn (*Hippophae rhamnoides L.*) berries grown in Turkey** **Times Cited: 50**
By: Ercisli, Semi; Orhan, Emine; Ozdemir, Ozlem; et al.
SCIENTIA HORTICULTURAE Volume: 115 Issue: 1 Pages: 27-33 Published: DEC 10 2007
12. **Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae rhamnoides L.*) during maturation** **Times Cited: 285**
By: Gao, XQ; Ohlander, M; Jeppsson, N; et al.
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY Volume: 48 Issue: 5 Pages: 1485-1490 Published: MAY 2000
13. **The conservation of genetic and chemical diversity in medicinal and aromatic plants** **Times Cited: 1**
By: Heywood, VH.
P 3 IUPAC INT C BIOD Published: 2001
14. **APPLICATIONS OF PRINCIPAL COMPONENT ANALYSIS TO HORTICULTURAL RESEARCH** **Times Cited: 137**
By: IEZZONI, AF; PRITTS, MP
HORTSCIENCE Volume: 26 Issue: 4 Pages: 334-338 Published: APR 1991
15. **Determination of vitamin losses and degradation kinetics during composting** **Times Cited: 9**
By: Ipek, U; Arslan, EI; Obek, E; et al.
PROCESS BIOCHEMISTRY Volume: 40 Issue: 2 Pages: 621-624 Published: FEB 2005
16. **The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals** **Times Cited: 2,299**
By: Jia, Z; Tang, MC; Wu, JM
FOOD CHEMISTRY Volume: 64 Issue: 4 Pages: 555-559 Published: MAR 1999
17. **The role of carotenoids in human health.** ([View record in MEDLINE](#)) **Times Cited: 164**
By: Johnson, Elizabeth J
Nutrition in clinical care : an official publication of Tufts University Volume: 5 Issue: 2 Pages: 56-65 Published: 2002 Mar-Apr
18. **Effects of different origins and harvesting time on vitamin C, tocopherols, and tocotrienols in sea buckthorn (*Hippophae rhamnoides*) berries** **Times Cited: 106**
By: Kallio, H; Yang, BR; Peippo, P
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY Volume: 50 Issue: 21 Pages: 6136-6142 Published: OCT 9 2002
19. **Variability and the genotypic effect on antioxidant activity, total phenolics, carotenoids and ascorbic acid content in seventeen natural population of Seabuckthorn (*Hippophae rhamnoides L.*) from trans-Himalaya** **Times Cited: 18**
By: Korekar, Girish; Dolkar, Phuntsog; Singh, Harvinder; et al.
LWT-FOOD SCIENCE AND TECHNOLOGY Volume: 55 Issue: 1 Pages: 157-162 Published: JAN 2014
20. **Characterization of carotenoids in juice of red navel orange (*Cara cara*)** **Times Cited: 92**
By: Lee, HS

21. Title: [not available] **Times Cited: 11**
 By: Lu, R.
 Sea buckthorn: A multipurpose plant species for fragile mountains Published: 1992
 Publisher: International Centre for Integrated Mountain Development (ICIMOD), Katmandu
22. **Quantification of tannins in tree foliage** **Times Cited: 121**
 By: Makkar, H P S.
 FAO/IAEA Working Document Published: 2000
 Publisher: A laboratory manual, Vienna, Austria.
23. **Radical scavenging activity and total phenolic content of medicinal plants used in primary health care** **Times Cited: 7**
 By: Savitree, M; Isara, P; Nittaya, SL; et al.
 J Pharmacol Sci Volume: 9 Pages: 32-35 Published: 2004
[\[Show additional data\]](#)
24. Title: [not available] **Times Cited: 1**
 By: Morsel, JT; Zubarev, Y; Eagle, D.
 Sea buckthorn. Research for a promising crop: A look at recent developments in cultivations, breeding, technology, health and environment Published: 2014
 Publisher: Books & Demand, Norderstedt
25. **Carotenoid composition of berries and leaves from six Romanian sea buckthorn (Hippophae rhamnoides L.) varieties** **Times Cited: 37**
 By: Pop, Raluca Maria; Weesepeel, Yannick; Socaciu, Carmen; et al.
 FOOD CHEMISTRY Volume: 147 Pages: 1-9 Published: MAR 15 2014
26. **Structural investigations of flavonol glycosides from sea buckthorn (Hippophae rhamnoides) pomace by NMR Spectroscopy and HPLC-ESI-MSn** **Times Cited: 59**
 By: Rosch, D; Krumbein, A; Mugge, C; et al.
 JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY Volume: 52 Issue: 13 Pages: 4039-4046 Published: JUN 30 2004
27. **THE GENUS HIPPOPHAE-D A TAXONOMIC STUDY** [\(View record in BIOSIS Citation Index\)](#) **Times Cited: 159**
 By: ROUSI A
 Annales Botanici Fennici Volume: 8 Issue: 3 Pages: 177-227 Published: 1971
28. **Morphological and biochemical variation in Sea buckthorn Hippophae rhamnoides ssp turkestanica, a multipurpose plant for fragile mountains of Pakistan** **Times Cited: 18**
 By: Sabir, SM; Ahmed, SD; Lodhi, N
 SOUTH AFRICAN JOURNAL OF BOTANY Volume: 69 Issue: 4 Pages: 587-592 Published: DEC 2003
29. **CONTENT OF PHENOLIC COMPOUNDS IN VARIOUS SEA BUCKTHORN PARTS** [\(View record in BIOSIS Citation Index\)](#) **Times Cited: 3**
 By: Sne, Elga; Seglina, Dalija; Galoburda, Ruta; et al.
 Proceedings of the Latvian Academy of Sciences Section B Natural Exact and Applied Sciences Volume: 67 Issue: 4-5 Pages: 411-415 Published: 2013
30. **Medicinal and therapeutic potential of Sea buckthorn (Hippophae rhamnoides L.)** **Times Cited: 105**
 By: Suryakumar, Geetha; Gupta, Asheesh
 JOURNAL OF ETHNOPHARMACOLOGY Volume: 138 Issue: 2 Pages: 268-278 Published: NOV 18 2011



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

A. Kuhkheil¹, H. Naghdi Badi², A. Mehrafarin^{2*}, V. Abdossi¹

¹Department of Horticulture, Science and Research Branch, Islamic Azad University, Tehran, Iran.

²Medicinal Plants Research Centre, Institute of Medicinal Plants, ACECR, Karaj, Iran.

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

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-polyrrolidone (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-polyrrolidone (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 NaNO₂ solution (10%). After 5 min, 0.3 mL AlCl₃ solution (10%) was added followed 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 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

Populations	Year	Fruit traits									
		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 ^c	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 ^c	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 ^c	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 ^c	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 ^c	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

Populations	Year	Fruit traits								
		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 ^{bcd}	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.

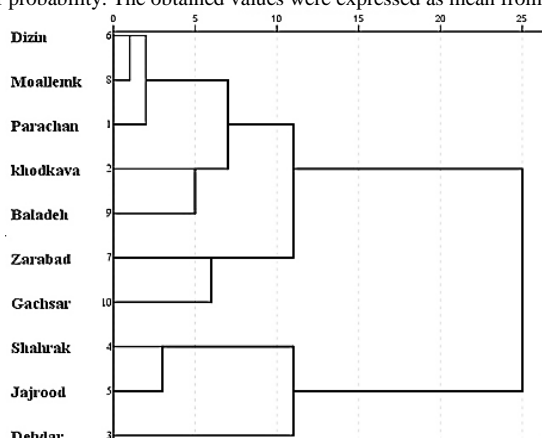


Figure 1. Ward's cluster analysis of *Hippophae rhamnoides* populations based on studied chemical constituents

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 ($r=-0.71$, $p\leq 0.05$). One hundred fruits dry weight exhibited positive correlation with fruit tannin ($r=0.74$, $p\leq 0.05$), carotenoids ($r=0.71$, $p\leq 0.05$), β -carotene ($r=0.73$, $p\leq 0.05$) and lycopene ($r=0.72$, $p\leq 0.05$) content. Fruit dry weight percentage had negative correlation with fruit vitamin C content ($r=-0.65$, $p\leq 0.05$). Also, fruit tannin had a negative correlation with fruit vitamin C ($r=-0.69$, $p\leq 0.05$). Fruit glucose had positive correlation with fruit total soluble solid ($r=0.98$, $p\leq 0.01$) and vitamin C ($r=0.93$, $p\leq 0.01$). It was evident that fruit β -carotene had negative correlation with linolenic acid of seed oil ($r=-0.63$, $p\leq 0.05$). Fruit TSS had positive correlation with fruit vitamin C ($r=0.96$, $p\leq 0.01$).

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	Carotenoid	Lycopene	β -carotene	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.

References

- [1] Rousi A. The genus *Hippophae* L. A taxonomic study. *Ann Bot Fennici*. 1971; 8(3): 177-227.
- [2] Rosch D, Krumbein A, Mugge C, Kroh LW. Structural investigations of flavonol glycosides from sea buckthorn (*Hippophae rhamnoides*) pomace by NMR spectroscopy and HPLC-ESI-MSn. *J Agric Food Chem*. 2004; 52(13): 4039-4046.
- [3] Yang B, Kallio H. Composition and physiological effects of sea buckthorn (*Hippophae*) lipids. *Trends Food Sci Technol*. 2002; 13(5): 160-167.
- [4] Arimboor R, Kumar SK, Arumughan C. Simultaneous estimation of phenolic acids in sea buckthorn (*Hippophae rhamnoides*) berries and leaves. *J Pharm Biomed Anal*. 2008; 47(1): 31-38.
- [5] Upadhyay NK, Kumar MSY, Gupta A. Antioxidant, cytoprotective and antibacterial effects of sea buckthorn (*Hippophae rhamnoides* L.) leaves. *Food Chem Toxicol*. 2010; 48(12): 3443-3448.
- [6] Morsel JT, Zubarev Y, Eagle D. *Sea buckthorn. Research for a promising crop: A look at recent developments in cultivations, breeding, technology, health and environment*. 3rd ed. Norderstedt: Books & Demand, 2014.
- [7] Kallio H, Yang B, Peippo P. Effects of different origins and harvesting time on vitamin C, tocopherols and tocotrienols in sea buckthorn (*Hippophae rhamnoides*) berries. *J Agric Food Chem*. 2002; 50(21): 6136-6142.
- [8] Pop R, Weesepeel Y, Socaciu C, Pintea A, Vincken JP. Carotenoid composition of berries and leaves from six Romanian sea buckthorn (*Hippophae rhamnoides*) varieties. *Food Chem*. 2014; 147(1): 1-9.
- [9] Andersson S, Olsson ME, Johansson E, Rumpunen K. Carotenoids in sea buckthorn (*Hippophae rhamnoides* L.) berries during ripening and use of pheophytin a as marker of maturity. *J Agric Food Chem*. 2009; 57(1): 250-258.
- [10] Suryakumar G, Gupta A. Medicinal and therapeutic potential of sea buckthorn (*Hippophae rhamnoides* L.). *J Ethnopharmacol*. 2011; 138(2): 268-278.
- [11] Heywood VH. The conservation of genetic and chemical diversity in medicinal and aromatic plants. Proceeding of the 3rd IUPAC international conference on biodiversity

- (ICOB-3). 2001 Nov 3-8; Antalya, Turkey.
- [12] Dong JE, Ma XH, Wei Q, Peng SB, Zhang SC. Effects of growing location on the contents of secondary metabolites in the leaves of four selected superior clones of *Eucommia ulmoides*. *Ind Crop Prod*. 2011; 34(3): 1607-1614.
- [13] Barl B, Akhoy L, Dunlop D, Jana S, Schroeder WR. Flavonoid content and composition in leaves and berries of sea buckthorn (*Hippophae rhamnoides*) of different origin. *Acta Hort*. 2003; 626: 397-405.
- [14] Beveridge T, Li TS, Oomah BD, Smith A. Sea buckthorn products: manufacture and composition. *J Agric Food Chem*. 1999; 47(9): 3480-3488.
- [15] Sne E, Seglina D, Galoburda R, Krasnova I. Content of phenolic compounds in various sea buckthorn parts. *Nat Exact Appl Sci*. 2013; 67(5): 411-415.
- [16] Savitree M, Isara P, Nittaya SL, Worapan S. Radical scavenging activity and total phenolic content of medicinal plants used in primary health care. *J Pharm Sci*. 2004; 9(1): 32-35.
- [17] Makkar HPS. *Quantification of tannins in tree foliage. A laboratory manual for the FAO/IAEA coordinated research project on use of nuclear and related technique to develop simple tannin assays for predicting and improving the safety and efficiency of feeding ruminants on tanniniferous tree foliage*. 1st ed. Vienna: FAO/IAEA Working Document, 2000.
- [18] Dubois M, Giles KA, Hamilton JK. Colorimetric method for determination of sugars and related substances. *Anal Chem*. 1956; 28(3): 350-356.
- [19] Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chem*. 1999; 64(4): 555-559.
- [20] Lee HS. Characterization of carotenoids in juice of red navel orange (Cara cara). *J Agric Food Chem*. 2001; 49(5): 2563-2568.
- [21] Barros L, Ferreira MJ, Queiros B, Ferreira ICFR, Baptista P. Total phenols, ascorbic acid, β -carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. *Food Chem*. 2007; 103(2): 413-419.
- [22] Ipek U, Arslan EI, Obek E, Karatas F, Erulas FA. Determination of vitamin losses and degradation kinetics during composting. *Proc Biochem*. 2005; 40(2): 621-624.
- [23] Carvalho RH, Galvao EL, Barros JA, Conceicao MM, Sousa EM. Extraction, fatty acid profile and antioxidant activity of sesame extract (*Sesamum indicum* L.). *Braz J Chem Eng*. 2012; 29(2): 409-420.
- [24] El-Adawy TA, Rahma EH, El-Bedawy AA, Gafar AF. Properties of some citrus seeds. Evaluation as a new source of protein and oil. *Mol Nutr Food Res*. 1999; 43(6): 385-391.
- [25] Korekar G, Dolkar P, Singh H, Srivastava RB, Stobdan T. Variability and the genotypic effect on antioxidant activity, total phenolics, carotenoids and ascorbic acid content in seventeen natural population of sea buckthorn (*Hippophae rhamnoides* L.) from Trans-Himalaya. *LWT Food Sci Technol*. 2014; 55(1): 157-162.
- [26] Ercisli S, Orhan E, Ozdemir O, Sengul M. The genotypic effects in the chemical composition and antioxidant activity of sea buckthorn (*Hippophae rhamnoides* L.) berries grown in Turkey. *Sci Hort*. 2007; 115(1): 27-33.
- [27] Zeb A. Chemical and nutritional constituents of sea buckthorn juice. *Pak J Nutr*. 2004; 3(2): 99-106.
- [28] Johnson EJ. The role of carotenoids in human health. *Nutr Clin Care*. 2002; 5(2): 56-65.
- [29] Zhang W, Yan J, Duo J, Ren B, Guo J. Preliminary study of biochemical constitutions of berry of sea buckthorn growing in Shanxi province and their changing trend. Proceedings of International Symposium on sea buckthorn (*H.*

- ramnoides* L.). 1989 Oct 19-23; Xian, China.
- [30] Gao X, Ohlander M, Jeppsson N, Bjork L, Trajkovski V. Changes in antioxidant effects and their relationship to phytonutrients in fruits of sea buckthorn (*Hippophae ramnoides* L.) during maturation. *J Agric Food Chem.* 2000; 48(5): 1485-1490.
- [31] Sabir SM, Ahmed SD, Lodhi N. Morphological and biochemical variation in Sea buckthorn *Hippophae ramnoides* ssp. *turkestanica*, a multipurpose plant for fragile mountains of Pakistan. *S Afr J Bot.* 2003; 69(4): 587-592.
- [32] Arif S, Ahmed SD, Shah AH, Hassan L, Awan SI, Hamid A, Batool F. Determination of optimum harvesting time for vitamin C, oil and mineral elements in berries sea buckthorn (*Hippophae ramnoides*). *Pak J Bot.* 2010; 42(5): 3561-3568.
- [33] Lu R. *Sea buckthorn: A multipurpose plant species for fragile mountains*. Katmandu: International Centre for Integrated Mountain Development (ICIMOD), 1992.
- [34] Iezzoni AF, Pritts MP. Applications of principal components analysis to horticultural research. *HortScience.* 1991; 26(4): 334-338.