Variations in phytochemical properties of seedy barberry (*Berberis integerrima* L.) grown in different habitats of Kerman

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Abstract

Seedy barberry (*Berberis integerrima* L.) has many applications in pharmaceutical, food, and health industries and grows naturally in Iran. To study the phytochemical properties of seedy barberry fruits and leaves in Kerman province, 5 habitats including Bam, Jiroft, Anbarabad, Rabar, and Baft were selected. The phytochemical properties of the fruit including pH, malic acid, soluble solids, anthocyanin, dry weight, antioxidant properties, and total phenol, as well as chlorophyll and carotenoid contents of the leaves were studied in a completely randomized design. Results showed that the sample from Anbarabad habitat had the highest pH (3.66), TSS (17.10%), fruit dry weight (3.66 g), total chlorophyll (0.99 µg g^{-1}), and carotenoids (1.82 µg g^{-1}), showing significant differences from the samples obtained from the other habitats. In addition, the antioxidant properties of Jiroft (0.42 mg g^{-1}) and Rabar (0.40 mg g^{-1}) samples were significantly 5%, respectively higher than those of the other habitats. The maximum and minimum levels of malic acid contents were recorded in the samples obtained from Jiroft and Bam, by 3.51% and 2.8%, respectively. Dendrogram analysis divided the biochemical properties of the seedy barberry populations into two main groups. Bam, Rabar, Baft, and Jiroft were grouped together and Anbarabad population made another group. The study concluded that the phytochemical properties of seedy barberry varies in different habitats.

Keywords: Anthocyanin, habitat, antioxidant properties, phytochemical properties, *Berberis integerrima* L. Geographic data

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Introduction

In recent years, there has been an increasing attention to functional foods, which have pharmaceutical properties in addition to nutritional values. Therefore, it is imperative to keep abreast of information on endemic plant species as a new food source. Iran enjoys a rich range of flora growing in various habitats around the country and one of these plants is the wild species of barberry (Chitgar et al., 2018).

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Side effects of chemical and industrial drugs have led to paying more attention to medicinal plant production and consumption of more natural products for health ((Van Wyk and Wink, 2018). The family *Berberidaceae* includes 15 genera and 650 species scattered around temperate climates of the northern hemisphere. The genus barberry includes 500 species, some of which such as *Berberis crataegina*, *Berberis khorasanica*, *Berberis orthobotrys*, *Berberis vulgaris* and *Berberis thunbergii* var. atropurpurea are found in Iran (Alemardan et al., 2013). *Berberis integerrima* L. has small fruits with nutritional and medicinal values. The bushes grow naturally in semidry and humid heights and their geographical distribution in Iran is mainly in north and northeast regions of the country as well as Azerbaijan and Kerman heights (Moqhaddam et al., 2018). *B. integerrima* L. fruits contain pigments, caffeic acid, chlorogenic acid, vitamins, anthocyanin, phenolic compounds, and flavonoids which are responsible for their pleasant taste and color. Various seedy barberry species are scattered in many mountainous regions of the country which have not been investigated properly.

In Persian traditional medicine, barberry fruit is considered as a painkiller and various organs of the plant such as roots, stems, leaves, flowers, and fruits are used as antibacterial, anti-fever, and anti-itching agents.

Acid chlorogenic and catechin compounds with antioxidant properties have been found in ethanolic extracts of barberry. The phenolic compounds and flavonoids contents in barberry genotypes depend on not only the species, but also the climatic conditions of various regions. investigated the phenol and flavonoid contents of various organs of barberry and reported that the maximum phenol content belonged to the fruits, flowers, and leaves in that order and the highest level of flavonoids was recorded in leaves. In another study, GHOLIZADEH et al., (2017) reported a direct relationship between antioxidant activities and total phenol and total flavonoid contents. Phytochemical analysis of the raw extract of *B. vulgaris* confirmed the presence of alkaloids, tannins, and phenolic compounds in this species (Rounsaville and Ranney, 2010). In another study on phytochemical properties of various organs of seedless barberry plants in Golestan province, including total flavonoids, total phenols, anthocyanin, and alkaloids, fruits and leaves were found to have the highest secondary metabolites (Mazandarani et al., 2013). Also, physical and chemical properties as well as effective components and antioxidant activities of 19 genotypes of *B. vulgaris* collected from northeast of Anatoly, in Turkey were examined in another study where significant differences were found among various genotypes in terms of phytochemical properties (Yildiz et al., 2014).

The difference in phytochemical properties of the genotypes collected from different regions has also been established for other medicinal plants. In their study on the antioxidant properties of various barberry fruits grown in different habitats, Khromykh et al. (2018) reported that the fruits of all barberry species can be considered as a source of antioxidant while the antioxidant capacity of fruits of different species varies.

Studies have reported the level of effective compounds under the influence of genotype and the collection site in a number of medicinal plants including *Laurus nobilis*, *Cinnamom verum* and *Achillea millefolium* (Marzouki et al., 2009); (Kumar et al., 2012); (Horwath et al., 2008); (Ebrahimi et al., 2012); (Chauhan et al., 2009). On the other hand, the phytochemical properties of seedy barberry have not been analyzed so far. The purpose of the present study was to analyze some phytochemical properties of seedy barberry fruits and leaves collected from Bam, Jiroft, Anbarabad, Rabar, and Baft habitats in Kerman, Iran.

The habitats under study

The study employed a common regional taxonomic research methodology. The habitats were selected based on Iranica flora and other reports on the distribution of seedy barberry in

Kerman province. The region was first divided into zones in terms of geographical and seasonal criteria. During several seasonal turns the plant samples were directly collected. To analyze the physical and chemical properties of the soils, soil samples were collected by a depth of 40 centimeters (Azarnivand et al., 2010)). Using an auger, 4 samples were collected and then dried in open air and at room temperature. Each sample was then crashed in a mortar and passed through a 2 mm sieve before the measuring the required parameters. Nitrogen, phosphorous, and absorbable potassium contents of the soils were assayed using Kjeldhal, spectrophotometry, atomic spectrophotometry (Gasparatos et al., 2011).

Plant samples

Ten healthy bushes of seedy barberry of approximately the same size were randomly selected from each habitat. When ripe, the fruits were selected and collected from each tree on early October before they were transferred to the laboratory. The leaves were collected on mid-September.

Extraction method

A mortar was used for extraction of seedy barberry fruit. The seeds were first removed from the pulp and the pulp was mashed into a relatively runny paste. The paste was put into a piece of cotton material and was put under direct pressure to obtain a clear juice. The juice from each sample was then passed through paper filter (MOQBELI et al., 2011).

Fruit pH and acidity (malic acid) assay

A pH-meter (Omega 744) was used to determine the pH level of the extracts. To improve the accuracy and reduce errors, pH measurements were repeated 4 times (MOQBELI et al., 2011).

To assay malic acid contents of the samples, first 10 ml of the clarified barberry extract was added to distilled water to obtain 100 ml solution. Then 2 drops of phenolphthalein were added to 10 ml of the diluted solution and titrated with 0.1 normal sodium hydroxide. Since phenolphthalein was used as an indicator, which needs to turn from no color to lilac at pH 7.3, and because its color was close to barberry, the change in color was not readily observable. Therefore, a pH-meter was used and its electrode was inserted into the solution while dropping and stirring 0.1 normal sodium hydroxide into the solution to achieve a pH of exactly 8.3. Eventually, the used sodium hydroxide was recorded and the percentage of acidity of malic acid was calculated using the following equation:

$$\text{Acidity \%} = \frac{miliequivalent\ acid \times S.H.\ normality \times used\ S.H.\ (ml)}{weight\ of\ the\ sample}$$

Where S. H. denotes sodium hydroxide and 1 ml 0.1 normal sodium hydroxide equals to 0.0067 g malic acid ((MOQBELI et al., 2011).

Soluble sugar (TSS) assay

A manual refractometer (Erma, Japan) was used for TSS assay. Two mm of the fruit juice was filtered and placed on the refractometer measurement board and the data were read and recorded MOQBELI et al., 2011

Fruit anthocyanin assay

The anthocyanin contents of the barberry fruits were assayed using a spectrophotometer at the visible zone of 510 nm in each repetition (Lo Piero et al., 2005).

Dry fruit weight measurement

Material and Methods

Fifteen (15) g barberry fruit from each sample was kept in an incubator set at 72 ℃ for 48 hours. When dried, the fruit samples were weighed using a digital scale (0.001 error of measurement) (MOQBELI et al., 2011).

Fruit antioxidant properties assay

The antioxidant activities of the barberry extracts were determined based on their capacity to neutralize DPPH free radical. Accordingly, 0.2 g of each fruit sample was ground in a porcelain mortar using liquid nitrogen adding 10 ml methanol. After stirring for a while, the solutions were put into small beakers and kept under room temperature for 1 h for better extraction. The extracts were filtered through paper filter and then centrifuged (3000 rpm) for 5 minutes. Then 50 µl methanolic extract was added to 950 µl DPPH and stirred immediately and kept in room temperature under dark condition for 15 to obtain a homogenous solution. Reduced absorption was determined at 515 nm wavelength. The experiment was repeated three times for each treatment. Afterwards, the antioxidant capacity of the sample extracts was determined using the following equation as the percentage of inhibition of DPPH (Dhifi et al., 2011):

$$\%DPPH = (A_{cont} - A_{samp}) \times 100/A_{cont}$$

Where %DPPH, A_{samp}, and A_{cont} are inhibition percent, absorption level sample + DPPH, and absorption level of DPPH, respectively.

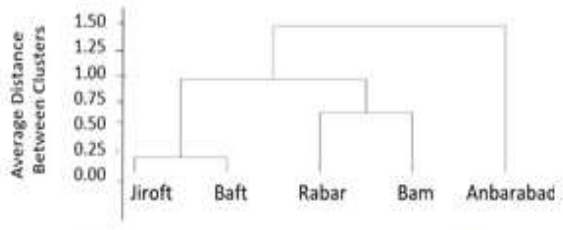

Fig.1. Cluster analysis of the populations of *Berberis integerrima* L. based on biochemical properties

Phenol assay

Total phenols of the fruits were assayed based on the method described by MOQBELI et al., 2011. For this purpose, 1 g sample was mashed in a porcelain mortar using liquid nitrogen. Then, 10 cc pure methanol was added to extract phenolic compounds and the solution was passed through filter paper. In order to obtain the standard solution, gallic acid solution, (0.1 g gallic acid in 100 ml pure methanol), Folin (5 ml Folin in 50 ml distilled water), and sodium carbonate 7.5% (1.5 g sodium carbonate in 20 cc distilled water) were mixed.

Leaf chlorophyll and carotenoid contents assay

Leaf tissue from each repetition (0.4 g) was ground in a mortar and 5 ml acetone 80% (20 distilled water: 80 acetone) was added. The mixture was centrifuged at 5000 rpm. Eventually, the transparent acetone extract was separated to obtain 5 ml solution with pure acetone. Leaf chlorophyll and carotenoid contents were measured using spectrophotometry at 646.2 nm and 470 nm, respectively. The following equations were used to determine chlorophyll a, b, and total and also carotenoids.

$$Chla\ (mg/ml) = 12.25\ A_{663.2} - 2.79\ A_{646.8}$$

$$Chlb\ (mg/ml) = 21.50A_{646.8} - 5.10\ A_{663.2}$$

$$TChl\ (mg/ml) = (8.2 * A_{663}) + (20.2 * A_{645})$$

$$C_{x+c} = (1000A_{470} - 1.8\ C_a - 85.02\ C_b)/198$$

Where Chla, Chlb, Chlt, and C_{x+c} is chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid. Chlorophyll and carotenoid concentrations were reported in mg/g fresh weight and mg/100 g fresh tissue (Sumanta et al., 2014)

Statistical Analysis

The experiment was carried out based on complete random design and the means were compared using Tukey test.

Table 1
Geographic and climatic conditions of the habitats and the soils properties

Habitat	Longitude	Latitude	Altitude (m)	Mean Annual Temperature	Mean Humidity (%)	Total Precipitation (mm)	Absorbable N (%)	Available P (ppm)	Available K (ppm)
Jiroft	28º 40′ 13″E	57º 44′ N13″	720	21.1	43	176.2	0.09	15.4	100.3
Anbarabad	28º 15′ 02″ E	N 57º 08′ 11″	601	21.2	41	175.3	0.15	16.3	130.1
Rabar	E 56º 45′ 19″	N 29º 27′ 25″	2342	15.6	39	247.1	0.04	9.12	110.8
Baft	E 56º 37′ 14″	N 29º 14′ 10″	2280	15.3	39	247.2	0.06	10.2	80.4
Bam	E 58º 21′ 32″	N 29º 06′ 26	1061	23	30	61.4	0.02	13.6	70.3

Table 2
ANOVA of phytochemical properties of seedy barberry (*Berberis Integerrima* L.) in various habitats of Kerman province

		Mean Squares								
Sources of Variance	df	pH	Acidity (Malic acid%)	TSS (%)	Anthocyanin (mg g^{-1})	Dry Weight of 100 fruits	Antioxidant Properties (%)	Total Phenol (mg g^{-1})	Chlorophyll (µg g^{-1})	Carotenoid (µg g^{-1})
Habitats	4	0.79	0.57*	38.40*	6.69*	0.89*	0.05*	0.40*	7.800 E-90*	0.40*
Error		0.04	0.06	0.04	0.14	0.18	0.0004	0.001	2.807E-0.5	0.0001
Coefficient of Variabilities (CV)		7.02	8.00	1.57	9.57	13.53	6.10	1.27	0.54	0.64

* shows significant at p≤0.05.

Results

Table 1 shows the features of Bam, Jiroft, Anbarabad, Rabar, and Baft habitats used for analysis of the phytochemical properties of seedy barberry.

Based on the analysis of the dendrogram, biochemical properties of the populations were classified into two groups (Fig.I). Populations of Bam, Rabar, Baft, and Jiroft were grouped together and Anbarabad population was kept in another group. Also, in this categorization, Baft and Jiroft populations were put in a subgroup and Bam and Rabar populations were put in another subgroup. Classifying populations in one group shows more homogeneity in the overall population of that group compared with the other populations. On the other hand, classifying populations into two separate groups means the genetic diversity of the populations and environmental conditions of the sampling sites.

Analysis variance of the phytochemical properties of seedy barberry in Kerman habitats is shown Table 2. As the table suggests, phytochemical properties including dry fruit weight, chlorophyll, phenol, anthocyanin, TSS, acidity (malic acid), and carotenoid showed a significant difference.

Comparisons of mean phytochemical properties of seedy barberry in various habitats are shown Table 3. Based on this table, the pH in Anbarabad habitat (3.66%) was significantly higher than that in the other habitats under study while there was no significant difference in the pH

of the other habitats. The highest acidity level was related to Jiroft (3.52%), showing no significant difference from that in Rabar (3.23%) and Baft (3.24%). On the other hand, the minimum malic acid content was recorded for Bam habitat (2.86%), which was significantly lower than that in the other habitats except Anbarabad (3.09%). TSS in Anbarabad (10.17%) was significantly higher than that in the other habitats followed by Bam, which also showed a significant difference from the TSS contents obtained in Baft (12.15%), Rabar (14.16%), and Jiroft (12.19%). Also, the highest anthocyanin level (4.84 mg g^{-1}) belonged to Jiroft habitat, showing a significant difference from that in Anbarabad, Rabar, and Bam while it was not significantly different from that in Baft habitat (4.64 mg g^{-1}). Anbarabad sample had the minimum anthocyanin content (2.74 mg g^{-1}) which was significantly lower than that in the habitats.

The maximum total chlorophyll content was recorded in Anbarabad (0.99 mg g^{-1}), which showed a significant difference from the other habitats. On the hand, the minimum total chlorophyll was recorded in Baft habitat (0.96 µg g^{-1}), which was significantly different from that in Jiroft, Rabar, and Bam habitats. Moreover, Carotenoid content of the samples obtained from Anbarabad (1.82 µg g^{-1}) was significantly different from, and higher than, those in Bam (1.78 µg g^{-1}), Rabar (1.75 µg g^{-1}), Baft (1.67 µg g^{-1}), and Jiroft (1.64 µg g^{-1}). Significant differences were observed between the carotenoid contents of the plant samples obtained from all habitats. Dry weight of 100 fruits in Anbarabad (3.66 g) was significantly higher in comparison with that in the other habitats except Bam (3.39 g) while no significant differences were observed for this trait among the other habitats. In addition, the total phenol contents of Jiroft (2.93 mg g^{-1}) and Rabar (2.91 mg g^{-1}) samples were significantly different from that in the other habitats. Also, the total phenol content of Baft sample (2.74 mg g^{-1}) was significantly different from that in Anbarabad (2.43 mg g^{-1}) and Bam (2.59 mg g^{-1}) habitats. On the other hand, the minimum phenol content was recorded in Anbarabad (2.43 mg g^{-1}), which was significantly different from the other habitats. Antioxidant properties of Jiroft sample (0.42 mg g^{-1}) was significantly higher than the other habitats while Anbarabad sample showed the minimum antioxidant properties (0.23 mg g^{-1}), which was also significantly different from the other habitats. Maximum and minimum malic acid contents belonged to Jiroft and Bam samples (3.51%) and (2.86%), respectively.

Discussion

Findings of the present study revealed that the seedy barberry enjoys a high phytochemical diversity in Kerman. Investigation of the phytochemical properties of seedy barberry plants showed that Anbarabad was superior to other

Table 3
Comparison of mean phytochemical properties of seedy barberry (*Berberis integerrima* L.) in various habitats of Kerman province

Habitat	pH	Acidity (Malic acid%)	TSS (%)	Anthocyanin (mg g^{-1})	Dry Weight of 100 fruits (g)	Antioxidant Properties (%)	Total Phenol (mg g^{-1})	Chlorophyll (µg g^{-1})	Carotenoid (µg g^{-1})
Jiroft	3.17b	3.51a	12.19d	4.84a	2.91b	0.42a	2.93a	0.97bc	1.64e
Anbarabad	3.66a	3.09bc	17.10a	2.74d	3.66a	0.23d	2.43d	0.99a	1.82a
Rabar	2.94b	3.32ab	14.16c	4.26b	2.97b	0.40a	2.91a	0.97bc	1.75c
Baft	2.92b	3.34ab	12.15d	4.64ab	3.07b	0.36b	2.74b	0.96c	1.67d
Bam	3.17b	2.86c	14.88b	3.54c	3.39ab	0.31c	2.59c	0.97b	1.78b

Similar letters in each column show no significant differences based on Tukey test ($p \leq 0.05$)

habitats in most of the phytochemical properties. In general, the seedy barberry plants in this habitat showed very favorable performance in terms of dry weight of fruits and chemical properties. This confirms the better environmental condition of the region in comparison with the other habitats under study.

Anbarabad samples contained the minimum levels of total phenols and antioxidant properties. Modi (2014) found a direct relationship between antioxidant activity and phenolic contents. Also a significant difference was found between anthocyanin contents of the samples from 5 regions, with the maximum and minimum concentrations recorded for Jiroft and Anbarabad samples, 4.84 mg g^{-1} and 2.74 mg g^{-1}, respectively. Khromykh et al., 2018 reported that all barberry species can be an available source of antioxidant while reduced antioxidant capacity in various species of barberry can be attributed to the differences in habitats and the effects of various environmental factors such as height, soil, light, etc. Sameh (2017) and Farhadi Chitgar et al. (2017)reported similar findings regarding phytochemical properties of barberries sampled from different habitats.

Studies of Motalleb et al. (2005)on various barberry species revealed that there exists a direct relationship between antioxidant activities and flavonoid and phenol contents. In the present study, the changes in anthocyanin and phenol contents followed a similar trend so that the highest levels of these compounds were recorded in Jiroft. Grassmann (2005) reported that in addition to phenolic compounds, other factors such as climate can also affect antioxidant activities, which is also in line with our findings. It seems that one of the reasons for higher antioxidant activity in Jiroft habitat is the high absorbable elements in soil which is in line with the study on the effects of soil elements on antioxidant activities reported by ALIBAKHSHI, et al. (2014).

In this study, Anbaranad stood at the lowest height from see level (601 m). TSS and pH in this habitat were significantly higher while anthocyanin contents were significantly lower compared with the other habitats. Height in this study affected plant phenotype, compounds, and photochemical properties directly and this is consistent with the findings of the study reported by Larti et al. (2013). Who also reported on the effects of habitat features on the plant phytochemical properties. Khayyat et al. (2018)and Chen et al. (2017)reported that in addition to genotypes, phytochemical properties are influenced by environmental factors including precipitation and mean temperature. Since the habitats under study differed in terms of precipitation and mean temperature, differences in phytochemical properties of the plants collected from these habitats might be attributed to effect of habitat.

Chlorophyll contents were in direct relationship with the dry weight of fruits and as chlorophyll levels increased, so did the photosynthesis and fruit weight. Generally, chlorophylls reduce under the influence of drought stress due to reduced synthesis or destruction. The differences in annual precipitation and heights of the habitats under study led to the observed differences in the chlorophyll contents and eventually, dry weight of fruits collected from different habitats and this confirms the findings of the study reported by Salehi et al.(2015).

The phytochemical properties of the plants collected from different habitats were different in the study. Confirming the findings of the present study, Ahmed et al. (2013)found stated that the habitat exerts heavy influences on quantitative parameters of the medicinal plant *Berberis aristata* DC. Nitrogen is a main element in plant nutrition (Salvagiotti et al., 2009). It is likely that higher nitrogen contents of the soil in

Anbarabad resulted in an increased level of photosynthetic pigments and eventually led to a significant increase in the chlorophyll content and dry weight of fruits in this habitat.

Total phenol contents of the samples obtained from different habitats in this study were different. Various levels of total phenols have been reported in different populations of basils which can be considered as a modification parameter (Kwee and Niemeyer, 2011)). In this study, plants collected from Jiroft and Rabar habitats had higher total phenol and antioxidant properties in comparison with the other habitats. Not only genotype, but also the place of growth is the main determining factor in growth, exploitation, and the nutrient components of barberry fruits. Kazemizadeh et al. (2008)in their analysis of the essential oil composition of two populations of *Teucrium hyrcanicum* found that the qualitative and quantitative difference in the essential oils can be due to the ecological properties of the habitats such as temperature, moisture, or other soil or geographical factors. Heydari and Salehi (2017)observed that one of the most important environmental factors that can exert heavy influences on the quality and quantity of the effective compounds of medicinal plants is the ambient temperature, height of the region, and chemical and physical properties of the soil. Hot and sunny climate can result in the production of more flower and through improved vegetative performance directly result in the production of more essential oils.

The study concludes that in nature, phytochemical properties are affected relatively highly by environmental factors such as climate, geographical features, fruit maturity, nutrition, precipitation, mean temperature, and other biotic and abiotic parameters.

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