International Journal of Horticultural Science and Technology
Vol. 7, No. 4; December 2020, pp 387-400
Print ISSN: 2322-1461 Online ISSN: 2588-3143
DOI: 10.22059/ijhst.2020.287117.314
Web Page: https:// ijhst.ut.ac.ir, Email: ijhst@ut.ac.ir

Investigation of Phytochemical Variability, Antioxidant Activity and Ecological Conditions of Native Iranian *Glycyrrhiza glabra* L.

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(Received: 10 August 2019, Accepted: 1 June 2020)

Abstract

Licorice (*Glycyrrhiza glabra* L.) as a strategic and highly valuable medicinal plant in Iran with numerous beneficial pharmaceutical properties contributes substantially to Iranian herbs exports. In the present study, a variation on the phytochemical and antioxidant activity of 25 populations of valuable and profitable medicinal plant of *G. glabra* was investigated. The climate variables and soil properties were evaluated in various habitates of *G. glabra.* Total phenol (Folin–Ciocalteu method), total flavonoid (Aluminum Chloride method), anthocyanin (pH difference method) and antioxidant activity (DPPH method) were studied. Glycyrrhizic acid, glabridin, liquiritigenin and liquiritin content of root were evaluated by liquid chromatography. The content of major components in *G. glabra* varied in different regions. Outstanding quantitative variability of glycyrrhizic acid, glabridin, liquiritin and liquiritigenin content were observed in roots of licorice from different regions of Iran. Glycyrrhizic acid content was varied from 93.89 (mg/g dry weight) for the Sepidan population to 20.68 (mg/g dry weight) for the Ahar population. The maximum and minimum amounts of glabridin were recorded in Kashmar and Bajgah populations, respectively. The highest levels of liquritin and liquiritigenin were in the population of Kazerun and Yasuj, respectively, and the lowest was observed in Kashmar. Also, the highest antioxidant activity (the lowest IC_{50}) for licorice root was associated with Semirom population. In conclusion, The populations with high amount of each active ingredient in licorice root and those with considerable antioxidant activity can be exploited depending on the purpose of breeding and cultivation.

Keyword: *Glycyrrhiza glabra*, environmental and soil analysis, phytochemical variation, antioxidant activity.

Introduction

Licorice (*Glycyrrhiza glabra* L.) is one of the most valuable medicinal plants that belong to Fabaceae family. *Glycyrrhiza* has its origins from two Greek words "glycos" which means sweet and "rhiza" which means root (Olukoga and Donaldson, 1998). Unpeeled dried roots and stolen is considered as the part of the plant which is economically

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valuable. This medicinal herb is among the most widely used herbs since a long time ago and former ages in different traditional systems of medicines worldwide (Asif et al., 2015). This plant is grown considerably in China, Germany, United Kingdom, France, Russia, India, Italy and USA (Parvaiz et al., 2014). *G. glabra* encompasses valuable and commercial constituents including glycyrrhizic acid, glabridin and other flavonoids in roots and leaves which has attracted the attention for use in food and pharmaceutical industries. Its active ingredients are used in beverage, confectionery and tobacco (to expose a sweet aromatic perfume) industries (Fenwick et al., 1990). Glycyrrhizic acid is known with excellent pharmacological effects such as stimulant, depletive, anti-inflammatory, anti-gastric ulcer, anti-hepatotoxic, and antivirus activities (Dehpour et al., 1995; Fujisawa et al., 2000; Cinatl et al., 2003; Fu et al., 2005). A species-specific flavonoid known as "Glabridin", contains numerous pharmaceutical properties such as antioxidant (Haraguchi et al., 2000), anti proliferative properties against human breast cancer cells (Tamir et al., 2000), anti-inflammation of the kidneys (Fukai et al., 2003), energy metabolism adjustment and many other activities (Simmler et al., 2013). Liquiritigenin and its aglycone liquiritin are flavonoids in licorice root with large consumption as herbal medicine (Kuang et al., 2018). It is proven that Liquiritigenin retains liver safe from harm triggered by some synthetic medicines (Kim et al., 2006; Park et al., 2015; Zhang et al., 2015) and has memory-enhancing effects (Ko et al., 2018).

Among endangered medicinal plant species in Iran, *G. glabra* is considered as one of the most important plants, which are only protected in few habitats and consequently is threatened by overexploitation and overharvesting. As a well-trusted medicinal herb, Licorice and its extract in liquid, solid and powdered form is exported from Iran to countries including Japan, Germany, Italy, India, France, Belgium Australia and also countries in the south of Persian Gulf every year (Khanahmadi et al., 2013).

Due to excessive harvesting, this plant is in danger of extinction. On the other hand, it is an economically important and profit making plant; therefore cultivation and domestication of the plant seem to be highly necessary. In line with the purpose of this study, firstly we investigate the natural habitat to exploring desirable areas for growing of licorice and evaluate the individuals and populations in phytochemical aspects. Introduction of species and also the development of germplasm conservation programs can be managed according to the areas predicted by habitat distribution modeling (Deka et al., 2017). It is very important to provide and develop cultivation conditions of *G. glabra* as a good alternative for the collection of wild resources. Therefore, the aim of this study was to investigate the environmental conditions and phytochemical variation of different Licorice plants and it was also tried to recommend the best populations for breeding and domestication.

Materials and Methods

Plant materials

In the fall season, roots of Licorice (*Glycyrrhiza glabra* L.) were collected from 25 distinct regional growing populations, from different parts of Iran with a diameter of 1.5 to 2 cm. Three replications from roots of each population were collected. Then, they were sent to the respective laboratory (Department of Agricultural and Medicinal Plants and Drug Research Institute) at Shahid Beheshti University. Once the samples were washed and divided into small pieces, they were dried in an oven with a temperature of 50 °C (Omidbaigi, 2006). The dried material was powdered in a grinder (TS-9500, Toos Shekan Khorasan Co.) for 10 min and passed through a 0.7 mm mesh screen and stored at 4 °C until extraction.

Ecological data

The samples collected from different localities (Table 1) and their climate data including precipitation, average temperatures, number of frost days annually and relative humidity were taken from the website of www.en.climate-data.org.

Soil data

Near the roots down at 20- 30 cm depth, the soil samples from each population were examined and we managed to determine soil characters applying the methods as follows. Through using the Bouyoucos Hydrometer Method the soil texture was determined (Gee and Bauder, 1979). In order to determine the amount of organic carbon (OC) content a modified Walkley and Black method (Allison et al., 1965) were used. The pH (acidity rate) and the EC (electrical conductivity) were calculated using a portable CPD-65N multi-meter (ISTEK, South Korea). In 100 g of dry soil, the amount of $CaCO_3$ as the total carbonates was determined using Calcimeter Bernard method. In order to determine the Phosphorus (p) content, Olsen method was used (Nelson and Sommers 1982). Through converting the various Nitrogen (N) forms into NH_4^+ the amount of N was determined using Kjeldahl method (Bremner and Mulvaney, 1982). By using IC (Ion chromatography) method, Chlorine in the soil was measured (Khym, 1974).

Sample preparation for analysis

For extraction, 500 mg of well- ground powder from the dried root of one sample by using 10 ml solvent (methanol: water 80:20) was added and ultrasonic bath for half an hour and Immediately was centrifuged. Extracts were filtered and stored at 4 °C till phytochemical and biological analysis. (Ahmadi-Hosseini et al., 2014).

Determination of total bioactive components

The total phenol content of the root extract was evaluated by Folin-Cictalo method. First, to 0.5 mL of each of the standards (20, 30, 60, 70, 100, 120 and 130 mg/mL) and methanol extract, 9 mL Folinic acid (10: 1) and 3 mL sodium carbonate 7.9% were added. After 19 min, absorbance at 769 nm was measured by a spectrophotometer (UV-vis 2800) and the standard curve was plotted in gallic acid at various concentrations and the amount of phenolic compounds equivalent to gallic acid was measured in mg per gram of dry powder (Pourmorad et al., 2006).

The use of aluminum chloride was used to measure the amount of flavonoids. Quercetin was used as the standard for plotting the calibration curve. Flavonoids were reported in terms of the equivalent of mg quercetin per gram of extract (Peluso, 2006).

DPPH assay

The free radical-scavenging ability of extracts were estimated by the potency of decolorizing purple colored methanolic solution of DPPH by Blois (1958) procedure. The antioxidant level of the extracts was calculated at 517 nm by using the following equation: Inhibition % = (Ac-As) /Ac*100 where ‘Ac’ and ‘As’ shows control absorbance and sample absorbance respectively. 50% inhibition concentration (IC_{50}) of extracts was evaluated with the curve drawn inhibition percentage versus extract concentration by linear regression analysis, using Origin software, version 9.0.

Quantitative analysis by HPLC

Glycyrrhizic acid, glabridin, liquiritigenin and liquiritin content of root were evaluated by the Waters Alliance 2695 Separations Module (USA) a high performance liquid chromatography device consisting of a, an Autosampler equipped with a 100 µL loop and Photodiode Array Detectors (PDA) using C18 column (Knauer, 25 cm × 4.6 mm Eurospher 100-5) with water containing 0.3% H3PO4 (as solvent A) and acetonitrile (as solvent B) in

the course of mobile phase. The flow rate was measured to be 1 mL/min with a linear solvent gradient of A–B as follows: 80% A for 10 min; reduce to 20% in 30 min and retained for 5 min. The samples monitored at wavelength of 276 nm for liquiritigenin and liquiritin, 230 nm for glabridin and 250 nm for glycyrrhizic acid.

Glycyrrhizic acid ammonium salt and glabridin from obtained from Sigma-Aldrich and liquritin and liquiritigenin was bought from Phytopurify, China. The stock solution (1000 ppm) prepared in MeOH. The samples were filtered with 0.45 µm diameter, and six concentration of standard preparation of Glycyrrhizic acid, Glabridin, Liquritin and liquiritigenin (7.8, 15.6, 31.2, 62.5, 125 and 250 ppm) were injected and the peak responses were recorded as directed under procedure (Table 1). Accurately weighed and transferred 5 mg dry extract to microtube 2 mL. 1.5 mL of DMSO was added, sonicated to dissolve for 20 minutes and centrifuged. The samples were filtered with 0.45 µm diameter, three sample of *Glycyrrhizic acid* were prepared and injected to the HPLC apparatus.

Table 1. Information of standard calibration curves for Glycyrhizic acid, Glabridin, Liquritin and Liquritigenin

Compound	Rang (ppm)	Equations	r
Glycyrhizic acid	10-250	y=10325x-10507	0.9997
Glabridin	10-250	y=101184x+66427	0.9993
Liquritin	10-250	y=60499x-98246	0.9995
Liquritigenin	10-250	y=38577x-365471	0.9831

Statistical analysis

The experiments were performed using a completely randomized design. Analysis of variance (ANOVA) was carried out with SPSS software version 18 (SPSS Inc., Chicago, IL, USA). Duncan's test was measured by utilizing differences between mean. As a result, the differences appeared to be significant at $P \leq 0.01$. Pearson correlation coefficient was applied to determine the relationships among the studied traits. Phytochemical variability among populations as represented through the coefficient of variation (CV%). Relationships among populations such as principal component analysis (PCA) and Cluster analysis according to UPGMA were investigated using Past statistics software.

Results

HPLC analysis

One-way ANOVA indicated that differences in contents of compounds regarding to the glycyrrhizic acid ($F = 223$, $p<0.01$), Glabridin ($F=388.87$, $p<0.01$), liquritin ($F=104.78$, $p<0.01$) and liquiritigenin ($F=62.35$, $p<0.01$) were significant. The highest amount of variation (C.V %) was observed for Glabridin and the lowest variation was observed in liquiritigenin trait.

Glycyrrhizic acid content was varied from 93.89 (mg/g dry weight) for the Sepidan population to 20.68 (mg/g dry weight) for the Ahar population (Table 1).

The maximum and minimum amounts of glabridin were recorded in Kashmar (22.87 mg/g dry weight) and Bajgah (Trace) populations, respectively (Table 1, Fig 1).

The highest level of liquritin was observed in population of Kazerun (6.09 mg/g dry weight). In contrast the population of Kashmar contained the lowest amount of this compound (liquritin=0.65 mg/g dry weight). The highest levels of liquiritigenin were in the population of Yasuj (8.57 mg/g dry weight), the lowest content was observed in the population of Kashmar (2.88 mg/g dry weight) (Table 1, Fig 1).

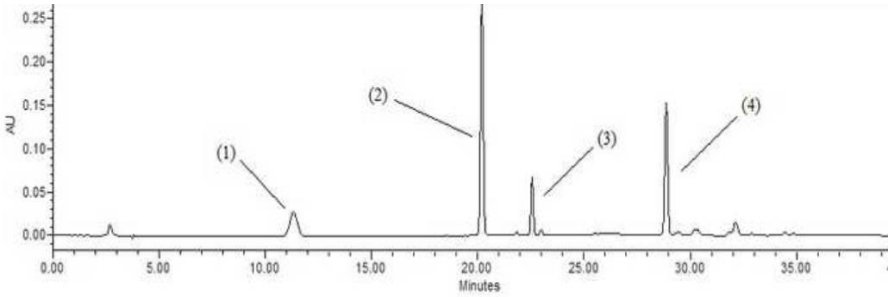

Fig. 1. The HPLC chromatogram of liquiritigenin (1), liquiritin (2), glycyrrhizic acid (3) and glabridin (4)

Environmental data and soil analysis

Results of soil analysis showed that the highest EC amount was recorded in Bojnord populations (5.44 ds/cm, Table 3) and the lowest one was recorded in Shahrebabak population (0.21 ds/cm). Ilam population demonstrated the highest pH (pH = 8.28) and Mahabad population showed the lowest amount (pH = 7.2). Localities had soil with sand, loam-sand, loam-silt, loam-clay, clay-sand and clay texture. Bojnord had the highest amounts of OC (2.47 %) and N_2 (0.25). The lowest amounts of OC (0.027%) and N (0.02 %) was observed in Nagadeh and Sirjan, respectively. The highest amount of P (19.99 ppm) was in the population of Saqqez and the highest amount of K (1015 ppm) in the population of Quchan, the smallest amount of P (5.26 ppm) and K (31 ppm) was observed in Taft population (Table 3).

The maximum average temperature was observed in Darab (24.35 °C) and the lowest one observed in Meshkinshahr (11.68 °C). Saqqez had the maximum annual precipitation (865 mm) and the lowest one observed in Bardsir (31 mm) (Table 2 and 3).

Table 2. Geographical characteristics of different *Glycyrrhiza glabra* populations

No.	Population	Province	Longitude(E)	Latitude(N)	Altitude (m)	Average Annual Precipitation (mm)	Average Annual Temperature (°C)	Number of ice days annually	Relative humidity (%)
1	Saqqez	Kordestan	51˚ 56' 67"	30˚ 71' 67"	1870	865	15.69	123	54.85
2	Quchan	Razavi Khorasan	58°24' 33.1"	37˚01' 57.5"	1678	308.4	12.7	97	57
3	Ahar	East Azerbaijan	47°03' 10.26"	38˚26' 10.72"	1403	285.2	11.99	91	59
4	Bojnord	North Khorasan	57°36' 54.38"	37˚27' 13.91"	970	267.8	13.3	83	58
5	Sepidan	Fars	52°00' 41.5"	30˚13' 21.5"	2157	678.3	14.8	58	40
6	Darab	Fars	54°25' 37.64"	28˚43' 3.95"	1081	257.4	22.1	30	33
7	Meshkinshahr	Ardabil	47˚ 39' 56"	38˚ 23' 39"	1400	333	11.68	98	63
8	Kashmar	Razavi Khorasan	58°27' 51.07"	35˚23' 59.70"	1632	197.2	17.8	37	40
9	Semirom	Isfahan	51°33' 20.32"	31˚22' 53.29"	2323	487.7	14.2	143	32.5
10	Mahabad	West Azarbaijan	45˚ 44' 51"	36˚ 54' 28"	1320	390	14.15	93	57.16
11	Piranshahr	West Azarbaijan	45˚ 11' 19"	36˚ 37' 15"	1502	453	13.81	100	57.83
12	Ilam	Ilam	46°17' 43.72"	33˚40' 49.64	1427	583.7	16.9	33	40.85
13	Sirjan	Kerman	55°43' 26.61"	29˚20' 41.76"	1708	133.3	17.4	64	38
14	Rabat	West Azarbaijan	45˚ 55' 13"	36˚ 20' 91"	1480	430	15.3	78	54.5
15	Yasuj	Kohgiluyeh and	51°36' 33.58"	30˚32' 11.95"	2002	823.3	15.2	79	41.45
16	Bardsir	Boyer-Ahmad	56°15' 21.94"	29˚52' 40.41"	2338	171	14.6	96	45
17	Shahrekord	Chaharmahal and Bakhtiari	50˚ 86' 49"	23˚ 21' 50"	2060	316	12.28	133	44.55
18	Marvast	Yazd	54°13' 51.9"	30˚26' 59.8"	1542	64.6	17.8	1	37
19	Nagadeh	West Azarbaijan	45˚ 15' 13"	36˚ 54' 40"	1299	380	14.85	81	62
20	Shahrebabak	Kerman	55˚ 36' 67"	30˚ 21' 67"	1845	163	16.07	90	34
21	Taft	Yazd	53°50' 59.3"	31˚39' 44.1"	2286	59.2	19.2	0	29
22	Bajgah	Fars	52°35' 17.98"	29˚43' 26.14"	1798	325.3	16.4	0	38
23	Hajiabad	Hormozgan	55°44' 38.4"	28˚17' 12.6"	897	179	23.3	10	32
24	Kazerun	Fars	51°50' 26.5"	29˚33' 59.4"	1258	291	22.9	0	39
25	Baft	Kerman	56˚ 60' 06"	29˚ 23' 30"	2300	249	15.86	48	42

Table 3. Physicochemical properties of the collection sites of *Glycyrrhiza glabra* populations

No.	Population	SP %	EC (ds/m)	pH	$CaCo_3$ TNV%	OC %	N %	P (ppm)	K (ppm)	Sand %	Silt %	Clay %	Soil tex
1	Saqqez	40	2.63	7.41	17.75	2.42	0.24	19.99	561	48	26	26	Loam-sand
2	Quchan	34	2.55	7.74	35.12	1.14	0.11	16.4	1015	37	43	20	Loam-clay
3	Ahar	43	2.32	7.52	1	1.94	0.19	13.42	783	33	39	28	Loam-silt
4	Bojnord	41	5.44	7.74	43.75	2.47	0.25	12	271	34	37	29	Loam- clay
5	Sepidan	57	0.7	7.78	17.87	0.82	0.08	11.72	541	24	35	41	sand
6	Darab	43	0.46	7.9	35.37	1.39	0.14	12.81	580	22	49	29	Loam-sand
7	Meshkinshahr	58	1.21	7.58	10.12	1.29	0.13	12.18	251	14	43	43	Loam-sand
8	Kashmar	39.6	0.7	7.7	24.5	0.62	0.08	7.1	123	52	26	22	loam-clay
9	Semirom	35	2.09	7.49	2.75	2.5	0.24	10	367	47	39	14	Loam-clay-sand
10	Mahabad	47	1.5	7.2	5.1	2.4	0.19	11	253	60	26	14	loam
11	Piranshahr	15	3.33	7.99	1.62	0.65	0.06	7.1	141	90	3	7	loam
12	Ilam	25	1.32	8.28	7	0.97	0.10	8.5	184	72	14	14	loam
13	Sirjan	35	2.82	7.55	21.75	0.33	0.02	7.83	377	29	49	22	clay
14	Rabat	42	0.65	7.43	18.5	0.19	0.03	5.9	86	20	19	61	Loam-clay-sand
15	Yasuj	56	2.34	7.44	40.5	0.34	0.03	8.9	416	13	49	38	Loam-sand
16	Bardsir	27	0.59	8.04	25.5	0.81	0.08	6.17	251	73	12	15	Loam-sand
17	Shahrekord	51	0.67	7.68	31.29	0.98	0.09	15.11	299	48.88	28.76	22.35	Clay-silt
18	Marvast	29	2.31	7.3	38.87	1.47	0.15	17.15	541	54	29	17	sand
19	Nagadeh	51	0.36	7.39	39	0.027	0.17	6.4	165	31.25	37.6	31.15	loam
20	Shahrebabak	44	0.21	7.4	41.32	0.65	0.05	6.5	168	84	6.1	9.9	loam
21	Taft	28	0.97	7.88	23.62	1.39	0.14	5.26	31	58	25	17	Loam-sand
22	Bajgah	43.2	0.39	7.74	29.8	1.21	0.09	7.7	153	22.9	39.5	37.6	Loam-clay-sand
23	Hajiabad	47	1.8	7.79	9	3.14	0.08	7.3	136	28	52	20	Loam-sand
24	Kazerun	29	0.45	7.92	46.62	0.82	0.08	10.5	382	34	51	15	loam
25	Baft	25	0.9	7.53	20.12	0.78	0.08	7.43	242	71	16	13	Loam-sand

Pearson correlation

Pearson correlation was conducted to show association among traits (Data not shown). In the present study, the content of glycyrrhizic acid was significantly correlated with Longitude, and it seems that Longitude plays a significant and influential role on the glycyrrhizic acid contents of this plant. Other studied climatic factors did not affect ($p \leq 0.05$) glycyrrhizic acid contents.

Glabridin had a significant positive correlation with total phenol content. Liquiritin had a significant negative correlation with Latitude. There was a significant positive correlation between antioxidant with liquritigenin, average annual precipitation, latitude and mean relative humidity. There was a significant and positive relationship between the phenol and phosphorus soil.

Total phenol, total flavonoid and antioxidant activity (DPPH assay)

The total phenolic content of the given plants was measured by using a method known as Folin-Ciocalteu. The results are given in Table 5. There was high variability of phenol concentrations among licorice populations ranging from 8.28–4.94 mg GAE/g DW. The highest and lowest contents were observed in Rabat and Quchan populations, respectively. Concerning the extracts of Liquorice, the contents of the total flavonoid compounds were closely evaluated (Table 4). TFC was varied from 10.22 to 22.93 mg QE/g DW in root extract of Shahrebabak and Darab populations, respectively.

Antioxidant properties of the mentioned extracts were calculated based on their efficient IC_{50} concentration. It must be noted that this value corresponds with the sample concentration and it needs to be decreased to the initial DPPH absorbance equal to 50%. Table 6 represents a variation in the scavenging activity of the DPPH in the plants, which ranged from 9.68 to 64.14 µg/ml. The highest and lowest antioxidant activities were obtained in the Semirom and Piranshahr populations, respectively.

Table 4. Glycyrrhizic acid, glabridin, liquiritin and liquiritigenin content in different studied populations of *Glycyrrhiza glabra*

No.	Population	Glycyrhizic acid (mg/g DW)	Glabridin (mg/g DW)	Liquritin (mg/g DW)	Liquritigenin (mg/g DW)
1	Saqqez	54.71±3.29^{e}	10.73±0.08^{c}	1.25±0.03^{gnı}	4.01±0.02^{d}
2	Quchan	35.63±3.18^{ıj}	3.29±0.02^{rg}	0.84±0.01^{ıj}	5.24±0.05^{c}
3	Ahar	20.68±1.39^{ı}	3.34±0.03^{rg}	1.27±0.02^{nı}	3.26±0.02^{gnı}
4	Bojnord	54.94±2.05^{de}	19.90±0.03^{b}	4.56±0.03^{bc}	2.95±0.03^{nı}
5	Sepidan	93.89±2.55^{a}	1.28±0.02^{ıjkı}	3.27±0.01^{e}	3.36±0.01^{gnı}
6	Darab	38.69±1.03^{ıj}	1.00±0.03^{jkım}	3.45±0.02^{de}	3.34±0.01^{rgnı}
7	Meshkinshahr	122.44±3.68^{c}	11.88±0.09^{c}	1.63±0.04^{gn}	6.47±0.02^{b}
8	Kashmar	60.58±1.79^{d}	22.87±0.58^{a}	0.65±0.02^{j}	2.88±0.04^{ı}
9	Semirom	43.95±2.25^{rg}	1.99±0.04^{nıj}	2.55±0.04^{r}	3.39±0.03^{gın}
10	Mahabad	38.68±1.30^{nıj}	1.43±0.02^{ıjkı}	0.95±0.01^{ıj}	3.10±0.02^{gnı}
11	Piranshahr	35.30±2.57^{rg}	4.63±0.03^{e}	1.76±0.02^{g}	3.95±0.021^{de}
12	Ilam	87.23±1.68^{b}	19.36±0.05^{b}	4.74±0.30^{b}	5.28±0.03^{c}
13	Sirjan	41.09±1.87^{gnı}	1.66±0.02^{nıjk}	2.59±0.04^{r}	3.32±0.007 ^{gtnı}
14	Rabat	43.89±2.54^{rgn}	0.91±0.002^{ıkım}	1.56±0.02^{gn}	3.95±0.021^{de}
15	Yasuj	65.79±1.44^{c}	0.41±0.003^{kım}	2.40±0.01^{r}	8.57±0.025^{a}
16	Bardsir	56.49±1.32^{de}	4.25±0.01^{er}	4.98±0.002^{b}	3.07±0.01^{gnı}
17	Shahrekord	59.68±1.22^{d}	3.29±0.01^{rg}	4.36±0.004^{c}	3.38±0.008^{gın}
18	Marvast	85.36±1.96^{b}	0.48±0.005^{kım}	2.50±0.012^{r}	2.95±0.02^{nı}
19	Nagadeh	34.68±0.01^{jk}	2.72±0.01^{gn}	2.39±0.013^{r}	3.37±0.011^{gın}
20	Shahrebabak	59.38±1.26^{d}	1.54±0.03^{nıjkı}	5.75±0.01^{a}	3.67±0.014^{derg}
21	Taft	44.41±1.44^{rg}	2.34±0.01^{gnı}	4.87±0.003^{b}	3.75±0.018^{der}
22	Bajgah	37.73±0.01^{ıj}	Trace	1.76±0.01^{g}	3.66±0.016^{der}
23	Hajiabad	31.93±2.05^{k}	6.69±0.08^{d}	3.86±0.01^{d}	3.44±0.019^{ergn}
24	Kazerun	38.45±1.80^{nıj}	0.09±0.01^{ım}	6.09±0.02^{a}	3.84±0.04^{der}
25	Baft	47.28±1.74^{r}	4.78±0.06^{ıjkı}	3.50±0.018^{de}	3.56±0.021^{derg}
	Mean	49.80	5.23	2.939	3.91
	SD	19.95	6.42	1.57	1.24
	C.V %	40.06	122.83	53.51	31.93

Means followed by same letters in each column are not significantly different based on Duncan at the 1% level of probability

Table 5. Total phenol content, total flavonoid content, antioxidant activity and extract yield in different studied populations of *Glycyrrhiza glabra*

No.	Population	Phenol (mg GAE/g DW)	Flavonoid (mg QUR/g DW)	Ic_{50} (µg/ml)	Extract yield (%)
1	Saqqez	5.855±0.043^{cde}	14.893±0.060^{ijk}	42.25±0.806^{cd}	21.463±0.821^{b}
2	Quchan	4.940±0.062^{g}	18.694±0.067^{cde}	52.54±0.370^{b}	9.480±0.396^{kım}
3	Ahar	6.377±0.053^{bc}	15.916±0.069^{nıj}	54.99±0.957^{b}	8.740±0.313^{m}
4	Bojnord	5.829±0.077^{cde}	21.100±0.070^{ab}	32.78±0.794^{erg}	9.220±0.466^{ım}
5	Sepidan	5.457±0.053^{derg}	12.317±0.086^{mı}	50.40±0.949^{b}	15.617±0.620^{rg}
6	Darab	6.827±0.078^{b}	10.220±0.077^{n}	32.27±0.885^{rg}	9.883±0.452^{kım}
7	Meshkinshahr	5.283±0.069^{erg}	17.827±0.061^{derg}	64.14±0.1286^{a}	13.807±0.699^{nı}
8	Kashmar	8.110±0.054^{a}	21.317±0.065^{ab}	36.22±1.164^{er}	15.283±0.462^{g}
9	Semirom	6.750±0.079^{b}	13.350±0.050^{kım}	9.68±0.287^{ı}	6.723±0.398^{n}
10	Mahabad	6.077±0.049^{cd}	13.123±0.094^{kım}	48.51±1.480^{bc}	14.517±0.384^{gn}
11	Piranshahr	6.377±0.087^{bc}	14.420±0.082^{ijk}	50.48±1.491^{b}	24.307±0.574^{a}
12	Ilam	5.467±0.084^{derg}	18.467±0.078^{cder}	50.26±1.897^{b}	10.723±0.498^{kı}
13	Sirjan	5.927±0.074^{cde}	13.117±0.061^{kım}	31.53±0.631^{rg}	19.793±0.640^{c}
14	Rabat	8.283±0.076^{a}	14.017±0.431^{jkı}	31.58±0.549^{rg}	10.575±0.693^{kı}
15	Yasuj	6.870±0.084^{b}	11.423±0.090^{mn}	49.15±1.474^{bc}	16.920±0.496^{er}
16	Bardsir	6.770±0.062^{b}	18.332±0.065^{cder}	10.04±0.675^{ı}	5.700±0.824^{n}
17	Shahrekord	5.143±0.050^{rg}	11.656±0.061^{mn}	39.46±0.842^{ed}	12.623±0.661^{ıj}
18	Marvast	6.439±0.057^{bc}	17.365±0.067^{ergn}	10.86±0.758^{ı}	13.350±0.497^{nı}
19	Nagadeh	7.059±0.345^{b}	16.759±0.054^{rgn}	60.82±0.902^{a}	10.200±0.765^{kım}
20	Shahrebabak	6.837±0.098^{b}	22.936±0.111^{a}	31.32±0.753^{rg}	10.857±0.696^{kı}
21	Taft	5.399±0.240^{derg}	13.153±0.074^{kım}	53.58±0.687^{b}	13.502±0.674^{nı}
22	Bajgah	5.858±0.102^{cde}	17.543±0.050^{ergn}	39.76±0.636^{ed}	17.420±0.696^{ed}
23	Hajiabad	5.852±0.066^{cde}	16.221±0.086^{gnı}	17.26±0.717^{n}	18.490±0.583^{cd}
24	Kazerun	5.636±0.084^{der}	19.928±0.090^{bc}	28.67±1.106^{g}	11.493±0.662^{jk}
25	Baft	7.925±0.088^{a}	19.534±0.109^{bcd}	38.38±1.159^{ed}	10.870±0.649^{kı}
	Mean	6.29	16.14	38.67	13.26
	SD	0.88	3.36	15.07	4.49
	C.V %	14.08	20.83	38.98	33.79

Means followed by same letters in each column are not significantly different based on Duncan at the 1% level of probability

Cluster analysis and principal component analysis

Cluster analysis of UPGMA based on euclidean distances from phytochemical and biological traits matrix placed the studied 25 populations into five main groups (Fig 2). The first group consisted of 5 populations that differed from other groups with common traits such as total phenol and glabridin. The second group of 5 spread populations that has similar amounts of total flavonoid put them in a separate group. Bojnourd, Ilam and Kashmar populations were placed in the third group that has the same values for the all traits. The fourth group consisted of three populations that differed from other groups with common traits such as antioxidant activity and liquritigenin. The fifth group consisted of 8 spread populations that had similar amounts of glycyrrhizic acid, liquritin and extract yield put them in a separate group.

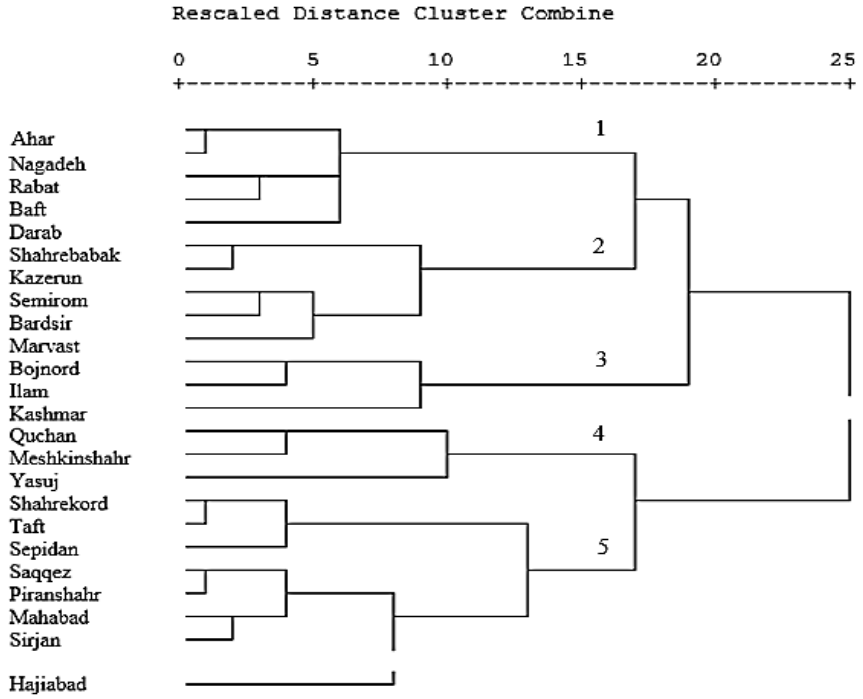

Fig. 2. The UPGMA dendrogram for the investigated traits of *Glycyrrhiza glabra* populations using Euclidean distance

Principal component analysis (PCA) was implemented by drawing from a correlation matrix of all traits, eigenvalues and cumulative variance for those factors which were obtained according to some characteristics for the evaluated populations of *G. glabra*. Eight phytochemical and biological traits were detected in three significant components with explicating 61.35 % of the total variance. The first to third factors were 24.02%, 20.01% and 17.32% of the total variance, respectively.

In the first component (PC1), elucidating liquritigenin, antioxidant and extract yield parameters of populations had the most portion of the variance. In the second component (PC2), glabridin and total flavonoid content of populations above had the highest amount of difference. In the third component (PC3), Glycyrrhizic acid, Liquritin and total phenol content traits had the most portion of the variance (Table 6, Fig 3).

Table 6. Eigenvalues and cumulative variance for factors obtained from the principal component analysis (PCA) based on phytochemical traits for the studied populations of *Glycyrrhiza glabra*

Variable	Component		
	1	2	3
Glycyrhizic acid	0.140	0.384	0.460
Glabridin	0.166	0.870	-0.059
Liqurtin	-0.520	0.122	0.725
Liquritigenin	0.650	0.050	0.295
Total phenol content	-0.293	0.175	-0.730
Total flavonoid content	-0.378	0.782	0.078
IC_{50}	0.786	0.109	0.092
Extract yield	0.579	-0.158	-0.096
Eigenvalue	01.92	1.60	1.37
% of variance	24.02	20.01	17.32
Cumulative %	24.02	44.03	61.35

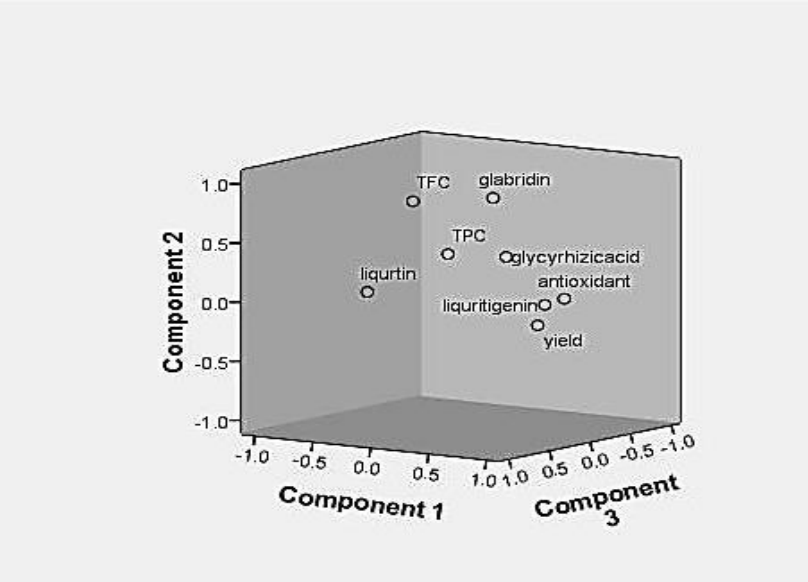

Fig. 3. Principal component analysis (PCA) of the Iranian populations of *Glycyrrhiza glabra*

Discussion

The results of this study showed that there was a high level of variation between populations for the glycyrrhizic acid, glabridin, liquritin and liquritigenin contents. Significant variation in glycyrrhizic acid was observed among licorice populations (93.98 to 20.68 mg/g dry weight). The quantity of Glycyrrhizic acid in this study was higher than that of previous reports in Italy (1.6 - 3%), Spain (0.7 - 4.4%) and Uzbekistan (4.76% to 6.13%) (Hayashi et al., 1998; Hayashi et al., 2003). Song et al. (2017) demonstrated that the distribution of glycyrrhizin was the same in the three studied species (40.5 ± 29.1, 49.3 ± 15.6, and 40.6 ± 26.4 mg/g in *G. uralensis*, *G. inflata*, and *G. glabra*, respectively), which are identified from their genetic information. The glycyrrhizic acid contents remarkably change based on genotype, climate condition, harvesting time and processing procedures, etc. (Hosseini et al., 2014). It has been reported that the amount of component in licorice roots are affected by growing environment (Kovalenko et al., 2004). Hosseini et al. (2014) reported that average content of

glycyrrhizic acid varied between 1.38% and 3.40% in their study with different populations from Iran. It has also been reported that the physicochemical characterization of soil is the most important factor concerning the accumulation of active ingredients in this plant (Zhang et al., 2011). In this study, range of glabridin content were varied from 22.87 (mg/g dry weight) to trace. Hayashi et al. (1998, 2003) estimated the amount of Glabridin in Italy, Spain and Uzbekistan with the ranges 0.07—0.27%, 0.21—0.80% and 0.08—0.35%, respectively.

The contents of liquritin and liquiritigenin were significantly different among populations with the highest values in Kazerun and Yasuj, respectively. Guannan et al. (2016) estimated the range of liquritin content from 1.90 to 8.14 mg/g dry weight. In another study, the amount of liquritin was reported to be 0.11-0.65% (Mareshige et al., 2011).

Environmental effects on the plant distribution, growth, development and reproduction are well known in plant ecology (Zhang et al., 2011). The relationships between plant biochemical contents and environmental variables have mainly been studied in cultivated plants (Zhou, 2003; Hayashi and Sudo, 2009). These data can be used for quality assessment of plant products (Zhang, 2003).

The content of glycyrrhizic acid was significantly correlated with Longitude; other studied climatic factors did not affect ($p \leq 0.05$) on contents of glycyrrhizic acid. Similar results were obtained by Oloumi and Hassibi (2011). Their research dealt with the relationship between climatic parameters and the content of glycyrrhizic acid in the root of *G. glabra*. They reported that temperature and soil parameters are the most critical factors affecting glycyrrhizic acid content in roots. In several species of medicinal plants, it has been reported that geographic variations lead to changes in the glycyrrhizin content in *Glycyrrhiza glabra* (Oloumi and Hassibi, 2011; Zhang et al., 2011; Ahmadi-Hossieni et al., 2014). Glabridin had a significant positive correlation with the total phenol content. Liquritin had a significant negative correlation with Latitude. There was a significant positive correlation between antioxidant with liquritigenin, average annual precipitation, latitude and mean relative humidity. There was a significant and positive relationship between the phenol and phosphorus soil. Although in our study bioactive compounds were not significantly correlated with soil variables, in populations with different soil characteristic, the bioactive compounds content was different from each other. It showed that the properties of physicochemical soil are important for the accumulation of active substances in habitat populations of *G. glabra*. Similarly, Zhou (2006), Zhang et al. (2011) and Ahmadi-Hosseini et al. (2014) reported the significant effects of soil variables on contents of bioactive compounds.

Phenols have immense importance in plant stability because of their scavenging ability on free radicals due to their hydroxyl groups. As a result, the phenolic content of the plant is in a close affinity with the antioxidant ability (Karami et al., 2013). The studied phytochemical traits such as total phenol and total flavonoids at the level of probability of one percent showed a significant difference between the estimated populations in different locations. This indicates the existence of high diversity in terms of studied phytochemical traits and the possibility of selection for these traits among the studied populations in different environmental conditions. In this study, the highest total phenol content was observed in the Rabat population and the lowest in the Quchan population. In other studies, total phenol content was reported to be 7.47 mg GAE/g (Husain et al., 2015), 71.29 mg GAE/g (Tupe et al., 2013) and 37.22 mg GAE/g (Sanja et al., 2018).

Flavonoids are considered to be one of the most influential bioactive components of plants, particularly in *G. glabra* L.

(Scherf et al., 2012). The highest total flavonoids were observed in the population of Shahrebabak. Sanja et al. (2018) reported that the amount of total flavonoid in the root extract of *G. glabra* is equal to 5.90 mg QE/ g DW. Many researchers evaluated the antioxidant activity of licorice extracts (Visavadiya and Narasimhacharya, 2006; Sultana et al., 2010; Siracusa et al., 2011; Saraf et al., 2013; Sanja et al., 2018) and suggested that these extracts can be used as safe antioxidants. The importance of the impact of different environmental conditions on different habitats on the quality and quantity of secondary metabolites of plants and even algae has already been reported by several studies (Becerro and Paul, 2004; Gairola et al., 2010; Gobbo-Neto et al., 2010; Jovancevic et al., 2011).

UPGMA cluster analysis based on phytochemical and biological traits of the 25 studied populations classified the populations into five main groups. The grouping of the populations was relatively unrelated to their geographical distribution, and the distribution of individuals in the cluster did not follow a specific geographical pattern, which was similar to the results of other studies on *G. glabra* (Ahmadi-Hosseini et al., 2014), *Satureja rechingeri* (Eghlima et al., 2018), *Satureja khuzestanica* (Hadian et al., 2011), *Anemopsis californica* (Medina-Holguin et al. 2007), which reported that the difference in chemical composition due to different genetic and environmental determinants.

Conclusion

Based on the obtained results, there is a correlation between the content of secondary metabolite production in licorice plants and the climate conditions. Remarkable variations in phytochemical and antioxidant properties were observed in the studied populations of *G. glabra*. According to the obtained results, populations with high amount of each active ingredient in licorice root and those with considerable antioxidant activity were identified, which can be exploited depending on the purpose of breeding and cultivation. Finally, the suitable regions for licorice cultivation were suggested based on some ecological parameters such as annual average temperature, annual average precipitation and elevation.

Acknowledgments

We are grateful to the University of Zanjan, Iran for the support of this study.

Conflict of interest

There is no conflict of interest for this research.

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