J Genet Resour2021;7(1): 87-105 *Homepage: http://sc.journals.umz.ac.ir/*

RESEARCH ARTICLE *DOI: 10.22080/jgr.2021.20033.1213*

J|G|R

Screen of Antioxidant Activity Leads to Recognition of High Valuable Medicinal Plants: A Case Study of Paveh and Ormanat, West of Iran

Mohammad Sanei[1], Azim Ghasemnezhad[*1], Alireza Sadeghi Mahounak[2], Mohammad Masoumi[3] and Khalil Ghorbani[4]

[1] Department of Horticulture, Faculty of Plant Production, Gorgan University of Agricultural Sciences and Natural Resources

[2] Department of Food Science Engineering, Faculty of Food Science, Gorgan University of Agricultural Sciences and Natural Resources

[3] Department of Biology, Faculty of Science, Razi University of Kermanshah

[4] Department of Water Engineering, Faculty of Water and Soil Engineering, Gorgan University of Agricultural Sciences and Natural Resources

ARTICLE INFO

Article history:
Received 29 August 2020
Accepted 01 November 2020
Available online 16 November 2020

Keywords:
ABTS
DPPH
FRAP
Kermanshah
Kurdistan
Phenols
TAC

Corresponding authors:
✉ A. Ghasemnezhad
ghasemnezhad@gau.ac.ir

p-ISSN 2423-4257
e-ISSN 2588-2589

ABSTRACT

Recently, the beneficial effects of the plants' secondary compounds for the treatment and improvement of diseases have been strongly interested. This study was conducted in a completely randomized design with three replications in the Paveh and Ormanat region of Kermanshah province. For this purpose, after studying the flora of the region, 10 high-widespread families (Apiaceae, Boraginaceae, Lamiaceae, Rosaceae, Solanaceae, Polygonaceae, Fabaceae, Brassicaceae, Liliaceae, and Asteraceae) were selected. In each case, three plant species with three replications were identified and randomly collected during flowering from April to September. Antioxidant potentials of the samples were evaluated by four methods of DPPH, FRAP, ABTS, and TAC. Results of analysis of variance showed significant differences between measured traits in plant species as well as plant family with 99% confidence. Among the studied plants, *Pimpinella kotschyana* Boiss. from Apiaceae, *Bellevalia dichroa* Hausskn. from Liliaceae, *Muscari neglectum* Guss, from Liliaceae showed the highest antioxidant activities using different methods. Results also showed that among the studied plant families, Liliaceae, Rosaceae, Brassicaceae, and Asteraceae showed considerable antioxidant potential and could be noticed in future research.

Please cite this paper as: Sanei M, Ghasemnezhad A, Sadeghi Mahounak A, Masoumi M, Ghorbani K. 2021. Screen of antioxidant activity leads to recognition of high valuable medicinal plants: A case study of Paveh and Ormanat, west of Iran. *J Genet Resour* 7(1): 87-105. doi: *10.22080/jgr.2021.20033.1213.*

Introduction

Plants and their natural products are the main sources of active pharmaceutical and therapeutic compounds that can be used as both drugs and valuable models for the production of similar synthetic compounds (Omidbaigi, 2005; Fathi *et al*., 2016). The scope of disease control by natural remedies has shown considerable progress in recent years. Physicians are keen to use natural remedies to treat illnesses (Kassa *et al*., 2020). Given the growing public awareness and understanding of the high benefits of using natural medicines as well as the tendency to consume useful natural compounds in plants (especially natural antioxidants), extensive research is underway to introduce new antioxidant sources (Kaviarasan *et al.*, 2007). Due to the different climates, Iran has diverse ecosystems, each with its characteristics. Understanding the enormous natural resources and achieving relationships among the plants and the factors affecting them is essential for their

conservation and sustainability (Mozaffariyan and Sanandaji, 2009; Afkar and Zand, 2020). Iran has a special geobotanical position among the Middle East countries. It plays as a bridge between the four major geographic regions, namely Irano-Turanian, Euro-Siberian, Saharo Arabian, and Sudanian (Zohary, 1973; Timurzadeh *et al*., 2015). The high diversity of topographic, geological, and climatic conditions has made Iran one of the most important areas of species diversity around the world (Safi Khani *et al*., 2006; Mortezaeinejad and Nowruz, 2011).

Kermanshah province is located in western Iran. The average long-term rainfall in the province is about 537 mm, which is much higher than the average rainfall in Iran (about 270 mm / year) (Nemati Pikhani and Jaliliyan, 2012). Due to geographical differences, Kermanshah province and Paveh and Ormanat region located in the north part of it, have a high diversity of plant species and so far few studies have been done on this area. Characteristics of plant growth and geographical location in nature are among the most important factors affecting the plant's essential oil content and constituents. There are several reports on the relationship between habitat conditions and chemical constituents of plants (Mohammad Nejadganji *et al*., 2014; Davazdahemami, 2017). The production of medicinal compounds in plants is largely influenced by the genetic process, but environmental conditions have a major impact on the growth of medicinal plants and the quality of their active ingredients including glycosides, alkaloids, volatile oils, and steroids (Maghsoudi *et al*., 2018). Research shows that oxidative reactions caused by the activity of free radicals in living cells stimulate many diseases, including cardiovascular disease, accelerated aging, depression, and cancer, as well as food spoilage and changes in the taste and smell of oils (Suhaj, 2006; Jamshidi *et al.*, 2010; Halliwell and Gutteridge, 2015; Mohadjerani *et al*., 2016). Antioxidants are compounds that capable to prevent biological systems from oxidative damage resulting from oxygen free radical known as reactive oxygen species (ROS) such as hydrogen peroxide (H2O2), hydroxyl radical (OH-), anion Superoxidase (O2-), and nitric oxide (NO-) (Halliwell, 2007; Khalili *et al*., 2015; Prakash *et al*., 2019; Kohli *et al*., 2019; Dipalma *et al*., 2020). Nowadays, due to the toxic and mutagenic effects of commonly used industrial (synthetic) antioxidants such as propyl gallate (PG), butyl hydroxyanisole (BHA), butylhydroxytoluene (BHT), tert-butyl hydroxyquinone (TBHQ) (Barlow, 1990; Hou, 2003; Khounani *et al*., 2020), many efforts have been made to find natural antioxidants in plants as an alternative to synthetic ones (Ismailzadeh *et al*., 2017; Taheri *et al.*, 2017). These include antioxidant compounds reported in *Cynara scolymus* L. (Ghasemnezhad *et al*., 2013), *Citrus sinensis* cv. Thomson Navel and Page mandarin (Ghasemnezhad *et al*, 2012), *Echium amoenum* L. (Fathi and Mohammadi, 2016), *Ficus carica* L. (Maghsudlu *et al*., 2016), *Myrtus communis* (Mozdstan *et al*., 2015), *Nigella sativa* L. (Kumbhakar *et al*., 2019), *Stevia rebaudiana* (Khatami *et al*., 2019) and *Matricaria recutita* L. (Al-Dabbagh *et al*., 2019). Natural antioxidant compounds that inhibit free radicals include substances such as phenolic and flavonoid compounds (flavonol, flavanone, flavon, anthocyanidin, and isoflavone), organic acids, steroids, terpenoids, vitamin E, and carotenoids (Pratt and Hudson, 1990; Halliwell *et al*., 1993; Lee *et al*., 2005; Gomes *et al*., 2012; Alamholo and Nazeri, 2014; Stagos, 2020). Due to the diversity of antioxidant substances a simple and universal method for the proper evaluation and quantification of antioxidants potency of a plant species is not introducible (Zulueta *et al*., 2009; Sarmadi and Ismaila, 2010). Also, the determination of antioxidant capacity in foods is associated with more complexities such as sample colloidal properties, conditions, and oxidation stage, natural properties such as color, pH, etc (Frankel *et al*., 2000). So, it is common to use several methods to validate research (Hosseini *et al.*, 2013). Research on native plants in an area is important from the point of view of their antioxidant potential. Due to the traditional consumption of medicinal plants and the presence of native spices in Paveh and Urmanat as food and medicine, research is needed to find valuable antioxidant sources in this region. Therefore, the present study was designed and conducted to investigate the antioxidant potential of major plant species grown in the Pave and Ormanat region.

Materials and Methods

In this study, initial information was obtained from local people. Then, to complete the research and ensure that the area's flora was investigated, some available resources were used (Mozaffariyan, 1996; Ghahraman, 2007; Mozaffariyan, 2011; Zargari, 2014; Rechinger, 1963-2005; Smsam Shariat, 2007). Around 10 widespread plant families were carefully identified (Hoshidari, 2009; Khezri, 2011; Nemati Paykani and Jaliliyan, 2012). Then, the plants were collected and information on each plant at the growing site was recorded. From each plant family, three species were randomly selected (Table 1). The present work was done based on the completely randomized design with three replications.

All selected plant species were harvested at full blooming stages and after preparation of the herbarium sample, their location information including latitude, longitude, and altitude was recorded using Garmin Etrex H GPS (Fig. 1). Identification and confirmation of the collected plants were carried out at the Razi University of Kermanshah. After drying in shade and room temperature conditions, the samples were transferred to the Horticultural Laboratory of Gorgan University of Agricultural Sciences and Natural Resources for antioxidant potency analysis.

Preparation of methanolic extract

The extraction was done using cold maceration (Enayati *et al.*, 2017). For this purpose, one gram of dried herbal powder was added to 10 ml of solvent (80% methanol with deionized water) and placed on the shaker at room temperature and dark conditions. After extraction, the contents of the extract were filtered using Whitman filter paper, and due to the sensitivity of the extracts to light and temperature, the samples were stored in a refrigerator at 4 °C until analysis.

Table 1. List of plants studied in Paveh and Ormanat region.

Plant family name	Plant Scientific name	Persian name	Sea level (m)
	Taraxacum officinale L.	Ghasedak kochak	974
Asteraceae	*Onopordon carduchrum* bornm. & beaur. DC.	Khar panbeh shahouei	1585
	Lactuca serriola L.	Kahou khardar	999
	Bellevalia dichroa Hausskn.	Tameshkin gol abi	1100
Liliaceae	*Muscari neglectum Guss*	Kalakhak	916
	Bellevalia longistyla (Miscz.)	Tameshkin Azari	979
	Descurinia sophia L.	Khakshir Irani	1378
Brassicaceae	*Cardaria draba* (L.) Desv	Ozmak	1020
	Lsatis kotschyana Boiss. et Hohen.	Vosmaei	1092
	Lamium amplexicaule L.	Kharbilak	1376
Lamiaceae	*Stachys lavandulifolia* Vahl.	Chay kouhi	1605
	Phlomis rigida Labill.	Goush bara tanaz	3125
	Ferulago stellata Boiss.	Chouyl shevidi	1432
Apiaceae	*Smyrnium aucheri* Boiss.	Avendol talkh	1433
	Pimpinella kotschyana Boiss.	Jafari kouhi kurdistani	995
	Anchusa italica Retz. Var. kurdica Gusuleac	Gol gavzaban	1024
Boraginaceae	*Onosma sericeum* Willd.	Zangoulei kork abrishami	924
	Heliotropium lasiocarpum Fisch. et C.A. Mey.	Aftab parast	1594
	Pyrus communis L.	Golabi vahshi	1726
Rosaceae	*AmyGdalus lycoides* spach	Tangars	1720
	Cerasus microcarpa Boiss.	Tameshk derakhti	1713
	Vicia sativa L.	Mashak (gavdaneh)	1534
Fabaceae	*Glycyrrhiza glabra* L.	Shirin bayan	1513
	coronilla varia L.	Yonjeh bakhi	1425
	Hyoscyamus Kotschianus pojark.	Bazr albanj	1697
Solanaceae	*Physalis alkekengi* L.	Arousak poshte pardeh	994
	Datura stramarium L.	Tatoureh tamashaei	1488
	Polygonum persicaria L.	Alafe haft band	955
Polygonaceae	*Rumex crispus* L.	Torshake mavaj	841
	Rheum ribes L.	Rivas	2300

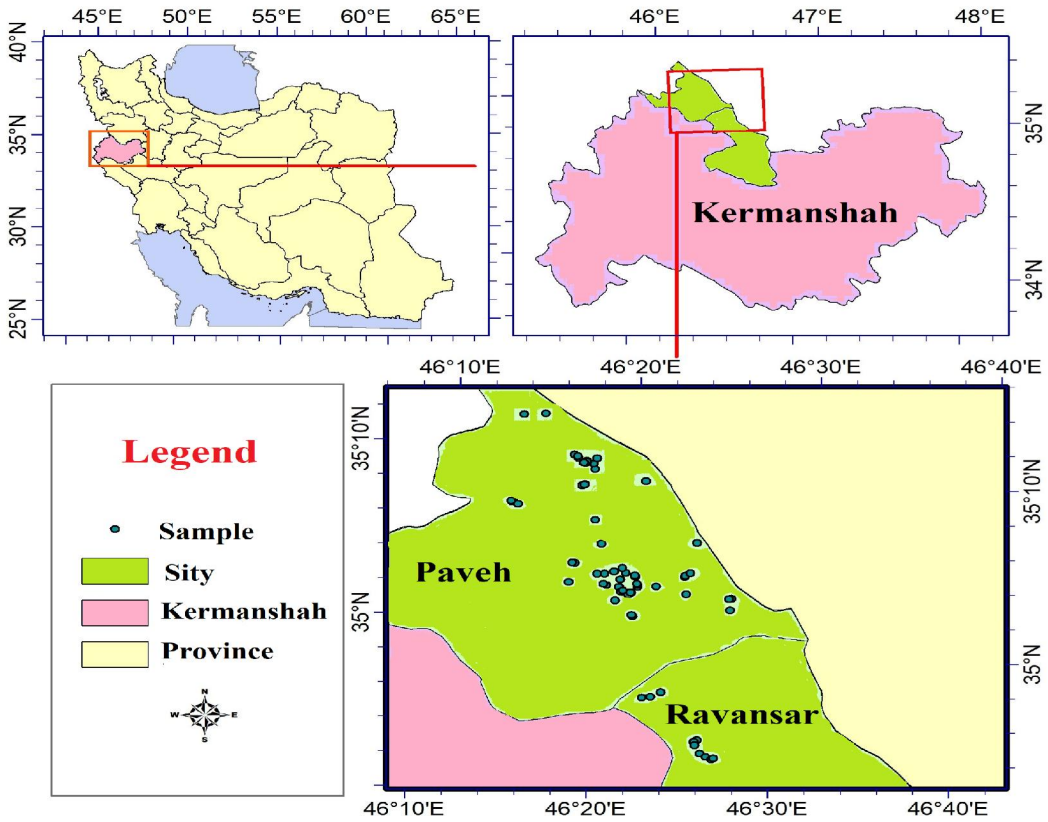

Fig. 1. Distribution map of the study area.

DPPH free radical scavenging assay

Determination of the antioxidant capacity of the extract by the DPPH free radical scavenging method was done according to Wu *et al*. (2003). This method is based on the free radical reduction of DPPH by antioxidants in the absence of other free radicals in the medium. In this process, color is formed in the medium whose intensity can be measured using spectrophotometry (Prevc *et al.*, 2013).To prepare the DPPH reagent, 0.004 g of this substance was dissolved in 100 ml of pure methanol. To one ml of the methanol extract (80% methanol), 1 ml of DPPH reagent was added and incubated for 30 minutes at room temperature in dark conditions. The absorbance of the samples was read after 30 minutes at 517 nm. The percentage of the DPPH free radical scavenging activity was calculated based on [DPPH Free radical scavenging activity (%) = (A control – A sample / A control) × 100] formula.
A control: Absorbance of control solution, A sample: Sample absorbance.

Ferric reducing antioxidant potential

Antioxidant potency was determined by the method of ferric reduction (FRAP) by Benzie *et al*. (1996). In this method, 0.1 g of sample was crushed in a mortar with 5 ml of distilled water and the contents were kept at room temperature for 30 min. The contents were then passed through Whitman's filter paper and the filtered extract was kept in the dark and refrigerated until use. For the preparation of FRAP reagents, sodium acetate ($CH_3COONa.3H_2O$), 10 mM glacial acetic acid, 4,2, and 6-tripyridyl-S-triazine (TPTZ), 37% hydrochloric acid, and ferric chloride ($FeCl_3$) were used. Around 1.5 ml of FRAP reagent was added to the 50 ml of the extract. After vortexing, it was incubated at 40°C in the water bath for four minutes.
The absorbance of the samples was read immediately after cooling against the control sample (all compounds except the extract) at the wavelength of 594 nm by a spectrophotometer. To prepare the standard curve, 1 mM ammonium ferrous sulfate $(NH_4)_2$ $Fe(SO_4)_2)$ .6H2O) was used. Results were expressed in terms of mg of

ferrous ion per gram of dry weight of the plant material.

Total Antioxidant Capacity

The amount of total antioxidant potential was evaluated according to the methods of Sun *et al*. (2011). To prepare the TAC reagent, 0.6 mM sulfuric acid (H_2SO_4), 28 mM sodium phosphate (Na_3PO_4), and 4 mM ammonium molybdate $(NH_4)_2MoO_4$ were used. One ml of TAC reagent was added to a test tube containing one ml of methanolic extract. After vortexing, the samples were incubated for 90 minutes at 95 °C in the water bath. Immediately after cooling, the absorbance of samples in comparison to the control (all compounds except extracts) was measured at 695 nm using a spectrophotometer. Ascorbic acid was used as a standard to prepare the standard curve and the obtained data was reported as milligrams of ascorbic acid per gram of dry weight of the plant.

Antioxidant potential by $ABTS^+$ method

The $ABTS^+$ (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) radical scavenging activity of the plant extracts was measured using the method described by You *et al*. (2010). $ABTS^+$ radical solution was prepared by combining the same volume of ABTS at 7 mM potassium persulfate at 2.45 mM. The mixture was placed in the dark at ambient temperature for 12-16 hours before use. During this time, $ABTS^+$ radical oxidation and production were carried out by potassium persulfate. Before the test, the $ABTS^+$ solution was diluted using PBS sodium phosphate buffer (pH = 7.4, 0.2 mM) to reach an absorbance of 0.7 ±0.02 at 734 nm. Then, 40 µl of each sample was added to four ml of diluted $ABTS^+$ solution. The mixture was stirred vigorously for 30 seconds and placed in the dark for six minutes. The absorbance of the final solution was measured using a spectrophotometer at 734 nm. The percentage of antioxidant potency in this method was calculated using [AA (%) = [A blank- A sample / A blank] × 100] formula. A blank is the absorbance of the control sample without an active compound and A sample is the absorption of the sample.

Total phenolic content

Ordonez *et al*. (2006) method was used to determine the total phenol content of the samples. In this method, 100 ml of methanolic extract, 2.8 ml of deionized water, 2 ml of sodium carbonate (2% Na_2CO_3), and 50 ml of 50% Folin reagent were added, respectively. The resulting solution was vortexed and stored at room temperature for 30 min. The absorbance of the samples was recorded against the control (all compounds except the extract) at 720 nm using a spectrophotometer. Gallic acid was used to prepare the standard curve and the value was expressed in milligrams of gallic acid in gram dry weight.

Total Flavonoid content

The total flavonoid content of samples was measured using the method of Kim *et al*. (2002). In this method, 500 ml of methanolic extract, 1.5 ml of 80% methanol, 100 ml of 10% aluminum chloride ($AlCl_3$), 100 ml of 1 M potassium acetate, and 2.8 ml of deionized water were added. The samples were vortexed properly and stored at room temperature for 40 min. Then the absorbance of the samples was recorded at 415 nm and compared with the control (including all compounds except the extract). Quercetin was used to prepare the standard curve and the data were expressed in mg of quercetin per gram dry weight.

Statistical analysis

The present study was based on a completely randomized design with three replications. In this study, SPSS software (SPSS 16.0 for windows) was used for data analysis, and mean values were compared using the LSD test at 5% probability.

Results

Table 2 shows the variance analysis of the interaction of plant family and plant species in the studied species. The results showed that the antioxidant capacity of the four applied methods of DPPH, FRAP, TAC, ABTS and the amount of phenol and flavonoid compounds of plants as affected by plant family was significant. Also, the simple effect of plant family regardless of plant species was significant with 99% of probability.

Table 2. Variance analysis of measured characteristics in studied plant species.

Variance source	df	Mean of square					
		DPPH	FRAP	TAC	ABTS	Flavonoid	Total phenol
Plant family (PF)	9	1188.809**	0.936**	16.959**	446.552**	21.654**	75.197**
Plant (P)	2	97.380^{ns}	0.611**	0.104^{ns}	3687.673**	3.299**	8.428**
PF × P	18	358.891**	0.343**	7.590**	402.122**	6.399**	25.250**
CV		40.230	21.480	29.180	5.602	45.101	21.269
Error		34.104	0.005	0.066	56.394	0.050	0.510

Antioxidant potential using DPPH method

As presented in Fig. 2, the antioxidant capacity of the plant species affected by the plant family concerning the ability to scavenge the DPPH radicals (2 and 2-diphenyl picryl hydrazyl). The results indicate that the highest antioxidant potential (around 61.9%) was observed in *Pimpinella kotschyana* Boiss, which belongs to the Apiaceae family. *Onopordon carduchrum* Bornm. & Beaur. DC., from the Asteraceae and *Glycyrrhiza glabra* L. from the Fabaceae family with 58.8% and 52.2% showed the highest DPPH radical scavenging ability, respectively. On the other hand, and among the studied plant species, the lowest amount of antioxidant potential (8.2%) was observed in *Rumex crispus* L. from the Polygonaceae family (Fig. 2).

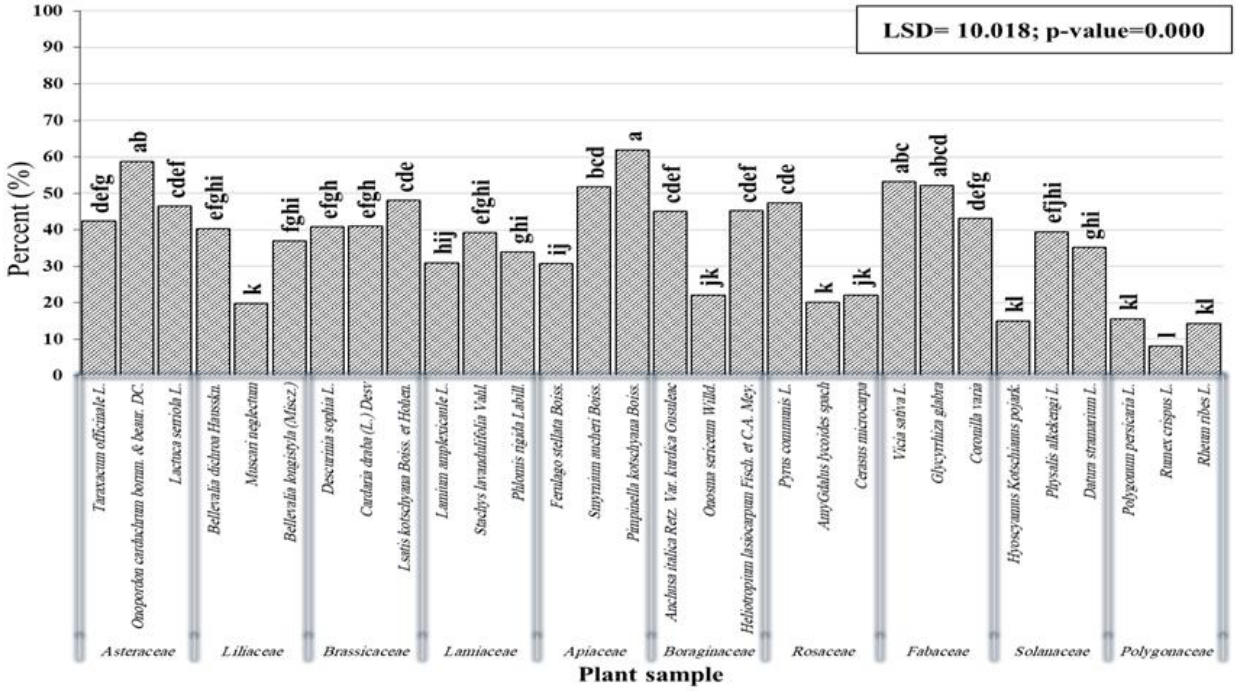

Fig. 2. Comparison between antioxidant capacities of studied plant species using DPPH method.

Fig. 3 shows the DPPH free radical scavenging potential of the studied plant families regardless of plant species. The highest amount of antioxidant activity was observed in the Asteraceae family, while there was no significant difference between Asteraceae, Fabaceae, and Apiaceae. The lowest amount of antioxidant potential was also observed in the Polygonaceae family (Fig. 3).

Antioxidant potential by FRAP method

The maximum antioxidant potential measured by ferric reducing power in plant species was observed in *Bellevalia dichroa* Hausskn. belongs to the Liliaceae family with 2.7 mg / g dry matter.

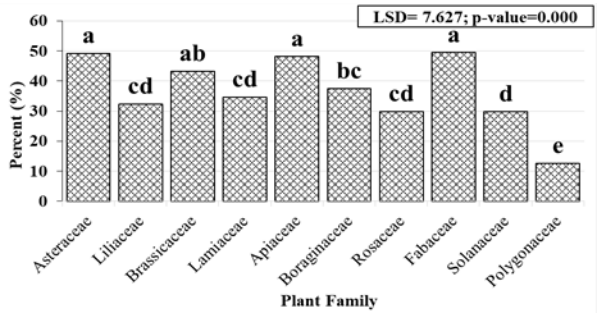

Fig. 3. Comparison between the antioxidant capacity of plant family regardless of plant species by DPPH method.

However, there was no significant difference between this plant and *Smyrnium aucheri* Boiss from Apiaceae and *Muscari neglectum* Guss from the Liliaceae family which were in the range of 2.6 and 2.59 mg/g dry matter, respectively (Fig. 4).

The lowest antioxidant potential was also observed in liquorice (*Glycyrrhiza glabra* L.) belonging to the Fabaceae family with 1.02 mg / g dry matter

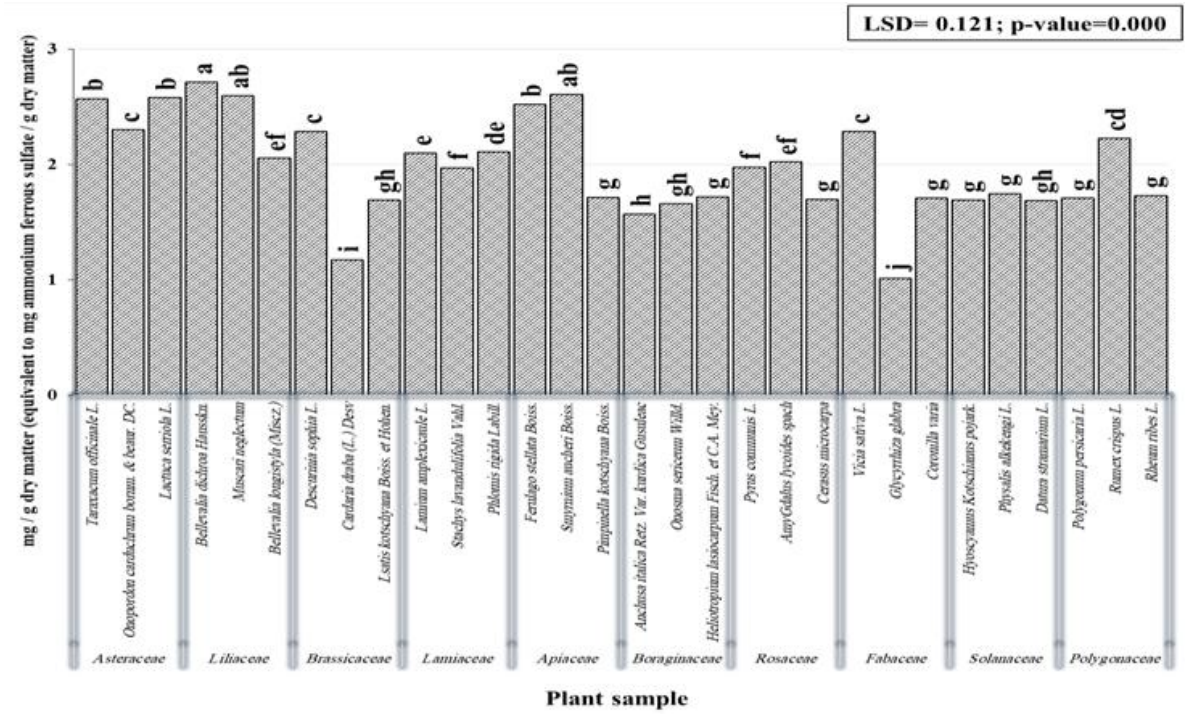

Fig. 4. Antioxidant capacity of studied plant species depending on plant family by FRAP method.

Fig. 5 shows that, regardless of plant species, the highest antioxidant activity belongs to the Asteraceae family. However, there was no statistically significant difference between this plant family and the Liliaceae family. As previously mentioned, the dominant constituents in Asteraceae and Liliaceae were reported as flavonoids and terpenoids, respectively. Most of the plants mentioned above are collected at higher altitudes and southern slopes.

Studies showed that the lowest amount of antioxidant potential was observed in the Boraginaceae family. Plants of this family are mostly known as plants with high flavonoid compounds and in some cases alkaloids. The production of these compounds in the plant is more dependent on light intensity. Most of the plants of this plant family were collected at lower altitudes or on the northern slope with less light intensity, which may be one of the reasons for their low antioxidant capacity. The decrease in light intensity results in a decrease in the production of flavonoid compounds, as indicated in the present study. However, there was no

statistically significant difference between the Boraginaceae, Fabaceae, Solanaceae, and Brassicaceae families.

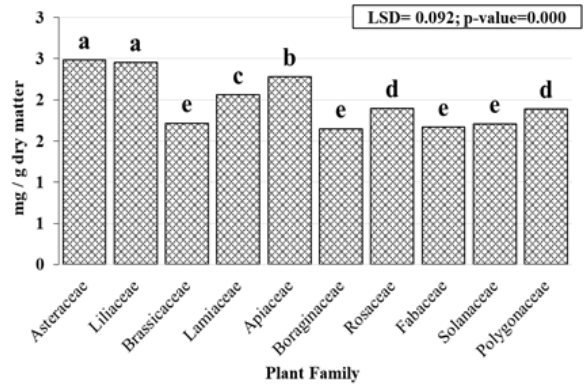

Fig. 5. Antioxidant capacity of plant family regardless of plant species by FRAP method (mg/g dry matter equivalent to mg ammonium ferrous sulfate/g dry matter).

Antioxidant potential by TAC method

The highest antioxidant potential based on the TAC method was observed in the *Muscari neglectum* Guss belongs to the Liliaceae family with 9.8 mg/g dry matter, which showed a significant difference compared to the other studied plants. In contrast, the lowest antioxidant potential was observed in *Anchusa italica* Retz and *Onosma sericeum* Willd., from the Boraginaceae family with 3.1 and 2.9 mg/g, respectively. However, no significant differences were observed between these plant species (Fig. 6). *Onosma sericeum* Willd collected from areas with alkaline soil (7.25). In contrast, the growth site of *Muscari neglectum* Guss showed a lower pH (6.22). Increasing soil pH reduces the absorption of elements involved in the structure of secondary compounds such as phosphorus, zinc, and manganese. On the other hand, increasing soil pH to neutral pH increases the uptake of elements such as nitrogen, potassium, magnesium, and calcium. The availability of important nutrients means reduced and minimized environmental stress and thus reduces the production of free radicals (Tabatabai, 2015). The pH is one of the most important factors in the uptake of elements, which may justify the differences in the amount of TAC observed in the above-mentioned plants.

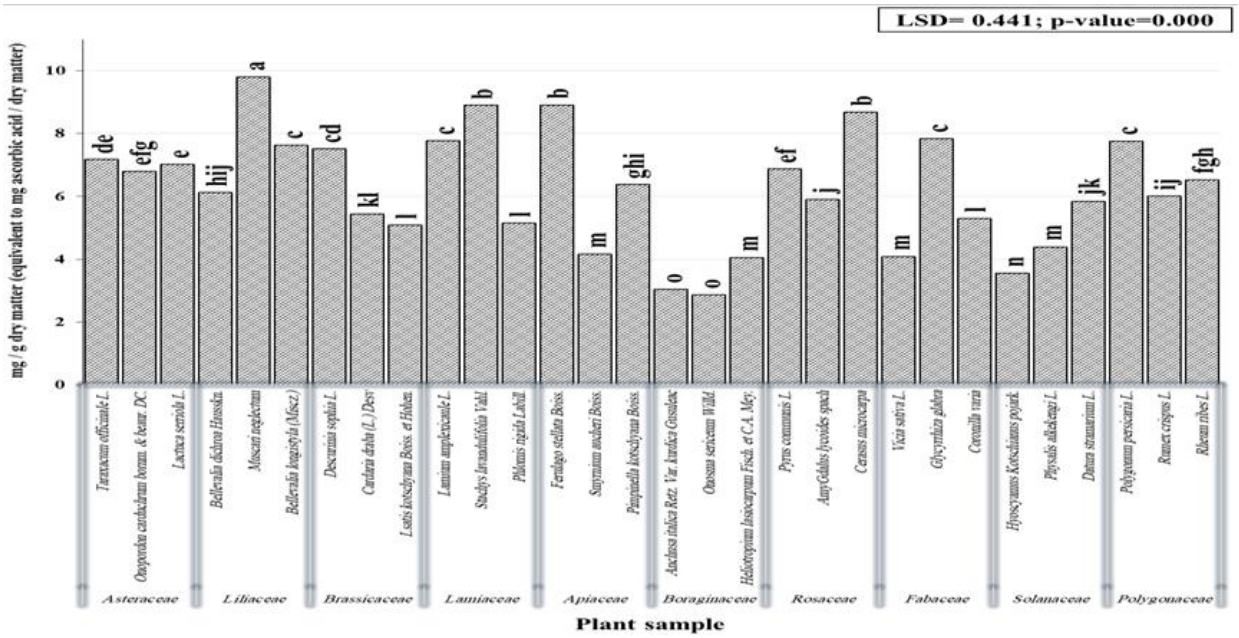

Fig. 6. Antioxidant capacity of studied plant species under the influence of plant family by TAC method.

Fig. 7 shows the antioxidant capacity of the plant family studied by the TAC method regardless of the plant species. According to these results, compared to the other studied plant families, the highest antioxidant activity was observed in the Liliaceae family following by the Lamiaceae and Rosaceae. The dominant constituents of Liliaceae, Lamiaceae, and Rosaceae are generally terpenoids. It seems that in the TAC method, the antioxidant potential of these plant

families mostly depends on the presence of terpenoids. In contrast, the lowest antioxidant potential was observed in the Boraginaceae family. This difference was statistically significant with the other plant families.

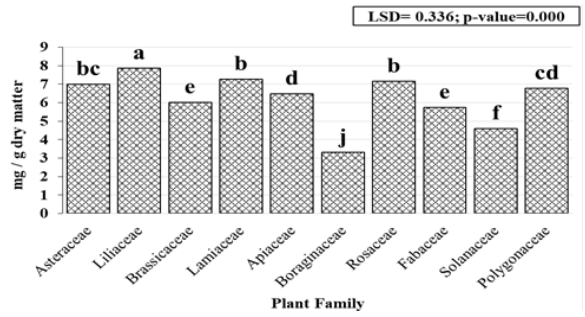

Fig. 7. The antioxidant capacity of plant family regardless of plant species by TAC method (mg / g dry matter equivalent to mg ascorbic acid /dry matter).

The antioxidant ability of plant samples by ABTS$^+$ method

Fig. 8 shows the ABTS$^+$ radicals scavenging potential of methanolic extract of studied plants that belong to different plant families. Accordingly, the highest antioxidant potential was recorded in the *Onopordon carduchrum* bornm. & beaur. DC. belongs to the Asteraceae family with 71.34%. The *Muscari neglectum* Guss and *Bellevalia long style* (Miscz.) from the Liliaceae family and the *Lactuca serriola* L. from the Asteraceae family with 70.6, 70.3%, and 70.2%, respectively were at the second places. However, no significant difference was observed between the two families. Also, the minimum antioxidant potential (19.0%) in this method was recorded in the *Anchusa italica* Retz. Var. Gusuleac kurdica belongs to the Boraginaceae family.

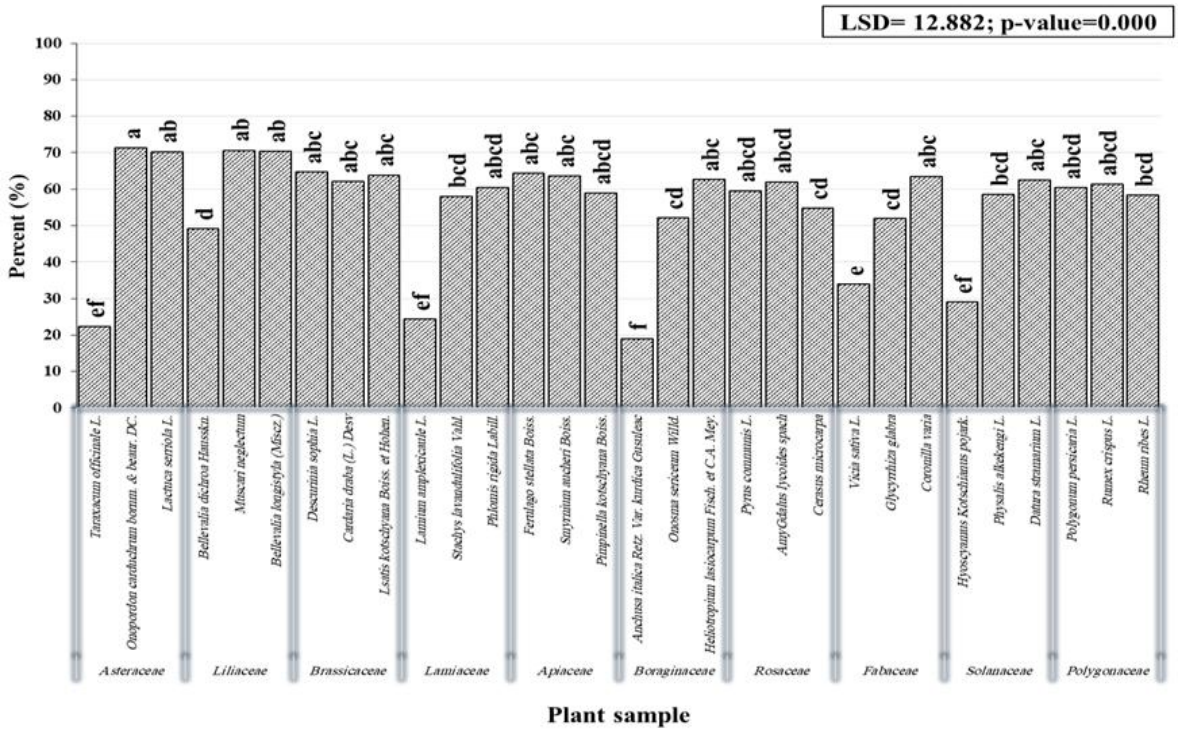

Fig. 8. Mean value of antioxidant capacity of studied plant species by ABTS method.

The antioxidant capacity of ABTS$^+$ in plant families, regardless of plant species, is illustrated in Fig. 9. Accordingly, the most potent ABTS$^+$ radical scavenging activity was observed in the Brassicaceae family, which is a constitutive nature of the most frequently glycosidic and alkaloid constituents. However, there was no significant difference between this family and the families of Liliaceae, Apiaceae, and Polygonaceae. Among the studied plant families, the lowest ABTS$^+$ radical scavenging ability was observed in the Boraginaceae family.

Investigation of studied plants in terms of total phenolic content

Phenol is a toxic organic and aromatic compound that is not freely available in plant tissue except in specific cases (Eiger, 2013). Most of the antioxidant compounds in biological systems have phenolic structures.

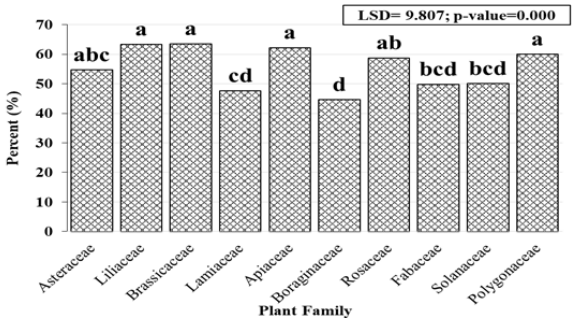

Fig. 9. Antioxidant capacity of plant families regardless of plant species using ABTS method.

These compounds are synthesized to counteract free radicals caused by a variety of stresses. The degree of accumulation of phenolic compounds in a plant indicates the ability to decrease or trap free radicals in the system. Fig. 10 shows the amount of phenolic compounds in the studied plant samples as affected by the plant family. The highest phenolic content was observed in *Glycyrrhiza glabra* L. from the Fabaceae family with 24.8 mg/g dry matter. However, the total phenolic content in this plant was not significantly different from that of *Stachys lavandulifolia* Vahl from Lamiaceae with 23.7 mg/g dry matter. The lowest amount of phenolic compounds was observed in the *Smyrnium aucheri* Boiss from the Apiaceae family with 9.7 mg /g dry matter.

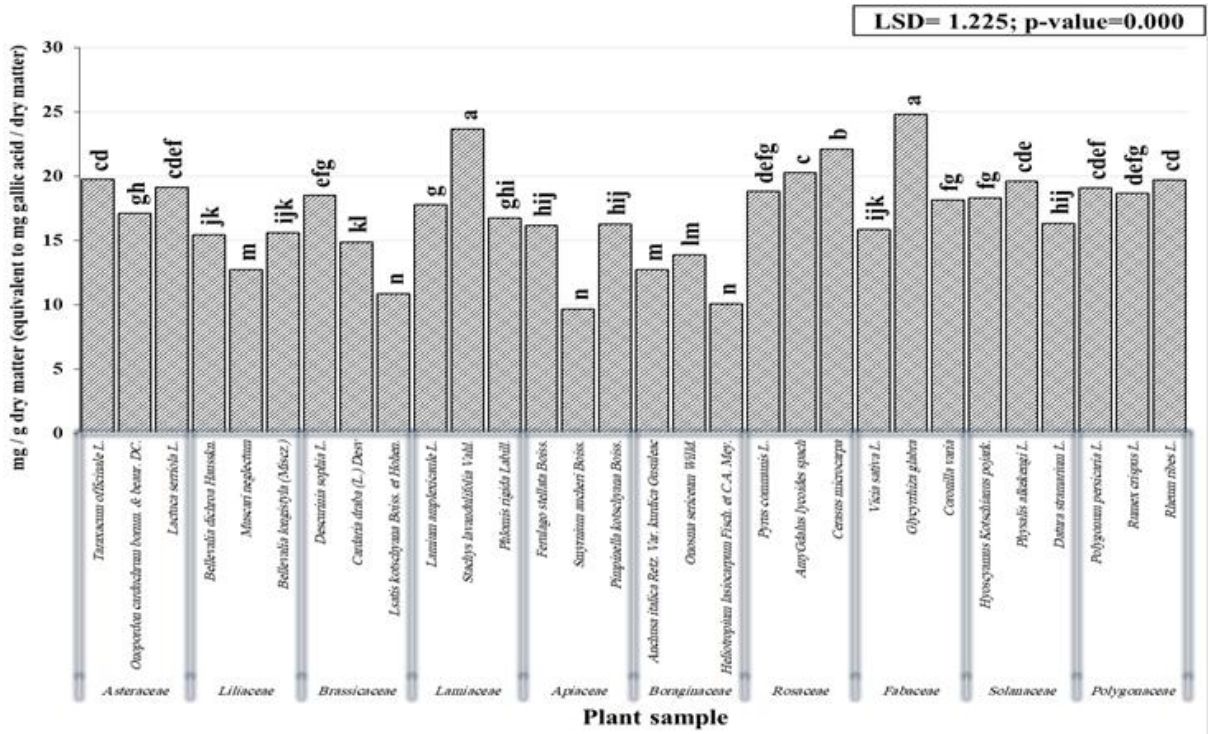

Fig. 10. Comparison of the average of phenolic compounds in the studied plant species under the influence of the plant family.

According to the results (Fig. 11) and regardless of plant species, the highest phenolic compound accumulation was observed in Rosaceae and Fabaceae families, respectively. These species are generally rich in flavonoid compounds. Flavonoids are components of phenolic compounds that play an important role in protecting plant tissues against the damage of UV light. Sunlight has a direct effect on the amount of synthesis of flavonoid compounds. The studied plants from these categories are more widely distributed at altitudes with high

light intensities. The amount of their flavonoid compounds seems to be directly related to the altitude of the habitat. Also, the lowest amount of phenolic compounds was observed in the Apiaceae, while there was no statistically significant difference between this plant family with the Brassicaceae and the Liliaceae families.

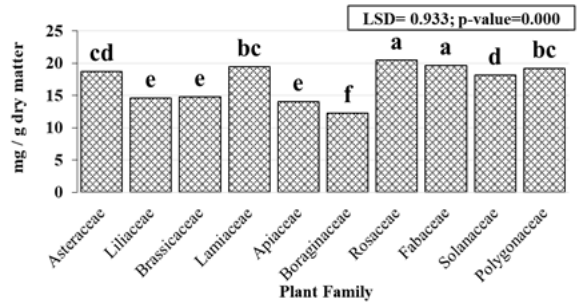

Fig. 11. Mean value for the accumulation of phenolic compounds in plant family regardless of plant species (mg/g dry matter equivalent to mg gallic acid/dry matter).

Evaluation of studied plants based on the amount of flavonoid compounds

Fig. 12 shows the amount of flavonoid compounds in the studied plant species as affected by the plant family. The results showed that the *Taraxacum officinale* L. from the Asteraceae with 10.3 mg/g dry matter showed the highest accumulation of flavonoid compounds. In contrast, the lowest (1.305 mg/g DM) accumulation of these compounds was observed in the *Ferulago stellata* Boiss from Apiaceae. As it has been mentioned before, one of the major roles of flavonoids in plants is to protect the plant against intense UV-B light (wavelength range of 320-380 nm), which absorbs this light range and permits the passage of visible and active light wavelengths for photosynthesis.

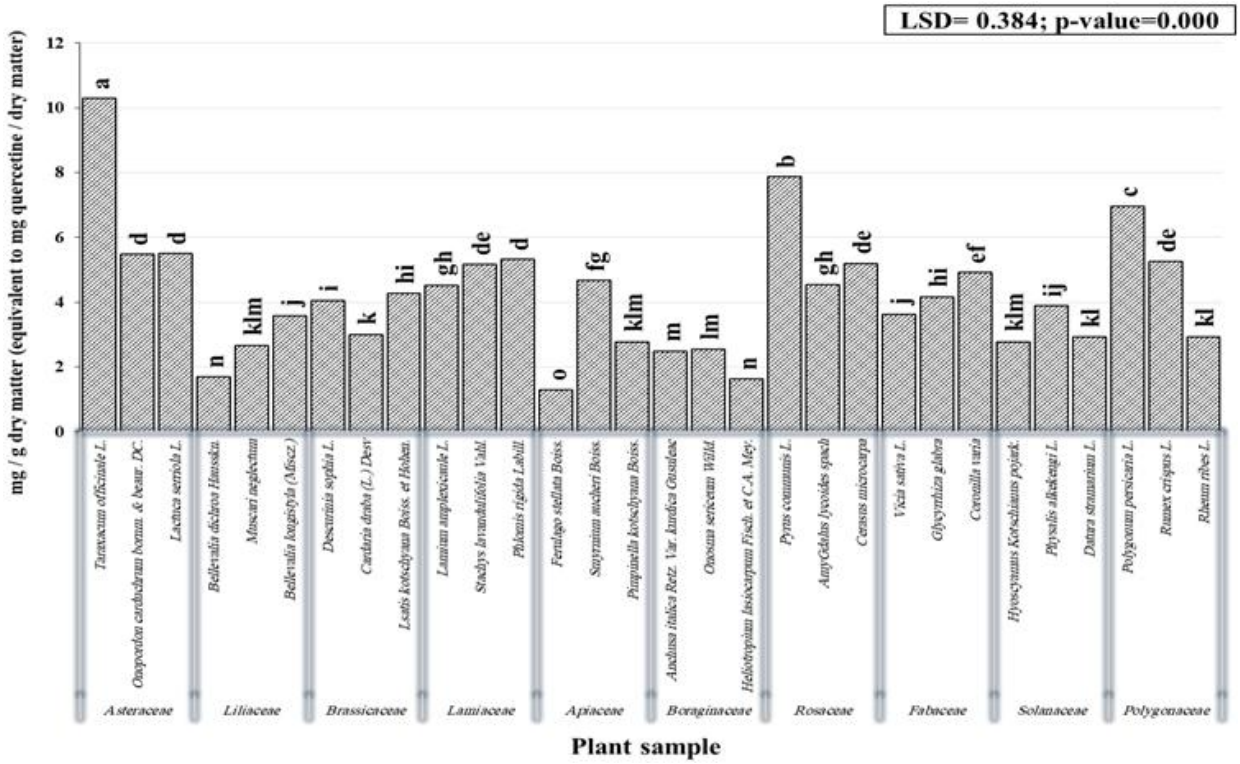

Fig. 12. Mean value of flavonoid of plant species affected by plant family.

The highest accumulation of flavonoid compounds in plant families, regardless of plant species, belonged to the Asteraceae family, and the lowest amount was observed in Boraginaceae (Fig. 13). Table 3 shows the correlation between measured traits. The results showed that there was a positive significant correlation between the antioxidant potential measured by the FRAP and TAC methods. There was also a positive significant correlation between TAC antioxidant

and phenol-flavonoid contents. The results showed that there is a significant and positive correlation between the results of TAC and ABTS measurement. There was also a significant positive correlation between phenolic and flavonoid compounds at 0.01 significant levels (Table 3).

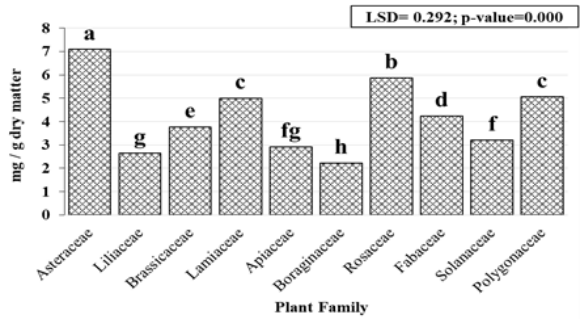

Fig. 13. Mean value of accumulation of flavonoid compounds in the plant family regardless of plant species (mg/g dry matter equivalent to mg quercetin / dry matter).

Table 3. Correlation between measured traits

	DPPH	FRAP	TAC	ABTS	flavonoid	phenol
DPPH	1					
FRAP	0.011	1				
TAC	-0.134	0.262*	1			
ABTS	0.004	0.071	0.285**	1		
Flavonoid	0.056	0.169	0.251*	-0.101	1	
Phenol	-0.18	-0.187	0.472**	-0.07	0.420**	1

* Correlation is significant at the level of 0.05, **. The correlation was significant at the 0.01 level.

Discussion

The Paveh and Oramanat area is a rich source of diverse plants and research to identify and study the medicinal plants in this area is recommended (Hoshidari, 2009). In this region by increasing the altitude the annual rainfall increases (Nemati Paykani and Jaliliyan, 2012). Due to the high-temperature variation and characteristics of the growing location, the study area is susceptible to plants with diverse secondary constituents.

In general, Apiaceae is one of the largest plant families in the world and also shows relatively high diversity in Iran. The diversity of secondary metabolites and the range of their biological activity are noticeable (Amiri and Joharchi, 2016). The antioxidant potential and medicinal importance of some plants of the Apiaceae family is reported (Zengin *et al.*, 2019). The DPPH method is based on electron transfer. One of the prominent features is its ability to react in hydrophobic and hydrophilic environments and measure the antioxidant potential in different extracts. Also, since the above method does not depend on the polarity of the reaction between free radicals and antioxidant compounds, a wide range of compounds can be incorporated into the DPPH radical scavenging reaction (Haghighat, 2016).

In a study by Guvenalp *et al.* (2010) on different species of Pimpinella spp., it has been shown that the highest DPPH radical scavenging potency was observed in *Pimpinella cappadocica* Boiss. & Bal. and *Pimpinella kotschyana* Boiss. plants, which were reported as valuable plants in inhibiting free radicals. In another study, it has been shown that P. kotschyana contains many phenolic compounds including flavonoids, terpenoids, and glycosides (Demirezer *et al.*, 2012). Little information is available on the phytochemical contents of many studied plants, especially concerning antioxidant compounds, for example, Onopordon carduchrum bornm. & beaur. DC, which has been used locally as a medicinal plant for many years (Hoshidari, 2009). The DPPH radical is a large nitrogen-containing compound that cannot readily participate in the reaction to the radical trapping by antioxidant compounds compared to hydrogen peroxide. Species and interspecific diversity in the studied plants have enabled many effective compounds to participate in the reaction, but some of them react sooner or later or sometimes do not react due to the lack of time. The DPPH method is reversible. Even, some compounds may have reacted and returned to their original state in less than the time specified in the protocol. Sarmadi and Ismaila (2010) stated in their research that achieving more transparent results and finding the indicator sample (s) in the study set would require the use of several antioxidant potency measurement methods. This point is also addressed in the present study.

In addition to genetic factors, many climatic factors (environmental and soil) play a role in the amount and structure of secondary constituents in the plant families (Ghorbanighojdari and Ladanmoghadam, 2005).

For example, the Asteraceae and Fabaceae families are mainly known as flavonoid-containing plants, the amount of flavonoid compounds of these plants is strongly related to the topographical conditions of their habitat and the nutritional potency of the soil. The higher the light intensity or the poorer the soil, the more stress the plant will suffer (Tabatabai, 2015). One of the mechanisms of plants against light stress is the production of flavonoid compounds that both absorb or reflect sunlight as an outer protective layer and act as an antioxidant in the plant. The DPPH method measures a wide range of secondary compounds and, at the top, phenolic and flavonoid compounds (Kedare and Singh, 2011). The lowest amount of antioxidant potential was also observed in the Polygonaceae family. Most of the collected plants of this family were collected at lower elevations in the lakes or rivers, or higher elevations in the northern slope. Secondary compounds mainly play a protective role in coping with stresses in the plants. It seems that the reduction of environmental stresses and the existence of ideal growing conditions in the habitat of this plant have reduced the formation of secondary compounds (Moharram and Youssef, 2014). However, achieving more accurate results in this area requires molecular and genetic evaluation.

Evaluation of plant growth habitat characteristics showed that the only common point of the above plants' habitat was the soil pH status (pH = 6.2-6.5). Given that the soils of the growth sites of these plants were acidic to neutral, it can be concluded that many of the macro and microelements required by the plant were available to plants under these conditions which could have a direct effect on the formation of antioxidant compounds. Sayedi *et al.* (2015) in their research reported a significant relationship between pH and the absorption of effective elements on the plant secondary compounds. They showed that phosphorus uptake in Nigella sativa L. decreased with an increase in the soil pH. When the phosphorus of soil is limited, reducing leaf area as well as the number of leaves reduces root canopy and consequently decreases water and nutrient uptake.

This causes drought stress and leads to the formation of more free radicals in the plant. The plant is also forced to make antioxidant compounds to inhibit them. In another study, the presence of sulfur in the soil has been reported as one of the effective factors in the uptake of microelements. Increasing the soil sulfur content increases the activity of micro-organisms of root media, resulting in lower pH and increasing phosphorus uptake (Karimnia and Shabanpour shahrestani, 2003). Many plants increase the accumulation of their secondary metabolites when exposed to stress to resist and cope with the stress condition. It should be noted that the presence of sufficient nutrients in the soil as well as the proper conditions for the uptake of elements such as pH and EC, promotes biomass yield, proper plant growth and creates ideal and stress-free conditions for plant life (Halliwell, 2007; Kafi *et al.*, 2018).

Other reasons for the high antioxidant potential of these plants can be found at the harvest time. Three top plants were harvested in March. Due to the topographic conditions of their habitat and high elevation at that time the weather temperature was low, which induces stress to the plants. Most stresses increase free radical production and force plant cells to produce secondary metabolites as radical scavenging compounds. In a study, Zhang *et al.* (2009) showed that salinity stress, high Na+ concentration of cytosol induces osmotic stress by producing free radicals and active some secondary metabolite pathways to control the ROS (reactive oxygen species) accumulation. The lowest antioxidant potential was also observed in liquorice (*Glycyrrhiza glabra* L.) belonging to the Fabaceae family with 1.02 mg / g dry matter. In addition to the genetic ability to produce antioxidant compounds, environmental factors play an important role in this process. For example, in the present study liquorice was harvested from a site where its soil EC was the lowest among all studied sites. As a result, it faced better-growing conditions than other habitats.

In other words, it can be stated that in this region the stress is in its deficit situation and is not strong enough to stimulate the antioxidant pathways. In non-saline soils, ideal conditions are provided for the growth of the plant, which reduces the production of antioxidant compounds in the plant (Ghorbanighojdari and Ladanmoghadam, 2005). There is not enough

information about the Bellevalia dichroa Hausskn. from the medicinal point of view. Based on the results of the present study and emphasizing the high antioxidant capacity of this plant, further investigation on the medicinal value seems to be necessary. It has been reported Smyrnium aucheri Boiss one of the other plants in this group with high antioxidant potential, shows a strong inhibitory effect on the bacteria and fungi due to the presence of compounds like nachsmyrin, smyrniorin, smyrnioridin, smyrinol, smyrindiol, and smyrindioloside (Faridi *et al.*, 2008).

Synthesis of flavonoid and essential oils depending on the amount of light irradiation and relative humidity, so that the higher the irradiation and the lower the moisture content especially in southern slopes, could be results in a higher amount of these compounds (Taiz and Zeiger, 2002).

Plant growth and function are affected by environmental factors in ecosystems and habitats (Habibi *et al.*, 2007). Altitude by affecting light intensity, temperature, and other environmental factors, soil pH, soil texture, nutrients, organic matter, soil depth, and soil moisture, directly and indirectly, plays an important role in the accumulation of plant secondary metabolites (Mahmoudzadeh Tilami, 2014; Davazdahemami, 2017).

It has been shown that the plants located in places with different UV-B light densities, show differences in the amount of flavonoids (Taiz and Zeiger, 2002). Flavonoid plays a key role in reducing short-wavelength absorption or reflection in plants by creating a protective barrier on the outer layers of the tissues (Ghorbanighojdari and Ladanmoghadam, 2005; Kafi *et al.*, 2018). The presence of Taraxacum officinale L. at low altitude and high temperature of this habitat in warm seasons can produce and synthesize more flavonoid compounds in the plant. However, genetic differences and topographic conditions of the habitat can cause differences in the amount of secondary compounds. In general, more studies need to be considered to obtain more information.

It has been reported that various antioxidant compounds perform specific functions in plants and animals. Until now, a single method that can provide direct and accurate results of all antioxidant compounds present in a sample has not been reported. For this reason, the best solution is to use different methods instead of one (Carocho *et al.*, 2013). Methods for measuring antioxidant and polyphenolic compounds are highly diverse. As stated, using a method to measure antioxidant potency is not available. For example, if using the same laboratory conditions, the FRAP method could yield different results. Because in this method the antioxidant potency is the ability of the compounds to reduce Fe (III) to Fe (II) and any electron-capable material can participate in the FRAP antioxidant reaction. Even these compounds may not be antioxidants. Thus the errors in this method can be very wide (Hosseini *et al.*, 2013).

Research has shown that some antioxidants, such as glutathione, which is a very important antioxidant in the body of living organisms, may not be able to reduce iron (Karadag *et al.*, 2009). It should be noted that unlike the ABTS method which reacts at neutral pH, the FRAP method is acid-dependent and the reaction can be performed at pH 3.6. Also, the antioxidant capacity of the iron-reduction method is oxidized-depletion, which will usually be accomplished in 4-6 minutes. Now some phenolic compounds react in less than four minutes and some in 30 minutes (Prior *et al.*, 2005). It does not appear to be a definite endpoint that indicates a reaction to this method. However, due to the ease of use and the low cost of the material, this method is commonly used to measure the antioxidant capacity of plant extracts (Huang *et al.*, 2005).

DPPH radical scavenging ability is one of the most applicable methods used to investigate the antioxidant capacity of plant extracts. The advantages of this method depending on substrate polarity, high speed, ability to react in lipophilic and hydrophilic environments as well as the ease of equipment required in this method (Hosseini *et al.*, 2013). However, DPPH is large and stable nitrogen radical, and many antioxidants that react immediately with peroxide radicals react very slowly with DPPH (Karadag *et al.*, 2009).

The solubility of this radical is also limited to organic solvents, especially alcohols, and does not dissolve in the aqueous medium (Nateghi,

2010). Another disadvantage of this method is that the reaction between DPPH and free radicals is a reversible reaction, which makes their antioxidant capacity in many cases less than their specified range (Sarmadi and Ismaila, 2010). Orbital access to the DPPH radical has made it more selective than other methods such as ABTS and does not react with any hydroxyl radical (Huang *et al.*, 2005; Haghighat, 2016). One of the most widely used methods in assessing the antioxidant capacity of plant extracts is their ability to trap ABTS+ radicals. The advantages of this method include high reactivity, the ability to react in a wide range of pH, solubility in different solvents, and lipophilic and hydrophilic environments. The ABTS method is a useful and applicable method for measuring the antioxidant potential of a variety of beverages, plant extracts (especially sources containing polyphenolic compounds), and foods (Cadenas *et al.*, 2002; Haghighat, 2016). But this method has high instability at the time of ABTS+ radical formation and thus there is a time limit for its use. ABTS+ radical formation requires a long time (12–24 h) which is relatively high time and also requires an additional pre-test step which is the weak point of this method (Huang *et al.*, 2005). Another disadvantage of this method is the failure to provide a standardized method for doing so, which has reported different results from researchers in relatively identical laboratory conditions (Prior *et al.*, 2005; Nateghi, 2010; Hosseini *et al.*, 2013).

The important point is that this radical can be reduced and deactivated by both two-electron transfer and hydrogen atom transfer mechanisms by the antioxidant compounds, but it will often react with the antioxidant compound via electron transfer mechanism (Rees *et al.*, 2008). One of the advantages of this method is its independence from pH, although it is often used at pH = 4.7 (Nateghi, 2010). Since this radical is soluble in aqueous and organic media, it has not been affected by ionic strength, thus measuring the antioxidant capacity of all hydrophilic and hydrophobic compounds (Zulueta *et al.*, 2009). Liu *et al.* (2014) examined the antioxidant capacity and major free radical scavengers in 110 edible fruits and vegetables in China, to determine the antioxidant power of fruits and vegetables and provide the right diet guide for the general public. Four DPPH, FRAP, ABTS, and TRAP tests were used to evaluate antioxidant capacity.

Conclusion

In this study, the antioxidant potency of 10 widely distributed plant families in the western Oramanat region of Iran was investigated. In general, considering the positive and significant correlation between measured antioxidant parameters in different applied methods, among studied plants *Pimpinella kotschyana* Boiss from the Apiaceae, *Bellevalia dichroa Hausskn* and *Muscari neglectum* Guss of Liliaceae, *Taraxacum officinale* L.*,* and *Onopordon carduchrum* bornm. & beaur. DC from Asteraceae, *Stachys lavandulifolia* Vahl from Lamiaceae, and *Glycyrrhiza glabra* L. from the Fabaceae families were selected as the rich sources of natural antioxidant compounds for future studies. Also, the plant families Liliaceae, Rosaceae, Brassicaceae, and Asteraceae were considered as the index plant family with high antioxidant potential without considering the plant species.

Conflicts of interest

The authors declare that there is no conflict of interest.

References

Afkar S, Zand R. 2020. Genetic relationships of some mint species using seed storage protein pattern. *J Genet Resour* 6: 12-19.

Alamholo M, Nazeri, S. 2014. Assessment of the antioxidant and antibacterial effects of stem and leaf alcoholic extracts of *Dendrostellera lesserti*. *J Microb World* 8: 298-289.

Al-Dabbagh B, Elhaty IA, Elhaw M, Murali C, AlMansoori A, Awad B, Amin A. 2019. Antioxidant and anticancer activities of chamomile (*Matricaria recutita* L.). *BMC Res Notes* 12:1-8.

Amiri MS, Joharchi MR. 2016. Ethnobotanical knowledge of Apiaceae family in Iran: A review. *Avicenna J Phytomed* 6: 621.

Barlow SM. 1990. Toxicological aspects of antioxidants used as food additives. *In Food Antioxidants* 1: 253-307.

Benzie IF, Strain JJ. 1996. The ferric reducing ability of plasma (FRAP) as a measure of

"antioxidant power": the FRAP assay. *Anal Biochem* 239*:* 70-76.

Cadenas E, Packer L. 2002. Handbook of antioxidants. 2nd ed. New York: Marcel Dekker, INQ. 732 p.

Carocho M, Ferreira IC. 2013. A review on antioxidants, prooxidants and related controversy: natural and synthetic compounds, screening and analysis methodologies and future perspectives. *Food Chem Toxicol* 51: 15-25.

Davazdahemami S. 2017. Production of Medicinal Plants Basics, Botany. Talk Publishing. Iran. 215 p.

Demirezer LÖ, Kuruüzüm-Uz A, Guvenalp Z, Simon A, Patocs T. 2012. Further secondary metabolites from *Pimpinella kotschyana*. *Planta Med* 78: PI 406.

Dipalma AA, Di Fino LM, Salvatore SR, D'Ambrosio JM, García-Mata C, Laxalt AM. 2020. Nitro-Oleic acid triggers ROS production via NADPH oxidase activation in plants: a pharmacological approach. *J Plant Physiol* 246: 153128.

Enayati N, Ghafarzadegan R, Hajiaghaie R, Vazirian M. 2017. Comparison of different extraction methods in the extraction of *Senna alexandrina* Mill. *J Med Plants* 16: 160-167.

Faridi P, Ghasemi Y, Gholami, A, Mehregan I, Mohagheghzadeh A. 2008. Antimicrobial essential oil from *Smyrniopsis aucheri*. *Chem Nat Compd* 44: 116-118.

Fathi H, Mohammad SN, Shaki F. 2016. Evaluation of different extraction methods from *Albizia julibrissin* leaves in reducing nausea in chickens. *Complement Med J Faculty of Nursing & Midwifery* 1: 1224-1233.

Fathi H, Mohammadi HR. 2016. Determination of in vitro total phenolic, flavonoid contents and antioxidant capacity of the methanolic extract of *Echium amoenum* L. *Complement*. *Med J Faculty of Nursing & Midwifery* 6: 1-3.

Frankel EN, Meyer AS. 2000. The problems of using one dimensional methods to evaluate multifunctional food and biological antioxidants. *Sci Food Agric* 80: 1925-1941.

Ghahraman A. 2007. Color Flora Iran, 26 vols. Publications of the Institute of Forestry and Rangelands, Tehran.

Ghasemnezhad A, Bagheri Fard A, Asghari A. 2013. Study of the effect of drying temperature on some phytochemical properties of *Cynara scolymus* L. *Eco-phytochem J Med Plants* 1: 10-21.

Ghasemnezhad A, Qasemi y, Hemmati Kh, Ebrahimzadeh M, Qasemi K. 2012. Effect of type of rootstock and fruit tissue on some chemical properties of page mandarin and thampson novel orange. *Plant Prod Res* 19: 43-53.

Ghorbanighojdari H, Ladanmoghadam AR. 2005. An introduction to oxidative stresses and plant strains. Davavin Publishing Institute, Tehran, Iran. 128 p.

Gomes EC, Silva AN, Oliveira MRD. 2012. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. *Oxid Med Cell Longev* 2012.

Guvenalp Z, Ozbek H, Yuzbasioglu M, Kuruuzum-Uz A, Demirezer LO. 2010. Flavonoid quantification and antioxidant activities of some *Pimpinella* species. *Rev Anal Chem* 29: 233-240.

Habibi H, Mazaheri D, Majnoon Hosseini N, Chaeechi MR, Bigdeli M. 2007. Effect of altitude on essential oil and components in wild thyme (*Thymus kotschyanus* Boiss) Taleghan region. Pajouhesh-Va-Sazandegi 19: 2-10.

Haghighat A., 2016. Investigation of antioxidant effect and determination of phenolic and flavonoid content of flowering aerial *Platychaete glaucescens Boiss. and Platychaete aucheri Boiss*. Doctoral thesis. Islamic Azad University of Pharmaceutical Sciences 25-25.

Halliwell B, Chirico S. 1993. Lipid peroxidation: its mechanism, measurement, and significance. *Am J Clin Nutr* 57*:* 715-725.

Halliwell B, Gutteridge JM. 2015. Free radicals in biology and medicine. Oxford University Press, USA.

Halliwell B. 2006. Oxidative stress and neurodegeneration: where are we now? *J Neurochem* 97: 1634-1658.

Halliwell B. 2007. Biochemistry of oxidative stress. *Biochem Soc Trans* 35: 1147-1150.

Hoshidari F. 2009. Medicinal plants of Kurdistan province. *Iran J Med Aromat Plants* 25: 92-103.

Hosseini S, Gharachurlou M, Ghiasitari B, Qavami M. 2013. A review of antioxidant capacity determination methods (basis of reaction, method of work, strengths and weaknesses). *Food Sci Nutr* 11: 89-112.

Hou DX. 2003. Potential mechanism of cancer chemoprevention by anthocyanin. *Curr Mol Med* 3: 149-159.

Huang D, Ou B, Prior RL. 2005. The chemistry behind antioxidant capacity assays. *J Agric Food Chem* 53: 1841-1856.

Ismailzadeh R, Mehdipour SZ, Razavi R. 2017. Investigation of changes in fatty acids and properties of a "oxidant" of Actinitia deliciosa extract in stabilization of sunflower oil. *Food Sci Technol* 14: 125-135.

Jamshidi M, Ahmadi Ashtiani HR, Rezazadeh Sh, Fathi Azad F, Mazandarani M, Khaki A. 2010. Study and comparison of phenolic compounds and antioxidant activity of some native plant species in Mazandaran. *J Med Plants* 9: 177-183.

Kafi M, Borzui A, Salehi M, Camandi A, Masumi A, Nabati J. 2018. Physiology of Environmental stresses in plants. Jihad Press, Mashhad University, Mashhad.

Karadag A, Ozcelik B, Saner S. 2009. Review of methods to determine antioxidant capacities. *Food Anal Methods* 2: 41-60.

Karimnia A, Shabanpourshahrestani M. 2003. Evaluation of sulfur oxidation potential by heterotrophic microorganisms in different soils. *Soil Water Sci* 17: 68-80.

Kassa Z, Asfaw Z, Demissew S. 2020. An ethnobotanical study of medicinal plants in Sheka Zone of Southern Nations Nationalities and peoples regional state, Ethiopia. *J Ethnobiol Ethnomedicine* 16: 7.

Kaviarasan S, Naik GH, Gangabhagirathi R, Anuradha CV, Priyadarsini KI. 2007. In vitro studies on antiradical and antioxidant activities of fenugreek (*Trigonella foenum graecum*) seeds. *Food Chem* 103: 31-37.

Kedare SB, Singh RP. 2011. Genesis and development of DPPH method of antioxidant assay. *Int J Food Sci Technol* 48: 412-422.

Khalili M, Ebrahimzadeh MA. 2015. A review on antioxidants and some of their common evaluation methods. *J Mazandaran Univ Med Sci* 24: 188-208.

Khatami M, Alijani HQ, Fakheri B, Mobasseri MM, Heydarpour M, Farahani ZK, Khan AU. 2019. Super-paramagnetic iron oxide nanoparticles (SPIONs): Greener synthesis using *Stevia* plant and evaluation of its antioxidant properties. *J Clean Prod* 208: 1171-1177.

Khezri Sh. 2011. Culture of medicinal plants in Kurdistan (Medicinal, Oral, Industrial). Kurdistan University Press, Sanandaj, Iran.

Khounani Z, Hosseinzadeh-Bandbafha H, Nizami AS, Sulaiman A, Goli SAH, Aghbashlo M. 2020. Data on environmental analysis of natural antioxidant production from walnut husk by a solar photovoltaic-driven system as a replacement for potentially carcinogenic synthetic antioxidants. *Data Brief* 28: 933-104.

Kim DO, Lee KW, Lee HJ, Lee CY. 2002. Vitamin C equivalent antioxidant capacity (VCEAC) of phenolic phytochemicals. *J Agric Food Chem* 50: 3713-3717.

Kohli SK, Khanna K, Bhardwaj R, AbdAllah EF, Ahmad P, Corpas FJ. 2019. Assessment of subcellular ROS and no metabolism in higher plants: multifunctional signaling molecules. *Antioxidants* 8: 641.

Kumbhakar DV, Datta AK, Das D, Ghosh B, Pramanik A, Gupta S. 2019. Assessment of oxidative stress, antioxidant enzyme activity and cellular apoptosis in a plant based system (*Nigella sativa* L.; black cumin) induced by copper and cadmium sulphide nanomaterials. *Environ Nanotechnol Monit Manag* 11: 100196.

Lee SJ, Umano K, Shibamoto T, Lee KG. 2005. Identification of volatile components in basil (*Ocimum basilicum* L.) and thyme leaves (*Thymus vulgaris* L.) and their antioxidant properties. *Food Chem* 91: 131-137.

Liu C, Zhao Y, Li X, Jia J, Chen Y, Hua Z. 2014. Antioxidant capacities and main reducing substance contents in 110 fruits and vegetables eaten in China. *Food and Nut. Sciences* 5: 293.

Maghsoudi A, Abbaspur H, Ghasemipirbalouti A, Saeedisar S. 2018. An overview of the environmental impact on secondary compounds and the regulation of the expression of terpenic synthases in medicinal plants. *International Conference on New Agricultural Findings* 1: 4.

Maghsudlu A, Esmaeilzadeh Kenari R, Raftani Amiri Z. 2016. Evaluation of antioxidant properties of fig and pulp extracts on oxidative stability of canola oil as a synthetic antioxidant replacement. *J Food Sci Tech* 3: 503-516.

Mahmoudzadeh Tilami Z. 2014. Effect of some ecological factors on the quantity and quality of essential oil of *Marrubium vulgare* in Chahar Bagh rangelands of Golestan province. Master thesis. Gonbad Kavous University. *Faculty of Agriculture and Natural Resources*. 4-25.

Mohadjerani M, Naqinezhad A, Aghasizadeh Sharbaf M. 2016. Platycladus orientalis extracts with antioxidant activity from north of Iran. *J Genet Resour* 2: 60-66.

MohammadNejad Ganji SM, Moradi H, Ghanbari A, Akbarzadeh M. 2014. Investigating the effect of Altitute on the quantity and quality of essential oils of *Rosmarinus officinalis* L. cultivated in two regions of Mazandaran province. *Echo-phytochem J Med Plants* (*EJMP*) 5 (1): 36-42.

Moharram HA, Youssef MM. 2014. Methods for determining the antioxidant activity: A review. *J Food Sci Technol* 11: 31-42.

Mortezaeinejad F, Nowruz M. 2011. Identification and introduction of rare and endangered plants in Isfahan province. First National Conference on Health, Environment and Sustainable *Development of Islamic Azad University, Bandar Abbas Branch* 1: 1-9.

Mozaffariyan V, Sanandaji S. 2009. Saral flora of Kurdistan. *Mag. Taxo.and biosystematics* 2: 59-84.

Mozaffariyan V. 1996. Culture of plants in iran. *Contemporary Publications*, Tehran. 740 p.

Mozaffariyan V. 2011. Recognition of Iranian Medicinal Plants. *Contemporary Publications*, Tehran. 1444 p.

Mozdstan Sh, Ebrahimzadeh MA, Khalili M. 2015. Comparison of the Importance of Different Extraction Methods on Antioxidant Activity of *Myrtus communis* Leaf. *J. Maza. Uni. of Medical Sci,* 25: 10-24.

Nateghi A. 2010. Optimization of extraction of natural antioxidants from rice bran by response surface method and antioxidant activity of some edible plants of Western Iran. Master thesis. Ilam University, Faculty of Basic Sciences. Iran 14-31.

Nemati Paykani M, Jaliliyan N. 2012. Medicinal plants of Kermanshah province. *J Taxo Biosystematics* 4: 69-78.

Omidbaigi R. 2005. 8- Production and processing of medicinal plants. Tehran University. 348 p.

Ordonez AAL, Gomez JD, Vattuone MA. 2006. Antioxidant activities of *Sechium edule* (Jacq.) Swartz extracts. *Food Chem* 97: 452-458.

Prakash V, Singh VP, Tripathi DK, Sharma S, Corpas FJ. 2019. Crosstalk between nitric oxide (NO) and abscisic acid (ABA) signalling molecules in higher plants. *Environ Exp Bot* 161: 41-49.

Pratt D, Hudson BJF. 1990. Natural antioxidants not exploited commercially. *Food antioxidants*. Springer, Dordrecht (pp.171-191).

Prevc T, Šegatin N, Ulrih NP, Cigić B. 2013. DPPH assay of vegetable oils and model antioxidants in protic and aprotic solvents. *Talanta* 109: 13-19.

Prior RL, Wu X, Schaich K. 2005. Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *J Agric Food Chem* 53: 4290-4302.

Rechinger KH. 2005. Flora Iranica, vol 1-176. Akademische Druck-u. Verlagsanstalt.-Graz.Austria.

Rees MD, Kennett EC, Whitelock JM, Davies MJ. 2008. Oxidative damage to extracellular matrix and its role in human pathologies. *Free Radic Biol Med* 44: 1973-2001.

SafiKhani K, Rahimi Nejad Ranjbar MR, Kolwandi R. 2006. Flouristic study and determination of biodiversity of plants of khangarms protected area in Hamedan

province. *Pajouhesh-Va-Sazandegi* 19: 70-78.

Sarmadi BH, Ismail A. 2010. Antioxidative peptides from food proteins: A review. *Peptides* 31: 1949-1956.

Sayedi SM, RezvaniMoghadam C, KhajehHosseini M, Shahande H. 2015. The role of sulfur, vermicompost and thiobacillus on some chemical properties of calcareous soils and black phosphorus use efficiency. *J Plant Physiol* 22: 205-220.

Smsam Shariat S. 2007. Selection of medicinal plants. Manny Publishing. Esfahan 610 p.

Stagos D. 2020. Antioxidant activity of polyphenolic plant extracts. *Antioxidants* 9: 19.

Suhaj M. 2006. Spice antioxidants isolation and their antiradical activity: a review. *J Food Compost Anal* 19: 531-537.

Sun L, Zhang J, Lu X, Zhang L, Zhang Y. 2011. Evaluation to the antioxidant activity of total flavonoids extract from persimmon leaves. *Food Chem. Toxicol* 49: 2689-2696

Tabatabai J. 2015. The principles of mineral nutrition of plants. Tabriz University. Tabriz. Iran. 562 p.

Taheri A, Ghaffari M, Bagherpour NS, Ata Ron Freiman G. 2017. Study of antioxidant properties of Cystoseira trinodis extracts from Chabahar beaches. *J Shahid Sadoughi Uni Medical Sci Health Services* 25: 658-669.

Timurzadeh A, Ghorbani A, Kavianpour AH. 2015. Investigation of Flora, Biological Form and Choreology of Plants in the South-East Forest of Namin County in Ardabil Province. *J. Plant Res* 28: 265-275.

Taiz L, Zeiger E, Plant Physiology. 2002. Plant physiology, sinauer associates.

Wu HC, Chen HM, Shiau CY. 2003. Free amino acids and peptides as related to antioxidant properties in protein hydrolysates of mackerel (*Scomber austriasicus*). *Food Res Int* 36: 949-957.

You L, Zhao M, Regenstein JM, Ren J. 2010. Changes in the antioxidant activity of loach (*Misgurnus anguillicaudatus*) protein hydrolysates during a simulated gastrointestinal digestion. *Food Chem* 120: 810-816.

Zargari A. 2014. Medicinal Plants. Volume 1-5. Tehran University Press, Tehran. 4274 p.

Zengin G, Mahomoodally MF, Paksoy MY, Picot-Allain C, Glamocilja J, Sinan KI. 2019. Phytochemical characterization and bioactivities of five Apiaceae species: Natural sources for novel ingredients. *Ind Crops Prod* 135: 107-121.

Zhang L, Tian LH, Zhao JF, Song Y, Zhang CJ, Guo Y. 2009. Identification of an apoplastic protein involved in the initial phase of salt stress response in rice root by two-dimensional electrophoresis. *Plant Physiol* 149: 916-928.

Zohary M. 1973. Geobotanical foundations of the Middle East, 2 Vols. Stuttgart. 739 p.

Zulueta A, Esteve MJ, Frígola A. 2009. ORAC and TEAC assays comparison to measure the antioxidant capacity of food products. *Food Chem* 114: 310-316.