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# Variation in Fruit Morphological Traits and Bioactive Compounds in Different Populations of *Ferula assa-foetida*, *F. gummosa*, and *F. ovina* Collected from Iran

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## ABSTRACT

**Variability in morphological traits, phenolic content, and antioxidant activity of 15 populations of Iranian *Ferula* species collected from natural habitats in different regions of Iran were investigated. Total Phenolic Content (TPC) of fruit extracts varied from 12.77 to 120.72 mg tannic acid per 1g dry weight. Total flavonoid of fruits extract varied from 5.45 mg quercetin per 1 g dry weight in *F. assa-foetida* to 8.09 mg QUE $g^{-1}$ in *F. ovina*. Antioxidant activity of fruits was assessed using three model systems. Fruits extract showed excellent radical scavenging activity as compared to BHT. Similar trend was also obtained in reducing power (FTC) and *β*-carotene-linoleic acid model systems. The cluster analysis subdivided the populations in three major groups. Group 1 possessed high inhibition of beta-carotene (> 60%), while group 2 showed low percent of inhibition (< 35%). Group 3 revealed the lowest TPC, TFC, and antioxidant activity. Most of *Ferula* populations (group 1) were more potent for scavenging of free radicals in lipid phases in comparison with aqueous phase. In this study, some fruit morphological traits were also measured in populations. Among the studied species, *F. gummosa* had the highest fruit length and thousand kernel weight and length/width ratio as well as high antioxidant activity. In overall, the results revealed the scientific basis for traditional usage of the studied *Ferula* species as spice plants and their potential as a rich source of natural antioxidant and flavonoid source.**

**Keywords**: Diversity, Environmental factors, Flavonoid, Phenolic correlation.

## INTRODUCTION

Nowadays, application of different medicinal plants has been widely studied for their preservative and pharmaceutical properties. Many of medicinal plants extracts can be used as natural compounds in food industry (Moghaddam and Mehdizadeh, 2015). Moreover, herbs and spices are amongst the most important targets to search for natural antioxidants from a safety point of view (Exarchou *et al.*, 2002; Jabri Karoui *et al.*, 2016). These properties are due to their many active phytochemicals such as phenolic compounds, flavonoids, terpenoids, and carotenoids (Gharibi *et al.*, 2013; Isbilir and Sagiroglu, 2013). Antioxidant activity is believed to be mainly due to redox properties of extracts (Zheng and Wang 2001) as they play an important role in absorbing and neutralizing free radicals, quenching singlet and triplet oxygen, or decomposing peroxides. Finally, to gain the herbal extracts or spices with acceptable criteria in food industry, the selection of the best populations in each plant species is crucial.

*Ferula* is a genus of perennial herbs belonging to the Apiaceae family. This genus comprises about 170 species distributed in central Asia, Mediterranean region, and Northern Africa (Sahebkar and Iranshahi, 2011; Jalali *et al*., 2013). Thirty

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two species of this genus have been represented in Flora Iranica (Rechinger, 1963)، in which some are endemic to Iran (Kanani *et al.*, 2011; Jalali *et al.*, 2013). Among the Iranian *Ferula* species, *F. assa-foetida, F. gummosa*, and *F. ovina* have wide distribution in many geographical regions of Iran (Rechinger, 1963). *F. assa-foetida* is a perennial herb with an unpleasant odor and is often considered to be the main source of oleo-gum-resin which has a characteristic of sulfurous odor and bitter taste (Mahendra and Bisht 2012; Kavoosi and Rowshan, 2013). In traditional medicine, the plant is used for the treatment of different diseases, such as asthma, epilepsy, stomachache, flatulence, intestinal parasites, weak digestion and influenza (Kavoosi and Rowshan, 2013). *F. gummosa* is rich in oleo-gum resin (Mortazaienezhad and Sadeghian, 2006). These exudates are commonly referred to as galbanum (Nadjafi *et al*., 2006). *F. ovina* is also rich in gums and it is considered as a spice in Iranian traditional medicine (Radulovic *et al.*, 2013).

Nowadays, the use of natural antioxidants instead of synthetic ones has been improved in food industries (Baharfar *et al.*, 2015; Kumar *et al.*, 2016). Many of medicinal plants can be added in the foods as natural spices. Phytochemical content and antioxidant properties are influenced by genetic variation, the degree of maturity at harvest and fruit size (Goyali *et al.*, 2013). In the genus of *Ferula,* the fruits are considered as valuable parts in respect to their flavonoids and antioxidant activity. So, the fruits of some species such as *F. ovina* are used in traditional Iranian medicine as flavoring agent or ingredients of spices and condiments (Radulovic *et al*., 2013). In Apiaceae family, the fruit morphological traits are highly important. Some of these traits can affect the total yield of product and the metabolites content (Bahrami *et al.*, 2013). The fruit morphological variation was reported in some Apiaceae plants such as the genus of *Heracleum* (Yu *et al.*, 2011), *Oxypolis* and *Ptilimnium* (Feist *et al.*, 2012) and *Trachyspermum ammi* (Chatterjee *et al.*, 2013). Higher antioxidant and flavonoid contents were reported in the fruits of Apiaceae plants compared with their vegetative parts (Rahimmalek *et al.*, 2014). Furthermore, the fruit morphology can also affect the total yield as well as secondary metabolites content (Sedláková *et al.*, 2003). In spite of high variation in fruit morphology, there are no comparative reports in *Ferula* species in Iran. Moreover, there are no comparative researches on antioxidant activity and flavonoid contents of *Ferula* species. Most of the researches in *Ferula* were conducted using one species originating from a limited geographical area (Nabavi *et al.*, 2011; Bahrami *et al.*, 2013). *F. assa-foetida, F. gummosa* and *F. ovina* are considered as the most important *Ferula* species in Iran, but most of the studies have focused on *F. assa-foetida* and *F. gummosa* oleo gum resin (Kavoosi and Rowshan, 2013) or few reports on antioxidant activity of other parts such as flower, stem, and leaves are available (Nabavi *et al.*, 2010) and there are no comparative reports among and within species in respect to phenolic, flavonoid content and antioxidant activity of fruits and their relationships with fruit morphological traits in these *Ferula* species.

The aims of the present study were: (1) To evaluate diversity among and within 15 *Ferula* populations belonging to three *Ferula* species including *F. assa-foetida, F. gummosa* and *F. ovina* in respect to their total phenolic and flavonoid content; (2) To evaluate the antioxidant activity of fruit extracts using three model systems in order to compare the extracts in aqueous and lipid phases; (3) To compare the fruit morphological traits, and (4) To assess the probable relationships of soil characteristics with evaluated metabolites.

## MATERIALS AND METHODS

All chemicals used in this study were obtained from Sigma–Aldrich or Merck.



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## Plant Materials

The fruits of fifteen populations of *Ferula* belonging to three species including *F. assa-foetida, F. gummosa* and *F. ovina,* were collected from Western and Central regions of Iran in full maturity stage (Table 1). Each sample was labeled and its location was recorded using a Global Positioning System (GPS, Vista Garmin) receiver. Climatic conditions of natural habitats were determined using data of the nearest meteorological station. Environmental properties, soil physical and chemical characteristics of different locations, including pH and organic matter content were determined and are presented in Table 1.

## Methanolic Extract and Total Phenolic Content

Phenolic extraction was carried out using 10 g of leaf powder and 200 mL of 80% methanol with orbital shaker (150 rpm) at 25°C for 24 hours. Then, the extracts were filtered through four layers of cheesecloth to remove the solid debris. The extraction was done four times. The total phenolics were determined colorimetrically using Folin-Ciocalteu reagent as described by Pinelo *et al.* (2004). In this regard, ten-fold diluted Folin-Ciocalteu reagent (2.5 mL), 7.5% sodium carbonate (2 mL), and methanolic extract (0.5 mL) were mixed; then, after heating at 45°C for 15 min, the absorbance was measured at 765 nm against a blank. The phenolic content was expressed as milligram tannic acid equivalent per gram dry weight of the sample.

## DPPH Scavenging Activity

The antioxidant activity of fruit extracts and standard antioxidant was assessed on the basis of the radical scavenging effect on 1, 1-DiPhenyl-2-PicrylHydrazyl (DPPH) free radical (Braca *et al.*, 2002). The BHT was used as the standard antioxidant in 1-100 µg $mL^{-1}$ solution. Radical scavenging activity of

**Table 1**. Collection site, geographical and soil characteristics of different ferula populations.

| No. | Accession name | Collection site | Latitude | Longitude | Altitude (m) | Clay | Silt | Sand | Organic matter% | Acidity |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Fa1 | Khoigan, Isfahan, Iran | 32° 39' 16" N | 51° 40' 4" E | 2440 | 18.4 | 26.1 | 24.9 | 1.4 | 7.5 |
| 2 | Fa2 | Fereydun Shahr, Isfahan, Iran | 32°56'28" N | 50°07'16" E | 2490 | 26 | 36.3 | 37.5 | 2.7 | 7.6 |
| 3 | Fa3 | Afus, Isfahan, Iran | 33°02'36" N | 50°09'27" E | 2450 | 22 | 41 | 37 | 2.21 | 7.8 |
| 4 | Fa4 | Padena, Isfahan, Iran | 30° 57' 59" N | 51°26'15" E | 2400 | 19 | 56 | 25 | 3.76 | 7.5 |
| 5 | Fa5 | Agche, Isfahan, Iran | 33° 4' 42" N | 50° 3' 21" E | 2696 | 29.2 | 34 | 36.4 | 1 | 7.7 |
| 6 | Fa6 | Yazd, Yazd, Iran | 31°53'49" N | 54°22'02" E | 1236.2 | 26.8 | 30.8 | 42.4 | 0.6 | 7.9 |
| 7 | Fo1 | Shikh Alikhan, Charmahal & Bakhtiari, Iran | 29° 36' 41" N | 50°45'29" E | 2360 | 34 | 45 | 21 | 2.1 | 7.4 |
| 8 | Fo2 | Yasuj, Kohgiluye va Boyerahmad | 30°40'05" N | 51°35'16" E | 2048 | 30 | 43 | 27 | 4.38 | 7.3 |
| 9 | Fo3 | Khoigan, Isfahan, Iran | 32° 39'16" N | 51° 40' 4" E | 2440 | 18.4 | 26.1 | 24.9 | 1.4 | 7.5 |
| 10 | Fo4 | Fereydun Shahr, Isfahan, Iran | 32°56'28" N | 50°07'16" E | 2490 | 26 | 36.3 | 37.5 | 2.7 | 7.6 |
| 11 | Fo5 | Meidanak, Isfahan, Iran | 32°65'46" N | 51°66'79" E | 2250 | 32 | 52 | 16 | 0.89 | 7.9 |
| 12 | Fo6 | Semirom, Isfahan, Iran | 31° 27'39" N | 51° 37'23" E | 2460 | 24 | 47 | 29 | 4.4 | 7.5 |
| 13 | Fo7 | Kooh Pardenjan, Charmahal & Bakhtiari, Iran | 32° 19'53" N | 50°51'86" E | 2300 | 42 | 39 | 19 | 2.9 | 7.7 |
| 14 | Fg1 | Shirmard, Charmahal & Bakhtiari, Iran | 29° 0' 45" N | 51° 21' 9" E | 2400 | 42 | 44 | 14 | 1.6 | 7.8 |
| 15 | Fg2 | Chogyurt, Isfahan, Iran | 32° 58' 40"N | 50° 0' 51" E | 2350 | 14 | 49 | 37 | 1.3 | 7.5 |



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the extracts was calculated by the following formula:

% Radical scavenging activity= (Control OD-Sample OD/control OD)×100

Methanol (80%) and DPPH solution (0.1 mM, 5 mL) were used separately as a blank and control sample, respectively.

### Antioxidant Activity Using *β*-Carotene-linoleic Acid Model System

This assay was conducted according to the method developed by Gursoy *et al.* (2009) with minor modifications. For this evaluation, 0.5 mg *β*-carotene was dissolved in 1 mL of chloroform and 25 µL linoleic acid and 200 mg Tween 80 were added to prepare the stock solution. The solvent was evaporated by a vacuum evaporator and 100 mL of oxygenated distilled water was added with vigorous shaking. Then, 2.5 mL of reaction mixture was dispersed intest tubes and 0.5 mL of various concentrations (0.5-5 mg per 1 mL) of the extracts and BHT was added and the mixture was incubated at 50°C. All solvents and chemicals were obtained from Merck (Darmstadt, Germany). The absorbance was measured at zero time (t= 0) at 490 nm. Absorbance reading was continued at an interval of 25 min until the color of *β*-carotene disappeared in the control tubes (t= 125 min). Antioxidant activity was expressed as the percentage of inhibition in relation to control according to an equation proposed by Kulisic *et al*. (2004):

% Inhibition$_{=}$ $[(A_{A(125)}-A_{C(125)})/(A_{C(0)}-A_{C(125)})]\times100$

Where, $A_{C(0)=}$ Absorbance of Control at the moment of solution preparation; $A_{C(125)=}$ Absorbance of Control after incubation for 125 minutes, and $A_{A(125)=}$ Absorbance of sample after incubation for 125 minutes.

### Reducing Power

The extracts (2.5 mL) and BHT were mixed with 2.5 mL of 1% potassium ferricyanide and 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and incubated at 50$^{o}$C for 20 minutes. Then, 2.5 mL of 10% trichloroacetic acid was added and the mixture was centrifuged at 200×g for 10 minutes. The upper layer (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. The absorbance at 700 nm was measured against a blank. The increased absorbance of the reaction mixture correlates with greater reducing power (Ardestani and Yazdanparast, 2007).

### Total Flavonoids Evaluation

Total flavonoids contents of different *Ferula* was evaluated by aluminium chloride colorimetric method as described by Braca et al (2002). In this assay, sodium nitrate 5% and sodium hydroxide 4% were prepared in different containers. Then, 10% aluminum chloride solution was also prepared. The extracts (0.25 mg) mixed with water (1.25 mL) and sodium nitrate (0.75 µL). Then, the test tubes were kept in the dark for six min. Later, 10% aluminum chloride (0.150 µL) was added and maintained for five minutes in the dark place to complete the reaction. Finally, the solution of 5% sodium hydroxide (0.5 mL) and water (0.275 mL) were also mixed. The absorbance of the samples was read at 510 nm. In this assay, quercetin standard was applied for the calibration curve. The estimation of total flavonoids contents in the extracts was done in triplicate and the results were stated as quercetin equivalent per gram dry weight of sample.

## Morphological Analysis

The major morphological traits of all populations were measured in three replicates at collection sites and the mean value was used for the analysis. Morphological traits that were assessed



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in this study were fruit length (cm), width (cm), thickness (cm), length/width ratio, and weight of 1,000 seeds (g). Data collection was carried out for five plants in each replicates.

### Statistical Analysis

In this research, treatments were performed in triplicate, and analysis of variance on data from the test measures of the total amount of phenolic compounds and the evaluation of antioxidant activity were performed in a completely randomized design. Mean separation was conducted according to the Least Significant Difference (LSD) at probability level of 5%. Cluster analysis and calculation of correlation were performed based on Ward's minimum variance method using SPSS ver. 18. All analysis was performed using SAS and Excel software.

## RESULTS AND DISCUSSION

### Total Phenols Content (TPC)

The extraction yield and TPC of fruits are presented in Table 2. High variation was observed among and within the three *Ferula* species. The extraction yield ranged from 10.94 (g 100 $g^{-1}$) in Fa5 to 13.7 (g 100 $g^{-1}$) in Fo1. TPC of fruit extracts of *F. ovina* varied from 26.86 to 120.72 mg tannic acid per 1 g dry weight of the samples. The lowest TPC was obtained in fruits extract (12.77 mg TAE $g^{-1}$ DW) in *F. assa-foetida* of Khoigan population. The similar ranges (55.56-90.14 mg GAE $g^{-1}$) for TPC were also reported by Kavoosi and Rowshan (2013) in *F. assa-foetida* oleo-gum resin. Furthermore, in other *Ferula* species such as *F. lutea* similar ranges (40.68-52.29 mg GAE $g^{-1}$) were also reported (Znati *et al.*, 2014). In overall, plant polyphenols possess high variation in their structure and general classification, but all

**Table 2.** Extraction yields, total phenolics, total flavonoids, inhibition of beta-carotene and reducing power (FTC) of seed extracts of *Ferula* populations.[a]

| populations | Extraction yield (g 100 $g^{-1}$) | Total Phenolic Content (TPC) (mg $g^{-1}$) | Total Flavonoids (mg QUE $g^{-1}$) | Inhibition (%) of $\beta$-carotene | Reducing power (FTC) absorbance at 700 nm |
|---|---|---|---|---|---|
| Fa1 | $12.29^{e}$± .28 | $12.77^{l}$ ± 0.45 | $5.45^{l}$ ± 0.11 | $20.77^{k}$± 0.11 | $0.23^{k}$± 0.34 |
| Fa2 | $12.75^{cd}$ ± 0.2 | $84.63^{d}$ ± 5.41 | $7.72^{cd}$ ± 0.21 | $80.21^{bc}$± 1.68 | $0.56^{c}$± 0.73 |
| Fa3 | $11.36^{f}$ ± 0.7 | $12.94^{l}$ ± 0.89 | $5.73^{k}$ ± 0.16 | $22.62^{k}$± 0.18 | $0.24^{jk}$± 0.21 |
| Fa4 | $12.92^{bc}$ ± 1.57 | $32.18^{hi}$ ± 0.67 | $6.8^{g}$ ± 0.18 | $63.26^{f}$± 0.71 | $0.29^{g}$± 0.17 |
| Fa5 | $10.94^{g}$ ± 1.64 | $34.96^{h}$ ± 1.26 | $7.28^{f}$ ± 0.14 | $73.33^{e}$± 1.42 | $0.32^{f}$± 0.41 |
| Fa6 | $11.43^{f}$ ± 1.23 | $78.19^{e}$ ± 0.16 | $7.6^{de}$ ± 0.07 | $78.92^{c}$± 1.54 | $0.4^{d}$± 0.51 |
| Fo1 | $13.7^{a}$ ± 0.64 | $26.86^{j}$ ± 0.65 | $6.22^{ij}$ ± 0.10 | $26.52^{j}$± 0.41 | $0.26^{hij}$± 0.38 |
| Fo2 | $12.42^{de}$ ± 0.47 | $27.85^{j}$ ± 0.23 | $6.43^{hi}$ ± 0.25 | $33.72^{i}$± 0.28 | $0.27^{ghi}$± 0.43 |
| Fo3 | $12.82^{c}$ ± 0.4 | $29.68^{ij}$ ± 0.28 | $6.65^{gh}$ ± 0.20 | $40.84^{h}$± 0.31 | $0.28^{gh}$± 0.36 |
| Fo4 | $12.09^{e}$ ± 0.54 | $90.98^{c}$ ± 0.3 | $7.85^{bc}$ ± 0.25 | $80.73^{bc}$± 1.61 | $0.55^{c}$± 0.72 |
| Fo5 | $13.26^{b}$ ± 0.44 | $39.44^{g}$ ± 0.36 | $7.36^{f}$ ± 0.13 | $75.83^{d}$± 0.67 | $0.38^{e}$± 0.45 |
| Fo6 | $12.21^{e}$ ± 0.47 | $47.74^{f}$ ± 0.6 | $7.48^{ef}$ ± 0.20 | $78.27^{cd}$± 1.83 | $0.39^{de}$± 0.52 |
| Fo7 | $11.61^{f}$ ± 0.5 | $120.72^{a}$ ± 0.39 | $8.09^{a}$ ± 0.15 | $92.28^{a}$± 1.11 | $0.9^{a}$± 1.12 |
| Fg1 | $13.64^{a}$ ± 0.55 | $99.03^{b}$ ± 0.71 | $8.02^{ab}$ ± 0.04 | $82.12^{b}$± 0.76 | $0.69^{b}$± 0.81 |
| Fg2 | $12.83^{c}$ ± 0.75 | $22.09^{k}$ ± 0.67 | $6.07^{j}$± 0.12 | $22.08^{k}$± 0.24 | $0.25^{ijk}$± 0.29 |
| BHT | - | - | - | $50.73^{g}$± 0.12 | $0.12^{l}$± 0.25 |
| Total | 12.41± 0.22 | 50.67± 2.09 | 6.98± 0.13 | 58.10±1.48 | 0.38± 0.46 |

[a] The results are expressed as means±*SD* (n= 3). In each column, different letters mean significant differences (P< 0.05).



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share the common feature of at least one aromatic ring and one or more hydroxyl groups (Moghaddam and Mehdizadeh, 2015). In the present research, high variation among and within species was obtained. This results can provide new insights for further selection in respect to phenolic content of *Ferula* species for food or pharmaceutical applications. So, in respect to TPC, Fo7, Fg1 and Fa2 can be introduced for future researches.

## Total Flavonoid Content (TFC)

TFC of fruits extract varied from 5.45 mg quercetin per 1g dry weight in *F. assa-foetida* of Khoigan (Fa1) to 8.09 mg QUE $g^{-1}$ in *F. ovina* collected from Chaharmahal and Bakhtiari (Fo7) (Table 2). This amount is lower than flavonoid content obtained in oleo-gum resin in *F. assa-foetida* (Kavoosi and Rowshan, 2013). Previous reports revealed that, in *Ferula* species, total phenol and flavonoid contents at the earlier growth stages were higher than the late growth stages and fruit ripening stage. This reduction of phenolic and flavonoid content could be attributed to biosynthetic pathway of this compounds and differential expression of the related enzymes in the different growth stages of the plants (Mamati *et al.*, 2006; Kavoosi and Rowshan, 2013). The antioxidant activity of flavonoids probably occurred through scavenging or chelation mechanism and their hydroxyl groups can confer scavenging ability and their effects on human nutrition and health (Prathapan *et al.* 2011). Thus, Fo7, Fg1 and Fa2 can be introduced as high flavonoid populations in each species.

## DPPH Scavenging Test

The ability of different extracts of *Ferula* populations to quench DPPH free radical was measured by the decrease in its absorbance at 517 nm induced by antioxidants. The effect of antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability. The extracts and BHT demonstrated a dose-dependent scavenging activity by reducing DPPH radical (Figure 1). Fruits extract showed excellent radical scavenging activity as compared to BHT, ranging from 14.89% in Fa1 to 95.98% in Fo7 (Figure 1). Previous studies revealed that methanol extracts from some *Ferula* species had moderate antioxidant activity and radical scavenging. The radical scavenging might be attributed to the presence of phenols, flavonoid, and

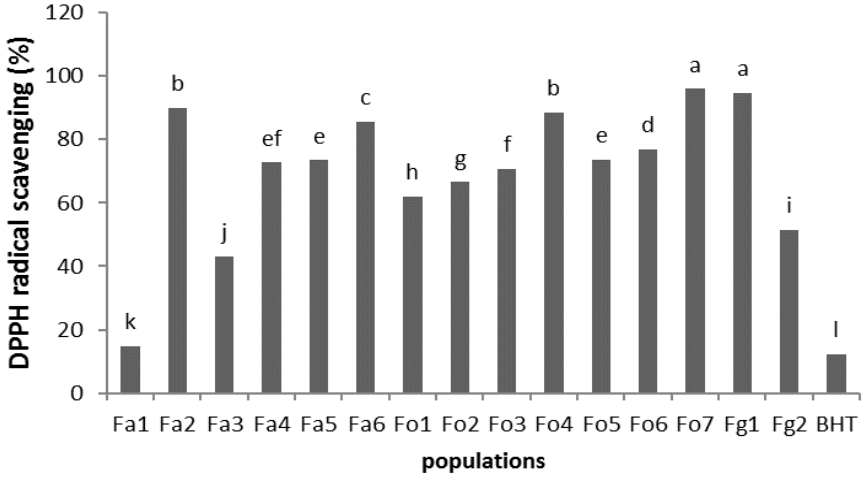


**Figure 1.** DPPH radical scavenging (%) of 15 *Ferula* populations compared with BHT. Different small letters represent significant difference at $P< 0.05$.



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sesquiterpenes in the extracts (Ibraheim *et al*., 2012). These compounds may be the main cause of its considerable radical-scavenging activity.

One of the major phenolic acids in *Ferula* species is ferulic acid. For the first time, ferulic acid was isolated from *Ferula foetida* for its structure determination, and its name was based on the botanical name of plant (Hlasiwetz and Barth, 1866). Like several other phenols, Ferulic Acid (4-hydroxy-3-methoxycinnamic acid, FA) also exhibits high antioxidant activity in response to free radicals via donating one hydrogen atom from its phenolic hydroxyl group (Natalia *et al.*, 2013). The resonance stabilization of ferulic acid is the main cause of its antioxidant nature (Graf, 1992). High antioxidant activity of the studied *Ferula* species might be attributed to high ferulic acid content of these species.

## Reducing Power

Reducing capacities of methanolic fruits extract of *Ferula* populations are shown in Table 2. The reducing power of the extracts and the standard were elevated with increasing the concentration of the samples. Similar to DPPH assay, in FTC model system the highest reducing power was obtained in Fo7 (0.9) and the lowest absorbance at 700 nm was observed in Fa1 (0.230) (Table 2). In this model system, the ability of the antioxidant fraction to reduce $Fe^{3+}$ to $Fe^{2+}$ represents the reductive power of the antioxidant (Gursoy *et al.,* 2009; Kosar *et al.,* 2011). All extracts, at the testing concentration, were capable of reducing $Fe^{3+}$. Among the species Fg1, Fo7, and Fa2 had the highest reducing power, while for DPPH assay in addition to these populations many others possessed acceptable antioxidant activity. It might be due to the mechanism of evaluation in each model systems. DPPH is mostly related to such phenolic compounds that participate in scavenging of free radicals, while in FTC assay the phenolic compounds that inactivate the metal ions are assessed (Gharibi *et al.,* 2013).

## Inhibition of Beta-carotene Bleaching System

The presence of different antioxidants can hinder beta-carotene bleaching by quenching free radicals in the system (Tosun *et al.,* 2009). The antioxidant effect of *Ferula* extracts as well as BHT in the model system of beta-carotene/linoleic acid is presented in Table 2. High variation was observed among and within species. The inhibition ranged from 20.77% in Fa1 to 92.28% in Fo7. In all three species, two groups were found. Group 1 revealed high inhibition of beta-carotene (> 60%), while group 2 showed low percent of inhibition (< 35%). In this method, most of methanolic extracts of *Ferula* populations (group 1) showed a greater ability in preventing fatty acid oxidation than BHT at the same concentration (Table 2). It can be described by the phenomenon of "polar paradox", in which polar antioxidants remaining in the aqueous phase of the emulsion are more diluted in the lipid phase and are, thus, less effective in protecting the linoleic acid (Gharibi *et al.,* 2013). The BHT probably possessed higher polarity than methanolic extracts of *Ferula* and, therefore, showed lower activity in quenching free radicals in the oil phase of beta-carotene/linoleic acid. The inhibition of lipid peroxidation by antioxidants might be due to high free radical scavenging activities of *Ferula* species.

So, it can be probably concluded that most of *Ferula* populations (group 1) were more potent for scavenging of free radicals in lipid phases in comparison with aqueous phase.

## Cluster Analysis of Metabolites

Cluster analysis was carried out using Ward's method to distinguish possible groups among the populations (Figure 2).



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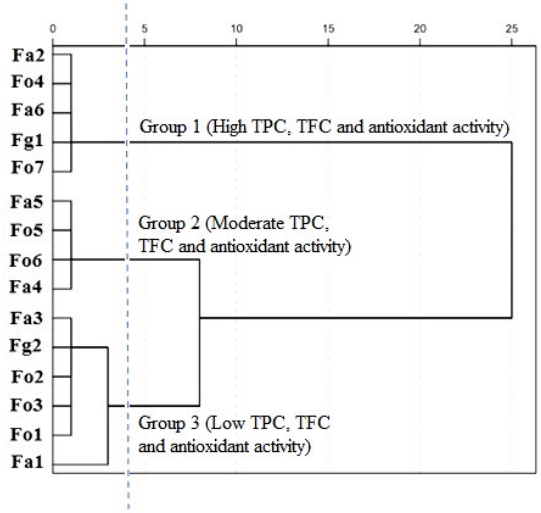


**Figure 2.** Grouping of 15 *Ferula* populations according to their phenolic and flavonoid content and antioxidant activity using Ward's minimum variance.

The cluster analysis subdivided the populations in three major groups. Group 1 included the populations with the highest TPC, TFC and antioxidant activity, while group 2 consisted of moderate TPC, TFC and antioxidant activity. Group 3 had the lowest values of the mentioned criteria. According to cluster analysis of metabolites, the classification of three *Ferula* species was mostly attributed to environmental changes and the metabolites were less affected by their genetics.

## Morphological Results

Analysis of variance showed significant differences among all evaluated traits. The results of the mean comparison, based on *LSD* test, are shown in Table 3. In overall, the highest fruit length and width belonged to *F. gummosa*, while the lowest length and width were observed in *F. assa-foetida* and *F. ovina*, respectively (Table 3). In respect to length/width ratio of the studied fruits, *F. ovina* and *F. assa-foetida* possessed the highest and the lowest ratio, respectively. The fruit thickness showed high variation among species and ranged from 0.69 (cm) in Fa6 to 1.67 in Fg2. The highest and the lowest thousand kernel weight were also obtained in *F. gummosa* and *F. assa-foetida*, respectively (Table 3).

A dendrogram was generated using Ward's method to reveal the relationships among populations. All populations were clustered into three main groups according to their fruit morphological traits (Figure 3). Fg2 was separated from all populations and classified in group 4. In many Apiaceae plant, fruit morphology was considered as an important factor for classification. Similar to the present study, fruit morphology was evaluated in Chinese *Heracleum* species (Yu *et al.* 2011), *Ferulago* (Urusak and Arslan, 2013) *Trachyspermum ammi* (Chatterjee *et al.*, 2013). Previous researches revealed that fruit morphology traits are considered as critical criteria for taxonomical classification of many Apiaceae plant as well as their importance for metabolite variation (Yu *et al.*, 2011).

## Correlations Analysis

In order to assess the relationships of environmental factors with metabolite variations in *Ferula* species, correlation analysis was carried out. Soil clay



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**Table 3**. Fruit morphology of 15 *ferula* population.

| Populations | Lenght (cm) | Width (cm) | Thickness (cm) | Lenght/Width | Thousand kernel weight (g) |
|---|---|---|---|---|---|
| Fa1 | 11.51 $^{fg}$ ± .28 | 6.4$^{bc}$ ± 0.56 | 0.75$^{fg}$ ± 0.11 | 1.81$^{fg}$± 0.12 | 11.65 $^{jk}$ ±0.14 |
| Fa2 | 13.65 $^{de}$ ± 0.2 | 8.09$^{a}$ ± 1.31 | 1.04$^{cde}$ ± 0.21 | 1.71$^{fg}$± 0.23 | 20.45$^{c}$ ± 0.77 |
| Fa3 | 13.61 $^{de}$ ± 0.7 | 6.35$^{bc}$ ± 0.89 | 0.87$^{efg}$ ± 0.16 | 2.16$^{de}$± 0.21 | 11.16$^{k}$ ± 0.22 |
| Fa4 | 10.67 $^{g}$ ± 1.57 | 6.74 $^{b}$ ± 0.67 | 0.7$^{g}$ ± 0.18 | 1.58$^{g}$± 0.09 | 12.86$^{ij}$ ± 0.22 |
| Fa5 | 11.61 $^{fg}$ ± 1.64 | 6.99$^{ab}$ ± 1.26 | 0.74$^{g}$ ± 0.14 | 1.68$^{fg}$± 0.30 | 12.53$^{ijk}$ ± 0.31 |
| Fa6 | 12.61 $^{ef}$ ± 1.23 | 6.25 $^{bc}$ ± 0.16 | 0.69$^{g}$ ± 0.07 | 2.02$^{ef}$± 0.15 | 15.39$^{fg}$ ± 2.00 |
| Fo1 | 15.17 $^{bc}$ ± 0.64 | 4.53 $^{de}$ ± 0.65 | 1.32$^{b}$ ± 0.10 | 3.38$^{a}$ ± 0.39 | 18.57$^{d}$ ± 0.91 |
| Fo2 | 16.33 $^{ab}$ ± 0.47 | 5.45 $^{cd}$ ± 0.23 | 1.17$^{bcd}$ ± 0.25 | 3.00$^{bc}$ ± 0.16 | 21.67$^{c}$ ± 1.46 |
| Fo3 | 14.2 $^{cd}$ ± 0.4 | 4.64 $^{de}$ ± 0.28 | 0.93$^{defg}$ ± 0.20 | 3.06$^{abc}$ ± 0.11 | 16.67$^{ef}$ ± 0.47 |
| Fo4 | 15.16 $^{bc}$ ± 0.54 | 4.92 $^{de}$ ± 0.3 | 1.23$^{bc}$ ± 0.25 | 3.08$^{abc}$ ± 0.09 | 17.4$^{de}$ ± 0.8 |
| Fo5 | 14.22 $^{cd}$ ± 0.44 | 4.27 $^{e}$ ± 0.36 | 1.28$^{bc}$ ± 0.13 | 3.34$^{ab}$ ± 0.18 | 14.67$^{gh}$ ± 0.57 |
| Fo6 | 13.63 $^{de}$ ± 0.47 | 4.61 $^{de}$ ± 0.6 | 1.03$^{cdef}$ ± 0.20 | 2.98 $^{c}$ ± 0.32 | 13.43$^{hi}$ ± 0.31 |
| Fo7 | 15.81 $^{b}$ ± 0.5 | 5.29 $^{cde}$ ± 0.39 | 1.42$^{ab}$ ± 0.15 | 3.00 $^{bc}$ ± 0.14 | 14.5$^{gh}$ ± 0.7 |
| Fg1 | 12.79 $^{ef}$ ± 0.55 | 7.37 $^{ab}$ ± 0.71 | 0.76$^{efg}$ ± 0.04 | 1.74 $^{fg}$ ± 0.10 | 24.43$^{b}$ ± 1.42 |
| Fg2 | 17.36 $^{a}$ ± 0.75 | 7.37 $^{ab}$ ± 0.67 | 1.67$^{a}$± 0.12 | 2.37 $^{d}$ ± 0.23 | 32.46$^{a}$ ± 1.62 |
| Total | 13.89± 1.95 | 5.95± 1.32 | 1.04± 0.32 | 2.46± 0.68 | 17.19± 5.64 |

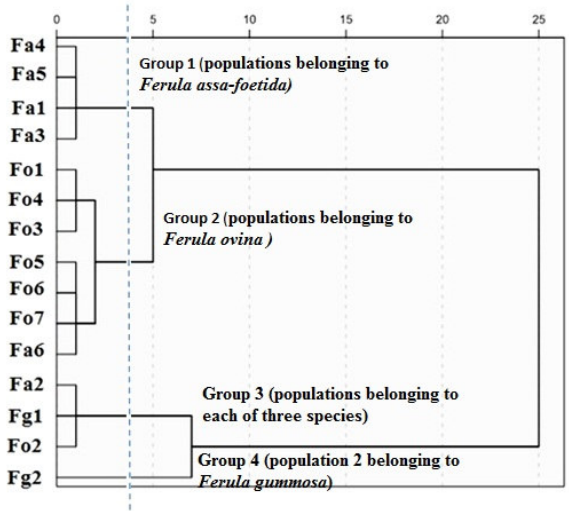


**Figure 3.** Grouping of 15 *Ferula* populations according to their fruit morphological traits using Ward's minimum variance.

percentage and phenolic content showed positive correlation (r= 0.649**) (Table 4). Other significant correlations were between soil clay percentage and antioxidant activity using three different model systems (reducing power (r= 0.714**), DPPH radical scavenging (r= 0.594*), *β*-carotene (r= 0.539*) and flavonoids (r= 0.608*). Sand percentage showed negative correlation with extraction yield (r= -0.616*) and clay percentage (r= -0.530*). Also, the correlation coefficient (r) was calculated in order to determine the relationship between phenolic content and antioxidant capacity in three model systems. In all of the 15 *Ferula* populations, there was a statistically significant and strong correlation of *r*= 0.823**, *r*= 0.820**, *r*= 0.954** and *r*= 0.878** between phenolic content and each inhibitory effect in beta-carotene bleaching, DPPH radical scavenging, reducing power, and total flavonoids, respectively (Figure 4).



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**Table 1**. Collection site, geographical and soil characteristics of different ferula populations.

| No. | Accession name | Collection site | Latitude | Longitude | Altitude (m) | Clay | Silt | Sand | Organic matter% | Acidity |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Fa1 | Khoigan, Isfahan, Iran | 32° 39' 16″ N | 51° 40' 4″ E | 2440 | 18.4 | 26.1 | 24.9 | 1.4 | 7.5 |
| 2 | Fa2 | Fereydun Shahr, Isfahan, Iran | 32°56′28″ N | 50°07′16″ E | 2490 | 26 | 36.3 | 37.5 | 2.7 | 7.6 |
| 3 | Fa3 | Afus, Isfahan, Iran | 33°02′36″ N | 50°09′27″ E | 2450 | 22 | 41 | 37 | 2.21 | 7.8 |
| 4 | Fa4 | Padena, Isfahan, Iran | 30° 57' 59″ N | 51°26'15" E | 2400 | 19 | 56 | 25 | 3.76 | 7.5 |
| 5 | Fa5 | Agche, Isfahan, Iran | 33° 4' 42″ N | 50° 3' 21" E | 2696 | 29.2 | 34 | 36.4 | 1 | 7.7 |
| 6 | Fa6 | Yazd, Yazd, Iran | 31°53′49″ N | 54°22′02″ E | 1236.2 | 26.8 | 30.8 | 42.4 | 0.6 | 7.9 |
| 7 | Fo1 | Shikh Alikhan, Charmahal & Bakhtiari, Iran | 29° 36' 41″ N | 50°45'29″ E | 2360 | 34 | 45 | 21 | 2.1 | 7.4 |
| 8 | Fo2 | Yasuj, Kohgiluye va Boyerahmad | 30°40′05″ N | 51°35′16″ E | 2048 | 30 | 43 | 27 | 4.38 | 7.3 |
| 9 | Fo3 | Khoigan, Isfahan, Iran | 32° 39'16″ N | 51° 40' 4″ E | 2440 | 18.4 | 26.1 | 24.9 | 1.4 | 7.5 |
| 10 | Fo4 | Fereydun Shahr, Isfahan, Iran | 32°56′28″ N | 50°07′16″ E | 2490 | 26 | 36.3 | 37.5 | 2.7 | 7.6 |
| 11 | Fo5 | Meidanak, Isfahan, Iran | 32°65'46″ N | 51°66′79″ E | 2250 | 32 | 52 | 16 | 0.89 | 7.9 |
| 12 | Fo6 | Semirom, Isfahan, Iran | 31° 27'39″ N | 51°37'23″ E | 2460 | 24 | 47 | 29 | 4.4 | 7.5 |
| 13 | Fo7 | Kooh Pardenjan, Charmahal & Bakhtiari, Iran | 32° 19'53″ N | 50°51'86″ E | 2300 | 42 | 39 | 19 | 2.9 | 7.7 |
| 14 | Fg1 | Shirmard, Charmahal & Bakhtiari, Iran | 29° 0' 45" N | 51° 21' 9" E | 2400 | 42 | 44 | 14 | 1.6 | 7.8 |
| 15 | Fg2 | Chogyurt, Isfahan, Iran | 32° 58' 40"N | 50° 0' 51" E | 2350 | 14 | 49 | 37 | 1.3 | 7.5 |

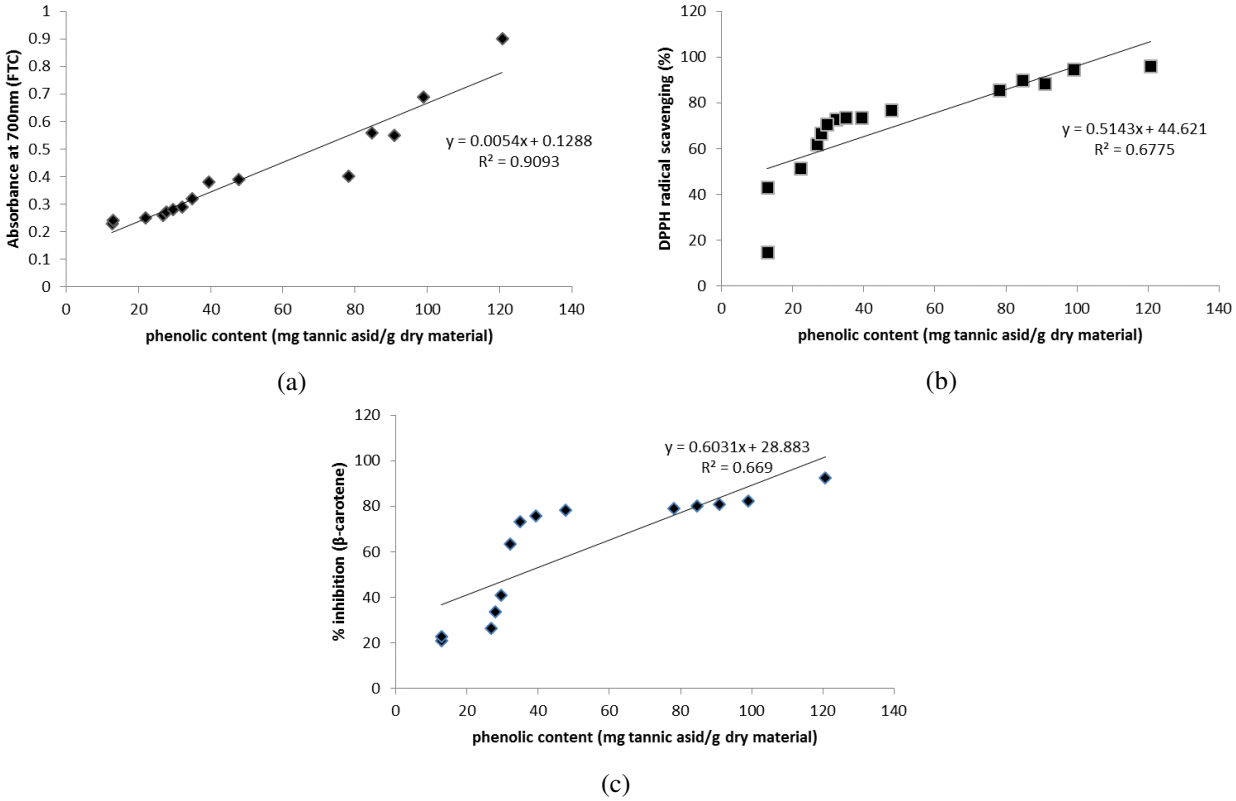


**Figure 4.** Correlation between total phenolic content and antioxidant activity (three model

Some researchers showed the positive correlation between phenolic content and antioxidant activity in other medicinal plants (Gharibi *et al.,* 2013; Gonçalves *et al.,* 2013), whereas Barros *et al.* (2009) found no such relationships. Mohammadi-Motamed and Naghibi (2010) reported a positive correlation between data of DPPH scavenging activity and total flavonoids (r= 0.64) and phenolic compounds (r= 0.59). Moreover, in this research, no strong correlation was observed between fruit morphological traits and antioxidant activity based on the three model systems.

The effect of soil characteristics and altitude on metabolites variation was also evaluated (Table 1). The results showed that soil and climatic factors were more effective than genetics on metabolite variation of *Ferula* species. The populations sampled in clay soils possessed higher phenolic content and antioxidant activity, while the populations collected from sandy soils had the lowest extraction yield. Previous reports also revealed that the environmental factors can affect the chemotypic variation of essential oils. Rahimmalek *et al*. (2013) reported that higher altitudes with loamy and clay soils lead to increase in the amounts of α-pinene and 1,8-cineole in myrtle, while the populations collected from sandy soils that were rich in organic matter possessed higher contents of limonene.

## CONCLUSIONS

In conclusion, the fruit extracts of *Ferula* species showed a relatively high antioxidant activity as compared to synthetic antioxidant. Among the studied populations, most of *F. ovina* populations revealed the highest total phenolic and flavonoid content and antioxidant activity as compared with the two other species. It can also be concluded that higher phenolic compounds might contribute directly to higher antioxidant in *Ferula* species and *F. ovina* seem to be the most promising species for further investigation in order to identify the compounds responsible for



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their activity. Finally, the populations that were collected in clay soils possessed higher phenolic content and antioxidant activity than the others, while the populations collected from sandy soils had the lowest extraction yield. Finally, it might be recommended to cultivate this plant in clay soils to gain higher phenolic, flavonoid, and antioxidant activity.

## ACKNOWLEDGEMENTS

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## تنوع صفات مورفولوژیک میوه و ترکیبات فعال زیستی جمعیت های مختلف آنقوزه، باریجه و کما جمع آوری شده در ایران

**ا. اکبریان، م. رحیم ملک، و م. ر. سبزعلیان**

### چکیده

تنوع صفات مورفولوژیک، محتوی فنولیک و فعالیت آنتی اکسیدانی ۱۵ جمعیت از گونه های آنقوزه و باریجه از مناطق جغرافیایی مختلف ایران، در این مطالعه بررسی شد. محتوای فنلی (TPC) عصاره میوه از 12.77تا ۱۲۰.۷۲ میلی گرم اسید تانیک در وزن خشک بذر متغیر بود .محتوای فلاونوئید، دامنه ای بین ۵.۴۵ تا ۸.۰۹ میلی گرم کوئرستین در وزن خشک-mgQUEg) ( 1به ترتیب در F. assa-foetida و F. ovina متغیر بود. فعالیت آنتی اکسیدانی میوه با استفاده از سه مدل سیستم آنتی اکسیدانی انجام شد .در این مطالعه، عصاره میوه ها فعالیت مهار کنندگی رادیکال آزاد بیشتری نسبت به آنتی اکسیدان مصنوعی (BHT) نشان دادند. روند مشابهی نیز در کاهش قدرت (FTC)و سیستم های مدل اسید β کاروتن-لینولئیک به دست آمد. تجزیه و تحلیل کلاستر جمعیت های مورد بررسی را در سه گروه اصلی قرار داد. گروه اول دارای قدرت مهارکنندگی بالایی از بتا کاروتن60 < ٪ (بود، در حالی که گروه ۲ درصد کمتری از مهارکنندگی35> ٪ (نشان داد. گروه ۳ دارای کمترین محتوای فنلی، فلاونوئید و فعالیت آنتی اکسیدانی بود. در این مطالعه، برخی صفات مورفولوژیکی میوه نیز در جمعیت های گیاهی اندازه گیری شد. بسیاری از جمعیت های کما (گروه ۱) قدرت مهارکنندگی بیشتری در فاز چربی در مقایسه با فاز آبی برای مهار رادیکال های آزاد نشان دادند. در میان گونه های مورد مطالعه، F. gummosaبالاترین طول میوه و وزن هزار دانه و نسبت طول به عرض میوه و همچنین بالاترین میزان فعالیت آنتی اکسیدانی را نشان داد. به طور کلی، نتایج تجزیه همبستگی نشان داد که جمعیت های گیاهی که در خاکهای رسی رشد نمودند دارای مقادیر بالاتری از ترکیبات فنولیک وفعالیت آنتی اکسیدانی بودند در حالی که جمعیت هایی که در خاک های شنی رشد یافته بودند دارای عملکرد بالاتر عصاره بودند.



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