



Comparison of Antioxidant Activity, Phenolic and Flavonoid Contents of *Zataria Multiflora* Populations in Iran

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Abstract

Medicinal plants are valuable sources of natural antioxidants such as some terpenoids, flavonoids and phenolic compounds. *Zataria multiflora* Boiss. is one of the species in the Lamiaceae family that is used in the medicine, cosmetic and food industries. It has high potential as a suitable alternative to synthetic antioxidants in reducing oxidative stress. Therefore, fifteen populations of *Z. multiflora* in five provinces of Iran were selected in new habitats to evaluate amounts of flavonoids, phenolic compounds and antioxidant activity for further examination in domestication, breeding and cultivation programs. The samples were air-dried at room temperature and then were powdered and macerated with aqueous pure methanol in a ratio 10:1 (v/w) by 48 h, resulting liquids were filtered and concentrated under vacuum evaporator to get the crude hydroalcoholic extracts. Subsequently, the total polyphenol, flavonoid and antioxidant content were determined. The total phenol content was measured according to Folin-Ciocalteu method. Total flavonoid content was quantified using the colorimetric method with aluminum chloride. Also antioxidant activity was evaluated using the method of DPPH free-radical scavenging activity. The maximum and minimum amounts of total phenol were observed in Tang-e-Zagh (378.9 mg GAE/g Extract) and Kerman (199.2 mg GAE/g Extract), respectively. The maximum difference flavonoid was 32.7 mg QE/g Extract which was observed between Jiroft and Pasargad populations. Results showed that the most antioxidant activity was obtained in Tashk and Faryab populations. This wide variation in biochemical parameters can be used in future researches in the medicine, cosmetic and food industries.

Keywords: Antioxidant activities, Population, Variation, *Zataria multiflora*.

Introduction

The use of natural antioxidants is one of the easiest ways to reduce cell destructive reactions. Plants are considered as rich sources of natural antioxidants [1]. The wide geographical and climatic distribution in Iran is indicative of the fact that there is a great biochemical diversity among native plants which needs to be identified and studied [2]. *Zataria multiflora* is the only known species of the *Zataria* genus in the Lamiaceae family that mostly growing on dry and mountainous areas in the south and southeast of the Iranian Plateau (Iran, Afghanistan, and Pakistan). Its genus name is taken from the Arabic word 'zaetar' meaning thyme and the common Persian name is "Avishan-e-Shirazi" [3]. In traditional Iranian medicine,

the leaves and flowers of *Z. multiflora* can be used for cold, inflation, antiseptic and continuous pain relief. Fresh and dry leaf powder is known as a spice for a wide variety of foods [4]. Recent studies have shown that the essential oils and extracts of this species are rich in antinociceptive, spasmolytic, anti-inflammatory, antimicrobial and antioxidant properties that can be used in the medicine, cosmetic and food industries [4, 6]. It seems that phenolic compounds of *Z. multiflora* play a key role in medicinal, aromatic and spice properties [7, 8]. In addition, a significant correlation between antioxidant properties and phenolic compounds has been proven in previous studies that can be used as natural antioxidants in dietary supplements [9, 10]. On the other, the amounts of these compounds in medicinal plants

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depend on genetic factors and environmental conditions [11]. Plants produce and store the phenolic compounds in vegetative and reproductive organs to be compatible with ecological conditions and maintain their survival [8]. Also, changes in environmental factors, similar the light and temperature can lead to changes in the morphological traits during the adaption process [12]. *Z. multiflora* has a wide geographical range in Iran that can make a high variation in biochemical parameters. Hence, the study of these variations in various populations of this species collected from different parts of Iran is an important step towards identifying desirable populations that can be used in future researches in the medicine, cosmetic and food industries.

Material and Methods

Plant Materials and Habitats Information

Iranian habitats of *Z. multiflora* were identified using the information in Flora Iranica [13], examination of scientific references, expert reports and direct observations. Following the identification of these habitats and direct observation of plant populations, 15 habitats were selected in five provinces (Table 1, Fig. 1). Identification of individuals for studied species was confirmed by a botanist and voucher specimens have been deposited in the Herbarium of Agriculture and Natural Resources Research Center of Hormozgan, Iran (Herbarium number: 2228).

Preparation of Methanol Extract

The aerial parts of plants dried in the shade and it was grounded to a fine powder. Fifty grams of dry powder of each sample were weighed into one-liter volumetric flasks, and then 200 ml of methanol were added to the plant samples. This mixture was left in a shaker for 48 h at room temperature and filtration through filter paper.

Table 1 Habitats information and climatic conditions of the studied *Z. multiflora* Boiss. populations

No	Habitats	Province	Altitude (m)	Longitude (E)	Latitude (N)	Mean annual temperature (°C)	Mean annual rainfall (mm)
1	Behabad	Yazd	1432	56° 01′	31° 87′	19.2	153.9
2	Abadeh	Fars	2056	52° 64′	31° 16′	14.8	307
3	Pasargad	Fars	1867	53° 18′	30° 19′	18.8	264
4	Tashk	Fars	1591	53° 72′	29° 80′	19.4	245
5	Fasa	Fars	1380	53° 64′	28° 94′	24.9	250
6	Khafr	Fars	1296	53° 20′	28° 97′	23.5	235
7	Jouyom	Fars	856	53° 98′	28° 25′	27.5	201.5
8	Khonj	Fars	668	53° 43′	27° 88′	28.4	180.6
9	Kerman	Kerman	1769	57° 07′	30° 28′	16.9	132
10	Jiroft	Kerman	603	57° 48′	28° 33′	25.6	198
11	Faryab	Hormozgan	492	57° 27′	27° 82′	27.3	180
12	Tang-e zagh	Hormozgan	894	55° 97′	27° 84′	26.8	188.7
13	Roudkhaneh	Hormozgan	485	57° 22′	27° 74′	28.3	188.5
14	Bashagard	Hormozgan	735	57° 89′	26° 45′	29	133
15	Fanouj	Sistan & Baluchestan	725	59° 38′	26° 35′	19.6	145

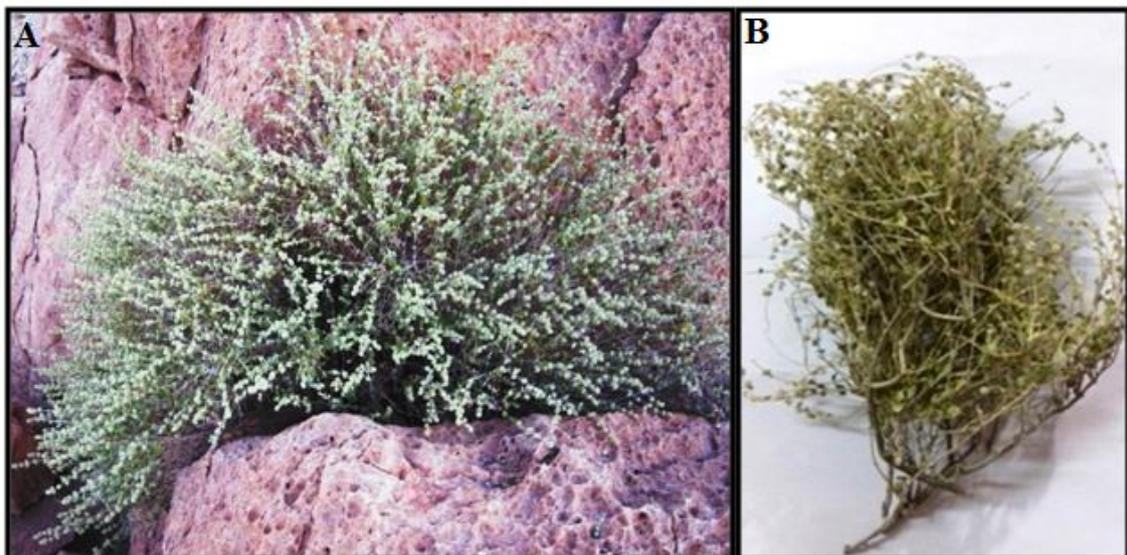


Fig. 1 The aerial parts of *Z. multiflora* Boiss. in natural habitat (A) and after drying in the laboratory (B).

The obtained extract was concentrated using a rotary evaporator under reduced pressure at 45 °C to eliminate the solvent. The final residues were dried by incubation at 50 °C and 10 mg of this extract was mixed with 1ml of distilled water to obtain dilute extract.

Total Phenolic Determination

The contents of total phenolic in methanol extracts were determined by the Folin-Ciocalteu method [14]. Nearly 500 µL of the diluted extract was mixed with 2 mL of Folin-Ciocalteu reagent. After 5 min incubate in a dark place, 2.5 mL of 7.5% sodium carbonate solution was added. The mixture was kept for 120 min in a dark place at room temperature. The absorbance was measured at 760 nm. The total phenolic content was expressed as mg gallic acid equivalents per gram of extract (mg GAE/g extract).

Total Flavonoid Determination

Total flavonoid contents of extracts were measured by the aluminum chloride colorimetric assay [15]. 500 µL of the diluted extract was added to volumetric flask containing 1.5 mL of methanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. After 30 min reaction at room temperature, the solution absorbance was measured 415 nm. Total flavonoid contents were expressed as mg of quercetin equivalent per gram of extract (mg QE/g extract).

Antioxidant Activity Determination

The ability of methanolic extracts to scavenge the DPPH (2,2-diphenyl-1-picrylhydrazyl) radical was used for the determination of antioxidant activity [16]. 1mL of the diluted extract was added with 1mL of the methanolic solution containing 0.1 mmol/ L DPPH and was kept for 15 min in a dark place. The absorbance was measured at 517 nm. The percentage inhibition of the DPPH radical was estimated by the following equation:

$$I(\%) = (A_0 - A_s / A_0) \times 100$$

Where A_0 is absorbance of control and A_s is the absorbance of tested sample. The IC_{50} values were calculated as the concentration of extract that could scavenge 50% of DPPH radical.

Statistical Analyses

The data were statistically evaluated by analysis of variance ($p < 0.05$) and significant differences between means were estimated by Duncan's multiple range test in SAS (version 9.1.3). Principal component analysis (PCA) was determined using IBM SPSS (version 23) software. Cluster analysis was performed using PAST statistics

software and the Ward method based on the Euclidean distances was used for drawing the dendrogram.

Results and Discussion

Total Phenolic Contents

The total phenolic contents of the methanolic extracts of the leaves are given in Table 2. The value of total phenolics varied in the different populations and ranged from 199.2 to 378.9 mg GAE/g extract for Kerman and Tang-e-Zagh populations, respectively (Fig.2). Sharafati *et al.* (2013) reported that the total phenol content in *Z. multiflora* extract was 283.43 ± 11.06 mg GAE/gr extract. Also formerly studies have reported that total phenolic content of *Z. multiflora* was 179.42 ± 80 to 322.0 ± 2.9 mg GAE/g DW. [4, 17]. Therefore, it is possible that widespread genetic variation in populations leads to various physiological interactions for the product and reserve of phenolic compounds.

Total Flavonoid Contents

The total flavonoid contents ranged from 35.1 to 67.8 mg/g as quercetin (QE) equivalent in Pasargad and Jiroft populations, respectively (Table 2, Fig.3). Different amount of total flavonoid contents has been reported in the *Z. multiflora* populations. As previously reported [18] the total flavonoid contents of *Z. multiflora* ranged from 54.6 to 137.0 mg rutin/g DW. Fatemi *et al.* (2012) reported that the total flavonoid content of *Z. multiflora* was determined to be nearly 32 mg of quercetin (QE)/g in the hydroalcoholic extract [19]. Hence, it has seemed that this difference in results of some reports depends on the type of solvent, methods of extractions and climatic conditions of plant growth.

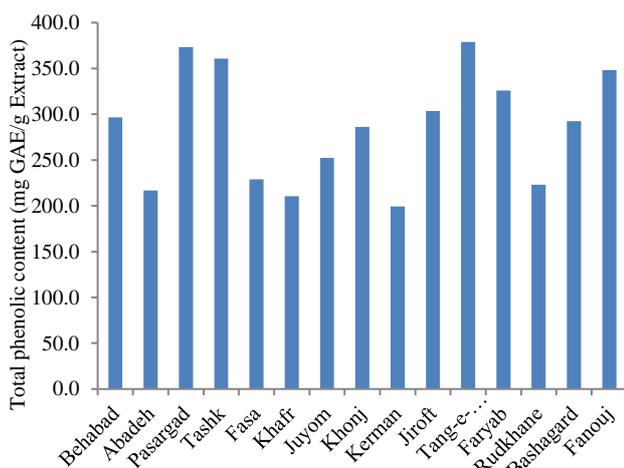
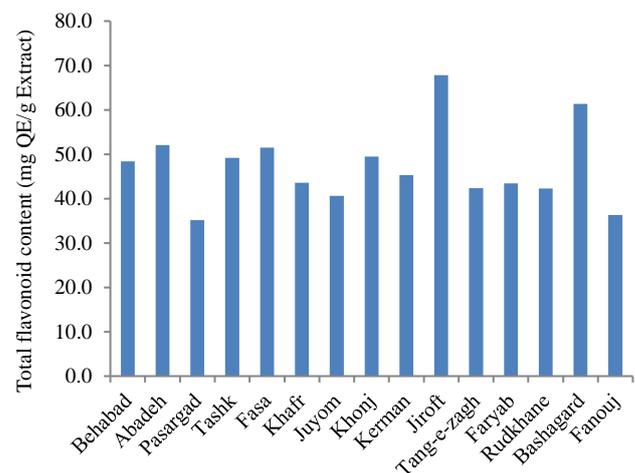
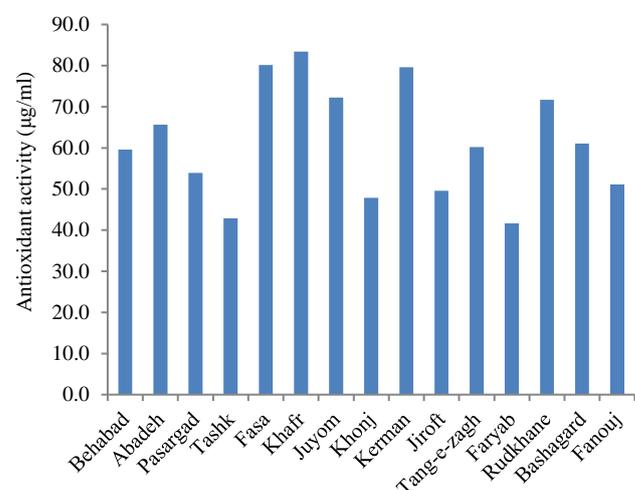
Antioxidant Activities

The antioxidant activities were measured by the DPPH assay and it was represented in IC_{50} values (µg/ml). It is estimated as the values of plant extract needed to decrease the initial concentration of DPPH radical by fifty percent. Thus, the highest antioxidant activity was obtained from the lowest IC_{50} of the extract. DPPH scavenging potential of the methanolic extracts of the leaves for all populations was variable from 41.6 to 83.4 µg/ml in Faryab and Khafr populations, respectively (Table 2, Fig.4). Also, a good correlation was observed between antioxidant activity and total phenolic content as well as total flavonoid content in some populations. In fact, it's possible that the phenolic compounds and flavonoids can react with lipid and hydroxyl radicals and retard or stop the oxidative reactions [20].

Table 2 Total phenolic total phenolic content, total flavonoid content and antioxidant activity in the studied populations of *Zataria multiflora* Boiss.

No	Population name	Total phenolic content (mg GAE/g Extract)	Total flavonoid content (mg QE/g Extract)	Antioxidant activity ($\mu\text{g/ml}$)
1	Behabad	296.7 d \pm 0.51	48.4 cd \pm 2.53	59.6 bcd \pm 2.78
2	Abadeh	216.8 fg \pm 0.62	52.1 bc \pm 4.27	65.6 cd \pm 1.02
3	Pasargad	373.4 a \pm 1.34	35.1 e \pm 0.60	53.9 abc \pm 5.58
4	Tashk	360.6 ab \pm 0.55	49.2 cd \pm 0.55	42.9 a \pm 2.96
5	Fasa	229.1 f \pm 0.90	51.5 bcd \pm 3.13	80.2 e \pm 1.17
6	Khafr	210.6 fg \pm 1.26	43.6 cde \pm 0.24	83.4 e \pm 0.66
7	Juyom	252.4 e \pm 0.89	40.7 de \pm 1.26	72.2 de \pm 2.44
8	Khonj	286.2 d \pm 1.07	49.5 cd \pm 0.66	47.9 ab \pm 3.66
9	Kerman	199.2 g \pm 0.26	45.3 cde \pm 0.48	79.6 e \pm 1.93
10	Jiroft	303.4 d \pm 0.56	67.8 a \pm 2.56	49.5 ab \pm 1.84
11	Faryab	325.9 c \pm 0.62	43.5 cde \pm 0.42	41.6 a \pm 1.40
12	Tang-e-Zagh	378.9 a \pm 3.23	42.3 cde \pm 2.12	60.2 bcd \pm 2.79
13	Rudkhane	222.9 f \pm 1.03	42.2 cde \pm 0.72	71.7 de \pm 1.58
14	Bashagard	292.6 d \pm 3.46	61.3 ab \pm 0.77	61.1 bcd \pm 0.92
15	Fanouj	348.2 b \pm 0.97	36.3 e \pm 3.67	51.2 ab \pm 2.10
CV (%)		21.69	18.41	22.35

These findings were consistent with previous reports about antioxidant properties in *Z. multiflora* extracts [10, 18, 19]. Recently, researchers have suggested that the extract of *Z. multiflora* can be replaced with synthetic antioxidants. For example, the dietary supplementation of *Z. multiflora* extract increased significantly the oxidative stability of chicken meat to lipid oxidation during frozen storage [21]. Another study showed that the essential oil of *Z. multiflora* has been effective in the preservation of the cakes [22]. In general, it is necessary to find natural replacements for synthetic antioxidants in the food industry because synthetic compounds have destructive effects on the human body.

**Fig. 2** Total phenolic content of leaves extract in the studied populations of *Z. multiflora*.**Fig. 3** Total flavonoid content of leaves extract in the studied populations of *Z. multiflora* Boiss..**Fig. 4** Antioxidant activity of leaves extract in the studied populations of *Z. multiflora* Boiss..

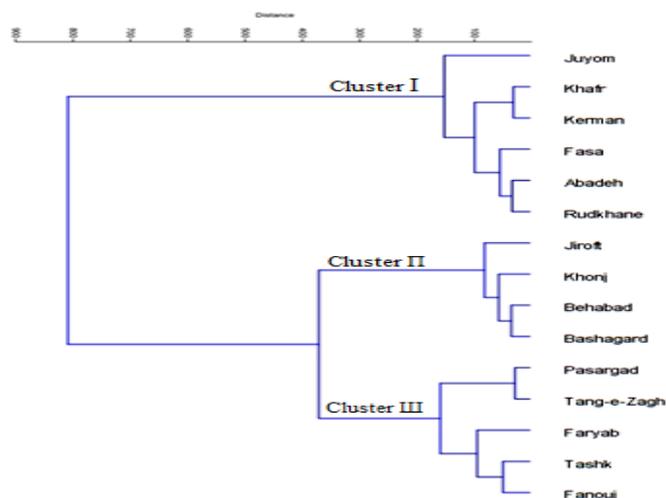


Fig. 5 Dendrogram of cluster analysis for the studied populations of *Z. multiflora* Boiss. based on biochemical traits.

Cluster Analysis

Cluster analysis based on all biochemical parameters by using the Ward method showed three separate clusters (Fig. 5). The first cluster included nine populations from Juyom, Khafr, Kerman, Fasa, Abadeh and Rudkhane. This cluster is characterized by low values of total phenolic content and unremarkable antioxidant activities. Second cluster included four population collected from Jiroft, Khonj, Behabad and Bashagard that were described with high values of total flavonoid content and moderate amounts of total phenolic content. The third cluster consists of Pasargad, Tang-e-Zagh, Faryab, Tashk and Fanouj populations with higher antioxidant activity and high value of total phenolic content. Also, cluster analysis showed that the populations originated from same province could not be placed with together completely, indicating high biochemical diversity. Based on previous reports, it seems conceivable that the potential effects of ecological and genetic backgrounds on the biosynthesis of phenolic compounds can be influence in this biochemical polymorphism among populations [23]. Also, our results showed that increasing the levels of antioxidant activity in these populations was related to values of total phenol content. It has already been proven that medicinal plants rich in phenolic content have high antioxidant activities [24]. In our study, comparing total phenolic content and antioxidant activities between the three clusters revealed that cluster III had much higher values than cluster I and cluster II. So, the populations of cluster III can be introduced as the superior populations that are used as a food supplement or in pharmaceutical applications to the replacement of synthetic antioxidants.

Finally, in comparison to other published articles, we evaluated some biochemical parameters of *Z. multiflora* in new habitats [10,18]. Although the similarity of some

habitats can be considered as a complement to previous research. Also, we should say that this plant is the only known species of the *Zataria* genus that only growing in the Iranian Plateau. Annually, a large quantity of these plants is harvested from nature and leads to the destruction of germplasm. Investigation of the biochemical diversity of this plant in different habitats is the first step to identify the accessions with the most useful traits for use in breeding programs to develop new suitable cultivars. Generally, helps to conserve and sustainable production of this native plant.

Conclusion

Increasing knowledge about the dangers of using synthetic materials has accelerated the use of medicinal plants in the pharmaceutical, cosmetic and food industries. Most plant raw materials are harvested from natural habits and lead to the destruction of these plants. Therefore, many studies should be done on medicinal plant domestication. Before entering plants into the domestication and cultivation process, native populations should be assessed in various aspects such as morphological, biochemical, and genetic diversity. *Z. multiflora* is one of the important native medicinal plants in Iran that can be widely used in the food, perfume, spice and medicine industries, so it is necessary to study the biochemical diversity among wild populations. Our study revealed the Jiroft and Tashk populations showed the highest total phenolic content and the antioxidant potential to scavenge DPPH radicals, which can be used as the possible new source for preservative ingredients in the food and pharmaceutical industry.

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Conflict of Interests

The authors have not declared any conflict of interests.

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