Chemodiversity of Volatile Oil Contents of Various Parts of 10 Iranian Prangos ferulacea Accessions, With Analysis of Antiradical Potential

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Abstract
The present study aimed at assessing the influence of ecological factors on volatile oil content and antiradical potential of Prangos ferulacea. The essential oil (EO) content and composition of different plant parts were also compared. Among 22 identified compounds by gas chromatography (GC) flame ionization detector and GC-mass spectrometry, monoterpene hydrocarbons as the major constituents contributed to 27.6% to 83.4% of the oil deriving from plants growing on the northern steeps of “Gandomkar” region at 2600 m (G.N-2600) and “Male-Amiri” at 2300 m height (MA.N-2300), respectively. Immature seed and leaf samples of “Male-Amiri” with 3.0% ± 0.16% and 0.79% ± 0.03% of EO content represented the samples with the highest and lowest EO yields, respectively. Whereas the EO of the leaves mostly contained δ-3-carene and α-bisabolol, other parts were rich in α- and β-pinene. Extracts of accessions “G.N-2600” (EC50 = 13.11 ± 0.69 μg/mL) and “M.S-2500” (10.55 ± 0.41 mmol TE/g) exhibited the most potent antiradical activities in the 1,1-diphenyl-2-picrylhydrazyl (DPPH) and Oxygen Radical Absorbance Capacity (ORAC) assays, respectively. Because of the extensive use of this species in traditional foods and the remarkable bioactivities of α- and β-pinene and δ-3-carene, the EO of the plant can be considered as a valuable raw material in phytopharmaceutical and food industries.

Keywords
Prangos ferulacea, chemical composition, DPPH, ORAC

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The approximately 30 species of the genus Prangos L. (Apiaceae family) are perennial herbs native to different parts of the world.¹ Of the 15 available species in Iran, 4 are endemic.² Prangos ferulacea (L.) Lindl. (syn. Cachrys ferulacea (L.) Calest., Cachrys goniocarpa Boiss., Cachrys prangoides Boiss.), which grows in Eastern Europe, Turkey, Caucasus, and Southwestern Asia,³ is the most popular species in Iran and is famed as “Jashir.” The aerial parts of P. ferulacea have been traditionally used in Iran as laxative and against ruminant parasites.⁴,⁵ Furthermore, P. ferulacea is consumed in Turkish folk medicine as digestive, antidiabetic, antihypertensive agent and is used to flavor cheese.⁶,⁷

In previous studies, monoterpene hydrocarbons were reported as the main oil (EO) components of P. ferulacea. Among them α- and β-pinene,⁸-¹² γ-terpinene,⁸ δ-3-carene,¹³ and β-phellandrene¹³,¹⁴ were the most significant ones. Former studies revealed antibacterial,¹⁶,¹⁴-¹⁷ phytotoxic, and fungistatic activities of P. ferulacea EO.⁹ Antioxidant⁶,¹⁷-²⁰ and antibiofilm¹⁷ activities were reported in addition to quantitative data on total phenolic⁶,¹⁹,²² and flavonoid contents of extracts.⁶,²⁰-²² Cytotoxic and antipheres potential of the isolated coumarins²³-²⁵ were previously evaluated. In addition, the prenylated coumarin osthol isolated from P. ferulacea protected oxidative stress and apoptosis induced by doxorubicin in PC12 as a neuronal model cell line.²⁶ A vaginal cream containing P. ferulacea extract accelerated the recovery from bacterial vaginosis.²⁷ Moreover, 3,5-nonadiyne isolated from its EO inhibited endogenous
accessions of this plant. The EO content and composition of previously unstudied folk medicine and as food, our study was planned to assess and determine the major EO constituents. In “MA.N-2300,” 44.2% of the identified EO components were pinenes. The lowest (8.1%) amount of δ-3-carene, as the subsequent main EO compound, were observed in “G.N-2600” and “DS.N-2000,” respectively. Furthermore, ecological effects on the variability of β-phellandrene content (0.9%-13.1%) (Table 1). A significant difference in EO yields of the various plant parts in the sample “Male-Amiri” collected from the northern steeps at 2300 m elevation was observed in immature seed and leaf samples (in vegetative period) with 3.0% ± 0.16% and 0.79% ± 0.03% of total oil content, respectively (Figure 2). The monoterpene hydrocarbon and oxygenated sesquiterpene content ranged from 26.0% to 79.0% and 2.7% to 20.4% in leaves harvested at flowering and vegetative phases, respectively. Monoterpene hydrocarbons, the major EO components, showed notable variation in the studied plant parts. The immature seeds and leaves were the richest and poorest in these compounds with 79.0% and 26.0%, respectively (Table 2). In contrast, the EO of leaves contained more δ-3-carene and α-bisabolol; other parts were rich in α- and β-pinene and δ-3-carene (hydrocarbon monoterpenes) (Table 2).

The extract of “G.N-2600” showed the most powerful antiradical agent with EC₅₀ = 13.11 ± 0.69 µg/mL; however, it was inferior than the positive control ascorbic acid (EC₅₀ = 0.3 ± 0.02 µg/mL). The most important phytoconstituents which are capable to scavenge free radicals are polyphenolic compounds; thus, the sample “G.N-2600” is probably rich in polyphenolics.

The flowers collected from “Male-Amiri” at 2300 m demonstrated the lowest antioxidant activity with EC₅₀ = 28.86 ± 4.29 µg/mL. The capacity of a wild sample harvested from Izeh (I.Z) to scavenge free radicals was higher than the cultured specimen with EC₅₀ = 13.48 ± 0.93 and 15.08 ± 1.58 µg/mL, respectively (Figure 3).

The accessions “M.S-2500” (10.55 ± 0.41 mmol TE/g) and “F.MA.N-2300” (3.53 ± 0.45 mmol TE/g) indicated the highest and lowest antiradical potential in the ORAC assay, respectively. However, the plant extracts possessed a weaker effect than ascorbic acid, rutin, and EGCG (6.98 ± 0.58, 20.22 ± 0.63, and 11.97 ± 0.02 mmol TE/g, respectively) as the controls. The wild plant gathered in Izeh (“I.W”) demonstrated the lowest antioxidant activity with EC₅₀ = 6.92 ± 0.04 mmol TE/g than the cultivated “I.C” with 4.57 ± 0.09 mmol TE/g (Figure 4).

Although the EO compositions of accessions “M.S-2500” and “F.MA.N-2300” were nearly similar, the antiradical activities of plant samples are highly influenced by the existence of polyphenolic compounds and hence the extract of sample “M.S-2500” is undoubtedly richer in these phytochemicals.

Gas chromatography (GC) flame ionization detector (FID) and GC-mass spectrometry (MS) revealed that α- and β-pinene were the major EO constituents. In “MA.N-2300,” 44.2% of the identified EO components were pinenes. The lowest (81.1%) and highest (21.0%) amounts of δ-3-carene, as the subsequent main EO compound, were observed in “G.N-2600” and “DS.N-2000,” respectively. Furthermore, ecological effects on the variability of β-phellandrene content (0.9%-13.1%) (Table 1). A significant difference in EO yields of the various plant parts in the sample “Male-Amiri” collected from the northern steeps at 2300 m elevation was observed in immature seed and leaf samples (in vegetative period) with 3.0% ± 0.16% and 0.79% ± 0.03% of total oil content, respectively (Figure 2).

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Hydrocarbon monoterpenes (α- and β-pinene, δ-3-carene, and β-phellandrene) were detected as the predominant EO compounds in almost all the studied accessions of P. ferulacea. γ-Terpinene (30.2%-33.3%) and α-pinene (16.7%-12.8%) were previously reported as the major EO constituents.
in crushed and whole fruits of *P. ferulacea*, respectively.\(^8\) Moreover, isolation of EOs led to identify α-pinene (57%) at vegetative and (E)-anethol (95.5%) at flowering stages of the species.\(^9\) By reason of various biological properties of pinenes, such as antimicrobial,\(^32\) sleep-enhancing,\(^33\) antinoceptive,\(^34\) and anti-inflammatory,\(^35\) besides the use of the oil as food flavoring and additive,\(^36-38\) the EO of *P. ferulacea* may be considered as valuable raw material in the food industry. Several studies report biological effects of 3-carene, such as anti-inflammatory,\(^39\) antibacterial,\(^40\) antifungal,\(^41\) and acetylcholinesterase inhibitory activities.\(^42\) Since, this monoterpene also stimulates the osteoblastic bone formation, it might be perspective in prevention of osteoporosis,\(^43\) the EOs of the plant, particularly samples “DS.N-2000” and “M.N-2500” with high 3-carene content (21.0 and 20.6%) may be of interest.

| No. | Compounds                          | RI   | RT   | M.S.-2000 | M.S.-2500 | M.S.-3000 | M.N.-2300 | T.S-2500 | G.N-2600 | DS.N-2000 | I.W | I.C |
|-----|-----------------------------------|------|------|-----------|-----------|-----------|-----------|----------|----------|-----------|-----|-----|
| 1   | α-Pinene                          | 932  | 5.06 | 11.8c     | 16.4a     | 10.1a     | 8.3a      | 16.3a    | 14.6b    | 4.8b      | 8.9f | 14.2bc |
| 2   | Sabinene                          | 969  | 5.82 | 1.6       | 0.5       | 1.3       | 0.5       | 2.4      | ND       | ND        | 1.2 | 1.3 |
| 3   | β-Pinene                          | 974  | 5.90 | 22.7c     | 22.9c     | 15.7a     | 14.6a     | 27.9c    | 22.7c    | 9.4d      | 17.7d |
| 4   | α-Phellandrene                    | 1002 | 6.47 | ND        | 2.4       | 0.5       | 1.6       | 1.4      | ND       | 4.3       | 2.7 | 2.9 |
| 5   | δ-3-Carene                        | 1008 | 6.61 | 14.5bc    | 14.1c     | 15.4b     | 20.6c     | 13.0d    | 11.0c    | 12.1a     | 8.1f | 21.0a |
| 6   | p-Cymene                          | 1020 | 6.93 | ND        | 0.6       | ND        | 1.4       | ND       | 1.3      | 1.2       | 1.5 | 1.5 |
| 7   | β-Phellandrene                    | 1025 | 7.03 | 1.2d      | 4.3d      | 1.6e      | 4.3d      | 13.1a    | 1.0f     | 0.9d      | 8.3bc |
| 8   | (Z)-β-Ocimene                     | 1032 | 7.20 | ND        | ND        | 0.5       | ND        | ND       | 0.5      | 1.4       | ND  | ND |
| 9   | p-Cresol                          | 1071 | 8.13 | 0.5       | ND        | 0.7       | ND        | ND       | 0.5      | 1.4       | ND  | ND |
| 10  | Terpinolene                       | 1086 | 8.52 | 3.2       | 2.6       | 3.7       | 3.6       | 3.1      | 1.9      | 1.7       | 6.8 | 3.3 |
| 11  | Alloocimene                       | 1140 | 9.98 | ND        | ND        | ND        | ND        | ND       | ND       | ND        | 1.4 | ND |
| 12  | Viridene                          | 1163 | 10.74| 4.2c      | 3.2c      | 7.6b      | 5.5a      | 2.8c     | 5.3b     | 3.6d      | 4.3c |
| 13  | (E)-Caryophyllene                 | 1423 | 17.89| ND        | ND        | ND        | ND        | ND       | ND       | ND        | 1.2 | ND |
| 14  | γ-Muurolene                       | 1478 | 19.14| 2.2       | 1.7       | 3.3       | 1.9       | ND       | 2.2      | 2.7       | 1.3 | ND |
| 15  | (Z)-Nerolidol                     | 1531 | 20.72| 3.1d      | 4.6c      | 2.6h      | 5.3c      | 1.0i     | 9.2a     | 7.9b      | 4.2f |
| 16  | α-Cadinene                        | 1537 | 21.03| ND        | ND        | 1.2       | ND        | ND       | 1.6      | ND        | ND  | ND |
| 17  | cis-Cadinene ether                | 1552 | 21.29| ND        | ND        | ND        | ND        | ND       | ND       | ND        | ND  | ND |
| 18  | Germacrene B                      | 1559 | 21.35| 4.3bc     | 2.6a      | 4.8b      | 4.0c      | 0.2d     | 2.9d     | 8.6a      | 2.1i |
| 19  | Caryophyllene oxide               | 1582 | 22.31| 3.6       | 1.2       | 3.1       | 2.5       | ND       | 2.0      | 7.8       | 1.3 | 1.8 |
| 20  | α-Bisabolol                       | 1685 | 24.66| 4.1d      | 4.0c      | 4.8c      | 3.8d      | 0.9d     | 3.3a     | 10.2a     | 3.5a |
| 21  | (2Z,6Z)-Farnesol                  | 1698 | 25.11| ND        | ND        | ND        | ND        | ND       | 1.2      | 3.5       | ND  | ND |
| 22  | (Z)-Ternine                       | 1844 | 22.82| 2.1       | 3.4       | 5.0       | 5.0       | ND       | 1.6      | 2.9       | 1.7 | ND |
|     | Monoterpenes hydrocarbons         |       |       |           |           |           |           |          |          |           |     |     |
|     | Sesquiterpenes hydrocarbons       |       |       |           |           |           |           |          |          |           |     |     |
|     | Oxygenated sesquiterpenes         |       |       |           |           |           |           |          |          |           |     |     |
|     | Others                            |       |       |           |           |           |           |          |          |           |     |     |
| Total|                                 |       |       |           |           |           |           |          |          |           |     |     |

RI, retention index; RT, retention time; ND, not detected.
The means were compared using Duncan comparisons test (P < 0.05).
\(^a\)Compounds listed in order of elution.
\(^b\)Retention indices relative to C8-C24 n-alkanes on Agilent 7890B capillary column.\(^31\)
\(^c\)Retention times

Table 1. Chemical Compositions of Essential Oils Obtained From the Leaves of 10 *Prangos Ferulacea* Accessions Harvested at Flourishing Period.
and 1,8-cineole (19.0%) in stem, α-pinene (42.2-63.1%), β-pinene (43.1%), β-phellandrene in leaf (11.1%) and flower (8.1%) and 20.4%, (E)-caryophyllene (48.2%), δ-3-carene (22.5%).

Investigation of antioxidant activities was reported with diverse potential of *P. ferulacea* extracts in free radicals scavenging from high to low effects. Also, in other studies exhibited slight to moderate activities. Because EOs of the samples “MA.N-2300” and “M.S-2500” were rich in pinenes (44.2% and 39.3%, respectively) and these monoterpenes demonstrated a good to moderate antioxidant effect, the EOs of these accessions can also be considered as an antiradical agent.

In conclusion, as *P. ferulacea* is extensively applied in traditional medicine and foods, the present study provides useful information about the volatile oil components of 10 different Iranian accessions harvested from the western parts of Iran. Our results explicitly demonstrated that the EO content and composition of *P. ferulacea* was quantitatively and qualitatively influenced by a variety of growth conditions. In fact, the geographical factors (such as variations of weather, humidity, soil composition, and sunlight) can alter the biosynthesis pathways of EO compositions in plants. Furthermore, the EO compositions of different plant parts were significantly different.

To choose a good genotype possessing the desired phytocochemical profile requires studying various plant populations to find out the optimal environmental circumstances.

In accordance with our findings, monoterpeno hydrocarbons (27.6%-83.4%) are the most dominant EO compounds of the selected *P. ferulacea* samples. Among them, pinenes (α- and β- isomers) and δ-3-carene were identified as the major components.

Immature seeds of “M.N-2300” are suggested to acquire the highest EO yield among all plant parts and populations. Moreover, “G.N-2600” and “M.S-2500” with the most potent antiradical activity are most probably the richest samples having polyphenolic compounds.

Further experiments are needed to elucidate other phytoneutrients of *P. ferulacea*, along with the characterization of pharmacological and biological activities of the extracts, in order to exploit this valuable plant in food and phytopharmaceutical industries.

**Experimental**

**Plant Materials**

The samples of *P. ferulacea* accessions were harvested at the beginning of flourish period (June) in 2017. The plant leaves were collected from different growth locations and altitudes of Khuzestan Province (Iran) (Table 3). Furthermore, various parts, including flowers (F.MA.N-2300), immature and mature seeds, and leaves in vegetative and in flowering periods (MA.N-2300), of the plant accession “Male-Amiri” were gathered at 2300 m.

The plants were identified by Dr Chehrazi at the Department of Horticultural Science, Shahid Chamran University of Ahvaz, and a voucher specimen of each sample was deposited in the herbarium of the department. For analysis, the samples were dried at shade and finely crushed by a grinder.

**Chemicals and Instruments**

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,2′-azobis-2-methylpropionamidine dihydrochloride (AAPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®) (Sigma-Aldrich, Hungary); fluorescein (Fluka Analytical, Japan); ascorbic acid, rutin, and Na₂SO₄ (Merck, Germany); and EGCG (Sigma-Aldrich, Germany) were purchased in analytical grade. Furthermore, all solvents of analytical grade were provided by Merck (Germany). Spectrophotometric measurements were carried out by using a UV-VIS spectrophotometer (FLUOstar Optima BMG Labtech, Germany).

**Essential Oil Extraction**

Powdered samples, 50 g each, were individually extracted by Clevenger apparatus (hydrodistillation method) for 3 hours. The obtained EOs were dried over anhydrous sodium sulfate and stored in refrigerator at 4°C until analysis.

**Gas Chromatographic Analysis**

In case of GC analysis, the EOs were analyzed by a Shimadzu GC-17A (Japan) gas chromatograph equipped with FID and a SGE BP-5 capillary column (30 m × 0.25 mm, 0.25 μm film thickness) (temperature range: -60°C to 250°C).
+340/360°C). Injector and FID temperatures were set at 250°C and 280°C, respectively. The oven temperature was kept at 60°C for 1 minute and then raised to 250°C at 5.0°C/min and held for 2 minutes, whereas the ambient oven temperature range was +4°C to 450°C. Helium gas was used at a flow rate of 1.1 mL/min as a carrier gas. The split mode in GC was in the ratio 1:100.

Gas Chromatography-Mass Spectrometric Analysis
Analysis of the samples was carried out using an Agilent 7890B GC-MS instrument equipped with a HP5-MS column (30 m × 0.25 mm, film thickness 0.25 µm) (temperature range: −60°C to +320/340°C). The GC instrument was equipped with split inlet, working in split ratio of 1:100 mode. The injection port temperature was 250°C. The

**Table 2.** Volatile Oil Compounds of Different Parts of *Prangos ferulacea* Harvested From the Northern Steep of “Male-Amiri” at 2300 m.

| NO. | Compounds       | RI  | RT  | Leaf | Flower | Immature seed | Mature seed |
|-----|-----------------|-----|-----|------|--------|---------------|-------------|
| 1   | α-Pinene        | 932 | 5.06| 6.6a | 12.5c  | 11.1d         | 18.0a       |
| 2   | Sabine           | 969 | 5.82| ND   | ND     | ND            | 1.1         |
| 3   | β-Pinene        | 974 | 5.90| 7.7c | 18.6c  | 20.2b         | 26.8c       |
| 4   | α-Phellandrene  | 1002| 6.47| ND   | 1.9    | 7             | 2.9         |
| 5   | δ-3-Carene      | 1008| 6.61| 9.7c | 12.2b  | 20.5a         | 12.1b       |
| 6   | p-Cymene        | 1020| 6.93| ND   | ND     | ND            | 2.5         |
| 7   | β-Phellandrene  | 1025| 7.03| 0.5d | 3.1c   | 12.9a         | 8.9c         |
| 8   | p-Cresol        | 1071| 8.13| 1.3  | 1.4    | ND            | ND          |
| 9   | Terpinolene     | 1086| 8.52| 1.5  | 2.5    | 7             | 2.3         |
| 10  | Virdene         | 1163| 10.74| 8.1a | 8.0a   | 8.3a          | 2.9b        |
| 11  | Bornyl acetate  | 1284| 13.82| ND   | ND     | ND            | 1.5         |
| 12  | (E)-Caryophyllene| 1423| 17.89| 2.0  | ND     | ND            | ND          |
| 13  | α-Humulene      | 1452| 18.76| ND   | ND     | ND            | 1.1         |
| 14  | γ-Muurolene     | 1478| 19.14| 3.2  | 2.8    | ND            | 1.4         |
| 15  | (Z)-Nerolidol   | 1531| 20.72| 4.2b | 6.3a   | 2.2d          | 3.2c        |
| 16  | α-Cadinene      | 1537| 21.03| 0.6  | 1.3    | ND            | ND          |
| 17  | cis-Cadinene ether | 1552| 21.29| 0.6  | ND     | ND            | ND          |
| 18  | Germacrene B    | 1559| 21.35| 6.5c | 2.5b   | 0.2e          | 0.3c        |
| 19  | Caryophyllene oxide | 1582| 22.31| 6.0  | 2.4    | ND            | ND          |
| 20  | α-Bisabolol     | 1685| 24.66| 8.2c | 4.0b   | 0.6c          | 2.2c        |
| 21  | (2Z,6Z)-Farnesol| 1698| 25.11| 1.4  | ND     | ND            | ND          |
| 22  | (Z)-Terpine     | 1844| 28.22| 8.2  | ND     | ND            | ND          |

**Figure 3.** Free radical scavenging potential of various *Prangos ferulacea* samples. Data represent the mean values of 3 experiments (± standard deviation).
oven temperature was kept at 60°C for 1 minute and next programmed from 60°C to 250°C at 5°C/min, then the temperature was kept at 250°C for 2 minutes. Helium (99.999%) was used as a carrier gas with a flow rate of 1.1 mL/min and inlet pressure 35.3 kPa. The mass spectrometer was operated in the electron impact mode at 70 eV. The inert ion source (High Efficiency Source [HES] Electron Ionization [EI]) temperature was set at 350°C, temperature of quadrupole was set at 150°C, and the MS interface was set to 250°C. A scan rate of 0.6 seconds (cycle time: 0.2 seconds) was applied, covering a mass range from 40 to 460 amu.

Identification of Essential Oil Composition

Most of the compounds were identified using 2 different analytical methods: (a) comparison of retention indices to those of n-alkanes (C₈-C₃₄)³¹ and (b) based on mass spectral data (comparison with authentic chemicals and Wiley spectral library collection). Identification was considered tentative when based on mass spectral data alone. In GC-FID and GC-MS, data acquisition and analysis were performed using Chrom-card and Xcalibur™ softwares, respectively.

Liquid extract preparation. 5 g of each accession was individually extracted with MeOH (3 × 75 mL) in ultrasonic bath (VWR-USC300D) at room temperature. After evaporating the solvent under reduced pressure at 50°C (Rotavapor R-114, Büchi), the concentrated extracts were assessed for antiradical activities.

Antiradical Capacity

DPPH Assay

Free radical scavenging activity of the plant extracts was assessed by DPPH assay.⁵⁰ The measurement was carried out on a 96-well microtiter plate. In brief, microdilution series of samples (1 mg/mL, dissolved in MeOH) were prepared starting with 150 µL. To gain 200 µL of sample, 50 µL of DPPH reagent (100 µM) was further added to each sample. The microplate was stored at room temperature in darkness. The absorbance was measured after 30 minutes at 550 nm using a microplate reader. MeOH and ascorbic acid (0.01 mg/mL) were used as blank control and standard, respectively.

Antiradical activity was calculated using the following equation:

\[
I_/\% = \left( \frac{A_0 - A_1}{A_0} \right) \times 100 ,
\]

where \( A_0 \) is the absorbance of the control and \( A_1 \) is the absorbance of the sample. Antiradical activity of the samples was expressed as EC₅₀ (concentration of the compounds that caused 50% inhibition). Each sample was measured in triplicate.

ORAC Assay

The ORAC assay was carried out on 96-well microtiter plates.⁵¹ In brief, 20 µL of extracts (0.1, 0.01, and 0.005 mg/mL) were mixed with 60 µL of AAPH (a peroxyl free radical initiator) and 80 µL of 50 µM DCFH-DA (2',7'-dichlorodihydrofluorescein diacetate) in a 96-well microtiter plate. The plate was incubated at 37°C for 30 min and then the fluorescence was measured at 520 nm. The ORAC activity of the samples was expressed as EC₅₀ (concentration of the compounds that caused 50% inhibition). Each sample was measured in triplicate.

Table 3. Geographic Locations and Voucher’s Codes of the Studied Iranian Prangos ferulacea Populations

| Plant location | Steep location | Abbreviated name | Voucher’s code | Altitude (m) | Latitude | Longitude |
|----------------|----------------|------------------|----------------|--------------|----------|-----------|
| Mongar         | South          | M.S-3000         | KHAU_450       | 3000         | 31°22'44.1" N | 50°12'12.2" E |
| Mongar         | South          | M.S-2500         | KHAU_451       | 2500         | 31°22'30.9" N | 50°11'55.3" E |
| Mongar         | South          | M.S-2000         | KHAU_452       | 2000         | 31°22'24.2" N | 50°10'16.8" E |
| Mongar         | North          | M.N-2500         | KHAU_453       | 2500         | 31°22'55.6" N | 50°11'50.5" E |
| Tagak          | South          | T.S-2500         | KHAU_454       | 2500         | 31°26'39.6" N | 50°12'15.8" E |
| Gandomkar      | North          | G.N-2600         | KHAU_455       | 2600         | 31°26'43.8" N | 50°12'18.3" E |
| Darreh-Siah    | North          | D.S.N-2000       | KHAU_456       | 2000         | 31°25'24.3" N | 50°12'00.3" E |
| Izeh (wild)    | North          | I.W              | KHAU_457       | 2600         | 31°44'57.5" N | 50°17'23.6" E |
| Izeh (cultivated) | South      | I.C              | KHAU_458       | 824          | 31°42'11.4" N | 50°17'55.6" E |
| Male-Amiri     | North          | M.A.N-2300       | KHAU_459       | 2300         | 31°24'59.9" N | 50°12'43.4" E |
generator, 12 mM) and 120 µL of fluorescein solution (70 mM). Then, the fluorescence was measured for 3 hours with 1.5-minute cycle intervals with a microplate reader. As standard, Trolox® was used. Activities of samples were compared with rutin, ascorbic acid, and EGCG as positive controls. Antioxidant capacities were reported as mmol TE (Trolox® equivalents)/g of dry matter.

**Statistical Analysis**

All the experiments were done in triplicate and the results expressed as mean ± standard deviation. The data were assessed with one-way analysis of variance using SAS Software and GraphPad Prism version 6.05. The means were compared using Duncan comparisons test ($P < 0.05$).

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