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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" (EC_{50} = 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, Caucasia, 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 essential oil (EO) components of *P. ferulacea*. Among them α- and β-pinene,[8-12] γ-terpinene,[8] δ-3-carene,[13] and β-phellandrene[13,14] were the most significant ones. Former studies revealed antibacterial,[10,14-17] phytotoxic, and fungistatic activities of *P. ferulacea* EO.[9] Antioxidant[6,17-20] and antibiofilm[17] activities were reported in addition to quantitative data on total phenolic[6,19-22] and flavonoid contents of extracts.[6,20-22] Cytotoxic and antiherpes potential of the isolated coumarins[23-25] 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

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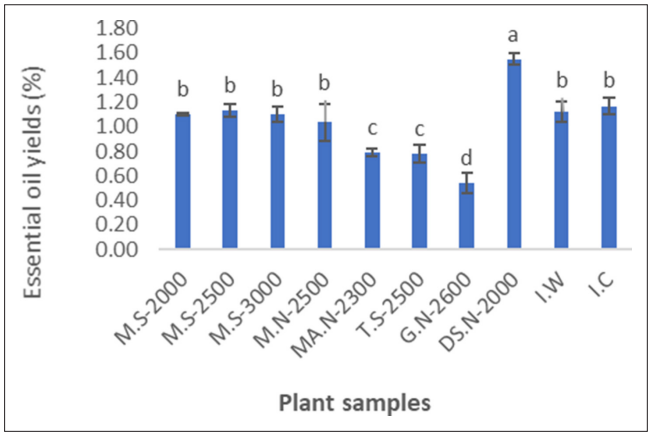

Figure 1. The yields of essential oils of 10 *Prangos ferulacea* accessions. Data represent the mean values of 3 experiments (±standard deviation). The means were compared using Duncan comparisons test ($P < 0.05$).

nitric oxide release of rat peritoneal macrophages.[28] The hydro-alcoholic extract of *P. ferulacea* prevented the histopathological changes of liver and pancreas in diabetic rats.[18,29,30]

Because *P. ferulacea* has been widely consumed in Iranian folk medicine and as food, our study was planned to assess the EO content and composition of previously unstudied accessions of this plant.

To identify the desired chemotypes of *P. ferulacea* for considering in phytopharmaceutical, food, and cosmetic industries, the ecological effects on volatile oil compositions and antiradical potential of the hydroalcoholic extracts from different accessions were investigated. The plant materials were harvested from 10 various locations with diverse geographical attributes. Furthermore, the cultivated and wild samples from "Izeh" and plant parts of "Male-Amiri" were also studied.

Essential oil yield as well as oil compositions of the selected accessions was significantly affected by ecological conditions. The plant samples "DS.N-2000" and "G.N-2600" with 1.55% ± 0.05% and 0.54% ± 0.08% EO content represented the highest and lowest EO yields, respectively (Figure 1).

Twenty-two fragrant compounds (76.7%-90.3% of total oil) were totally identified. Although, among accessions, monoterpene hydrocarbons were characterized as the predominant EO constituents, their ratio changed within a wide range (27.6%-83.4% of the identified compounds) in samples "G.N-2600" and "MA.N-2300."

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 (8.1%) 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 chemodiversity of EOs in different accessions were reflected in 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 EC_{50} = 13.11 ± 0.69 µg/mL; however, it was inferior than the positive control ascorbic acid (EC_{50} = 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 EC_{50} = 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 EC_{50} = 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 more potent antiradical activity with 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.

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

Table 1. Chemical Compositions of Essential Oils Obtained From the Leaves of 10 *Prangos Ferulacea* Accessions Harvested at Flourishing Period.

No.	[a]Compounds	[b]RI	RT	M.S-2000	M.S-2500	M.S-3000	M.N-2500	MA.N-2300	T.S-2500	G.N-2600	DS.N-2000	I.W	I.C
1	α-Pinene	932	5.06	11.8^{d}	16.4^{a}	10.1^{e}	8.3^{g}	16.3^{a}	14.6^{b}	4.8^{h}	8.9^{f}	14.2^{bc}	14.0^{c}
2	Sabinene	969	5.82	1.6	0.5	1.3	0.5	2.4	ND	ND	ND	1.2	1.3
3	β-Pinene	974	5.90	22.7^{c}	22.9^{c}	15.7^{e}	14.6^{f}	27.9^{a}	22.7^{c}	9.4^{g}	17.7^{d}	24.0^{b}	22.4^{c}
4	α-Phellandrene	1002	6.47	ND	2.4	0.5	1.6	6.1	1.4	ND	4.3	2.7	2.9
5	δ-3-Carene	1008	6.61	14.5^{bc}	14.1^{c}	15.4^{b}	20.6^{a}	13.0^{d}	12.1^{e}	8.1^{f}	21.0^{a}	12.4^{de}	12.7^{de}
6	*p*-Cymene	1020	6.93	0.6	ND	ND	ND	1.4	ND	1.3	1.2	1.5	1.5
7	β-Phellandrene	1025	7.03	1.2^{f}	4.3^{d}	1.6^{e}	4.3^{d}	13.1^{a}	1.0^{g}	0.9^{h}	8.3^{bc}	7.3^{c}	7.6^{bc}
8	(*Z*)-β-Ocimene	1032	7.20	ND	ND	ND	ND	ND	2.6	ND	ND	ND	ND
9	*p*-Cresol	1071	8.13	0.5	ND	0.7	ND	ND	0.5	1.4	ND	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	3.3
11	Alloocimene	1140	9.98	0.6	ND	ND	ND	ND	ND	1.4	ND	ND	ND
12	Viridene	1163	10.74	4.2^{c}	3.2^{d}	7.6^{b}	5.5^{a}	2.8^{e}	5.3^{b}	3.6^{d}	4.3^{c}	2.1^{f}	2.7^{e}
13	(*E*)-Caryophyllene	1423	17.89	ND	ND	ND	ND	ND	ND	1.2	ND	1.1	0.6
14	γ-Muurolene	1478	19.14	2.2	1.7	3.3	1.9	ND	2.2	2.7	1.3	ND	ND
15	(*Z*)-Nerolidol	1531	20.72	3.1^{g}	4.6^{e}	2.6^{h}	5.3^{d}	1.0^{i}	9.2^{a}	7.9^{b}	4.2^{f}	6.1^{c}	5.7^{cd}
16	α-Cadinene	1537	21.03	1.2	ND	1.2	ND	ND	ND	1.6	ND	ND	ND
17	*cis*-Cadinene ether	1552	21.29	ND	ND	ND	ND	ND	ND	ND	ND	2.4	ND
18	Germacrene B	1559	21.35	4.3^{bc}	2.6^{de}	4.8^{b}	4.0^{c}	0.2^{g}	2.9^{d}	8.6^{a}	2.1^{f}	2.4^{e}	2.4^{e}
19	Caryophyllene oxide	1582	22.31	3.6	1.2	3.1	2.5	ND	2.0	7.8	1.3	1.8	1.9
20	α-Bisabolol	1685	24.66	4.1^{c}	4.0^{c}	4.8^{b}	3.8^{cd}	0.9^{g}	3.3^{e}	10.2^{a}	3.5^{de}	2.8^{f}	3.1^{ef}
21	(2*Z*,6*Z*)-Farnesol	1698	25.11	ND	ND	ND	ND	ND	ND	1.2	3.5	ND	ND
22	(*Z*)-Ternine	1844	28.22	2.1	3.4	5.0	5.0	ND	1.6	2.9	1.7	ND	ND
Monoterpene hydrocarbons				52.4	63.2	48.4	53.6	83.4	56.4	27.6	68.4	66.7	65.7
Sesquiterpene hydrocarbons				7.7	4.3	9.3	5.9	0.2	5.1	14.1	3.4	3.5	3.0
Oxygenated sesquiterpenes				10.8	9.9	10.8	11.7	1.9	14.5	27.1	12.5	13.2	10.8
Others				6.8	6.6	13.3	10.5	2.8	7.4	7.9	6.0	2.1	2.7
Total				81.5	84.0	81.6	81.7	88.4	83.4	76.7	90.3	85.6	82.0

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 C_8-C_{24} *n*-alkanes on Agilent 7890B capillary column.[31]
[c]Retention times

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] antinociceptive,[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.

The main EO components of aerial parts and seeds of *P. ferulacea* were characterized as *β*-pinene with 22.9% and 33.0%, respectively.[11] In literature the following compounds were also recorded as the predominant volatile compounds of *P. ferulacea*: linalool in leaf (36.7%) and flower (19.0%)

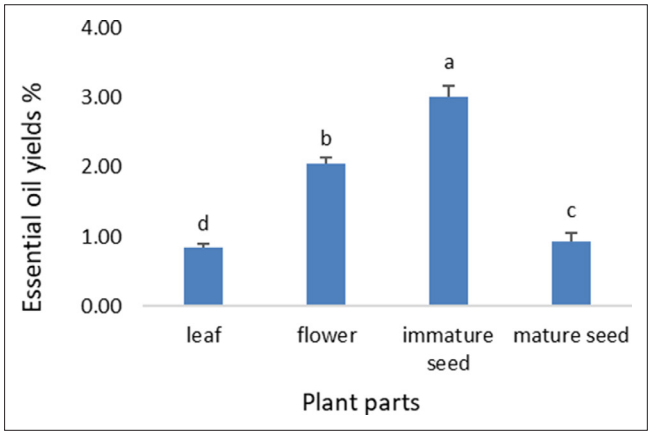

Figure 2. The yields of volatile oils from different plant parts of "Male-Amiri" from the northern steeps at 2300 m. Data represent the mean values of 3 experiments (± standard deviation). The means were compared using Duncan comparisons test ($P < 0.05$).

and 1,8-cineole (19.0%) in stem,[44] *α*-pinene (42.2-63.1%),[9] *β*-pinene (43.1%),[12] *β*-phellandrene in leaf (11.1%) and flower (8.1%)[44] and 20.4%,[14] (*E*)-caryophyllene (48.2%),[45] *δ*−3-carene (22.5%).[13]

Investigation of antioxidant activities was reported with diverse potential of *P. ferulacea* extracts in free radicals scavenging from high[6] to low effects.[21] Also, in other studies exhibited slight to moderate activities.[6,17,19,20,46]

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,[31,47-51] 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 phytochemical profile requires studying various plant populations to find out the optimal environmental circumstances.

In accordance with our findings, monoterpene 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 phytonutrients 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 Na_2SO_4 (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

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

NO.	[a]Compounds	[b]RI	RT	[c]Leaf	Flower	Immature seed	Mature seed
1	α-Pinene	932	5.06	6.6[e]	12.5[c]	11.1[d]	18.0[a]
2	Sabinene	969	5.82	ND	ND	ND	1.1
3	β-Pinene	974	5.90	7.7[d]	18.6[c]	20.2[b]	26.8[a]
4	α-Phellandrene	1002	6.47	ND	1.9	7	2.9
5	δ-3-Carene	1008	6.61	9.7[c]	12.2[b]	20.5[a]	12.1[b]
6	*p*-Cymene	1020	6.93	ND	ND	ND	2.5
7	β-Phellandrene	1025	7.03	0.5[d]	3.1[c]	12.9[a]	8.9[b]
8	*p*-Cresol	1071	8.13	1.3	1.4	ND	ND
9	Terpinolene	1086	8.52	1.5	2.5	7.3	2.3
10	Viridene	1163	10.74	8.1[a]	8.0[a]	8.3[a]	2.9[b]
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	4.2[b]	6.3[a]	2.2[d]	3.2[c]
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	6.5[a]	2.5[b]	0.2[e]	0.3[c]
19	Caryophyllene oxide	1582	22.31	6.0	2.4	ND	ND
20	α-Bisabolol	1685	24.66	8.2[a]	4.0[b]	0.6[e]	2.2[c]
21	(2*Z*,6*Z*)-Farnesol	1698	25.11	1.4	ND	ND	ND
22	(*Z*)-Ternine	1844	28.22	8.2	ND	ND	ND
Monoterpene hydrocarbons				26.0	50.9	79.0	74.6
Sesquiterpene hydrocarbons				12.2	6.7	0.2	2.8
Oxygenated sesquiterpenes				20.4	12.7	2.7	5.4
Others				17.6	9.4	8.3	4.4
Total				76.1	79.7	90.2	87.2

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 C_8-C_{24} *n*-alkanes on Agilent 7890B capillary column.[31]
[c]Harvested at vegetative stage.

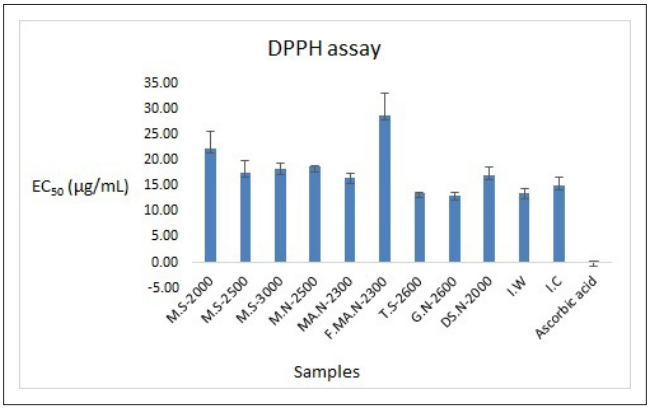

Figure 3. Free radical scavenging potential of various *Prangos ferulacea* samples. Data represent the mean values of 3 experiments (± standard deviation).

+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

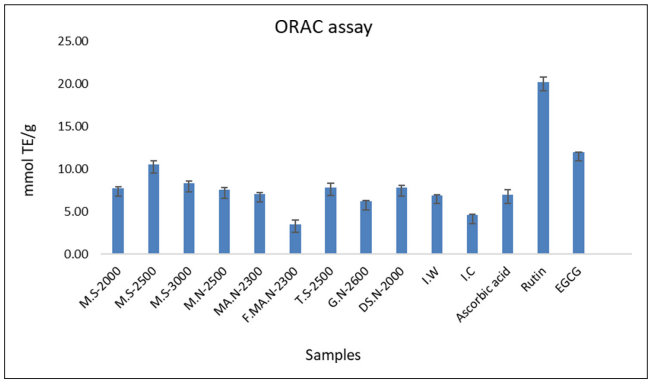

Figure 4. Antiradical activities of various plant samples of *Prangos ferulacea* evaluated by ORAC assay. Data represent the mean values of 3 experiments (± standard deviation).

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 (C_8-C_{24})[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:

$I\% = [(A_0 - A_1/A_0) \times 100]$,

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 EC_{50} (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

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	DS.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	MA.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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