

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

Natural Product Communications
 May 2019: 1–9
 © The Author(s) 2019
 Article reuse guidelines:
sagepub.com/journals-permissions
 DOI: 10.1177/1934578X19851985
journals.sagepub.com/home/npx



Soleyman Bagherifar¹, Mohammad Mahmoodi Sourestani¹, Maryam Zolfaghari¹, Javad Mottaghipisheh², Zoltán Péter Zomborszki², and Dezső Csupor²

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 $3.0\% \pm 0.16\%$ and $0.79\% \pm 0.03\%$ 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” ($EC_{50} = 13.11 \pm 0.69 \mu\text{g/mL}$) and “M.S-2500” ($10.55 \pm 0.41 \text{ mmol TE/g}$) 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

Received: January 12th, 2019; Accepted: February 25th, 2019.

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} phytoxic, and fungistatic activities of *P. ferulacea* EO.⁹

Antioxidant^{6,17-20} and antibiofilm¹⁷ 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 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

¹ Department of Horticultural Science, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Iran

² Department of Pharmacognosy, University of Szeged, Hungary

Corresponding Author:

Mohammad Mahmoodi Sourestani, Department of Horticultural Science, Faculty of Agriculture, Shahid Chamran University of Ahvaz, Ahvaz, Iran.
 Email: m.mahmoodi@scu.ac.ir



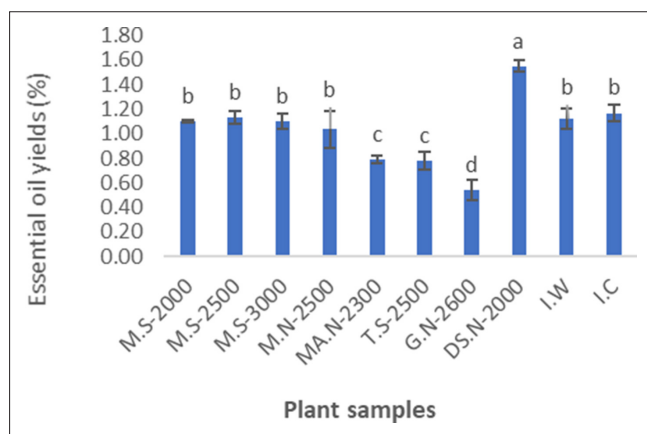


Figure 1. The yields of essential oils of 10 *Prangos ferulacea* accessions. Data represent the mean values of 3 experiments (\pm standard deviation). The means were compared using Duncan comparisons test ($P < 0.05$).

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 $1.55\% \pm 0.05\%$ and $0.54\% \pm 0.08\%$ 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 $3.0\% \pm 0.16\%$ and $0.79\% \pm 0.03\%$ 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 $EC_{50} = 13.11 \pm 0.69 \mu\text{g/mL}$; however, it was inferior than the positive control ascorbic acid ($EC_{50} = 0.3 \pm 0.02 \mu\text{g/mL}$). 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 $EC_{50} = 28.86 \pm 4.29 \mu\text{g/mL}$. The capacity of a wild sample harvested from Izeh (I.Z) to scavenge free radicals was higher than the cultured specimen with $EC_{50} = 13.48 \pm 0.93$ and $15.08 \pm 1.58 \mu\text{g/mL}$, respectively (Figure 3).

The accessions “M.S-2500” ($10.55 \pm 0.41 \text{ mmol TE/g}$) and “F.MA.N-2300” ($3.53 \pm 0.45 \text{ mmol TE/g}$) 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 $11.97 \pm 0.02 \text{ mmol TE/g}$, respectively) as the controls. The wild plant gathered in Izeh (“I.W”) demonstrated more potent antiradical activity with $6.92 \pm 0.04 \text{ mmol TE/g}$ than the cultivated “I.C” with $4.57 \pm 0.09 \text{ mmol TE/g}$ (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	<i>p</i> -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	<i>p</i> -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 -Muuroolene	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	<i>cis</i> -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	(2Z,6Z)-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 ($P < 0.05$).

^aCompounds listed in order of elution.

^bRetention indices relative to C₈-C₂₄ *n*-alkanes on Agilent 7890B capillary column.³¹

^cRetention 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%)

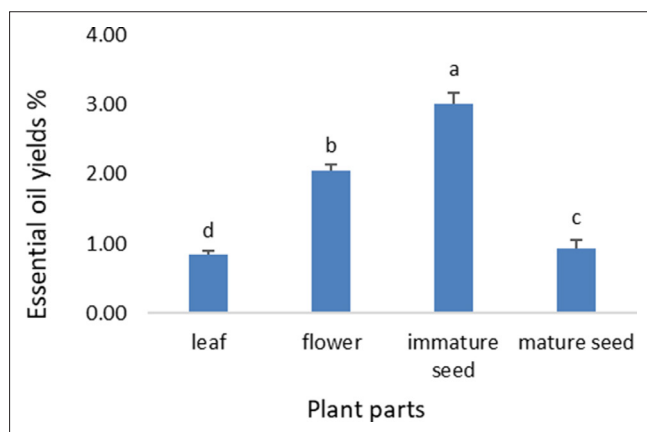


Figure 2. The yields of volatile oils from different plant parts of “Male-Amiri” from the northern steeps at 2300 m. Data represent the mean values of 3 experiments (\pm standard deviation). The means were compared using Duncan comparisons test ($P < 0.05$).

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 high⁶ 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 Chehrizi 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-methylpropionamide 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 \times 0.25 mm, 0.25 μ m 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	<i>p</i> -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	<i>p</i> -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	(<i>E</i>)-Caryophyllene	1423	17.89	2.0	ND	ND	ND
13	α -Humulene	1452	18.76	ND	ND	ND	1.1
14	γ -Murolene	1478	19.14	3.2	2.8	ND	1.4
15	(<i>Z</i>)-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	<i>cis</i> -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 <i>Z</i> ,6 <i>Z</i>)-Farnesol	1698	25.11	1.4	ND	ND	ND
22	(<i>Z</i>)-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 ($P < 0.05$).

^aCompounds listed in order of elution;

^bRetention indices relative to C₈-C₂₄ *n*-alkanes on Agilent 7890B capillary column.³¹

^cHarvested at vegetative stage.

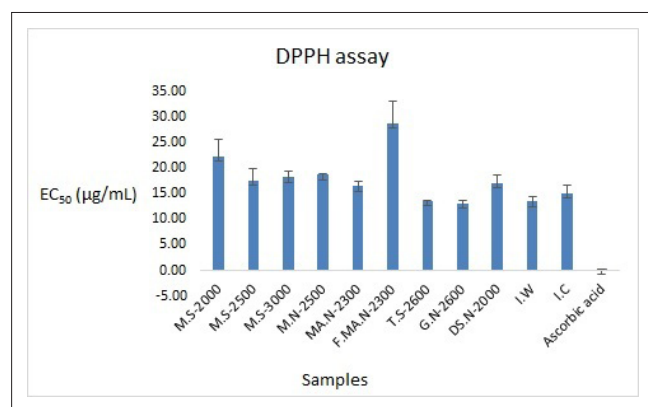


Figure 3. Free radical scavenging potential of various *Prangos ferulacea* samples. Data represent the mean values of 3 experiments (\pm standard deviation).

+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 \times 0.25 mm, film thickness 0.25 μ m) (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

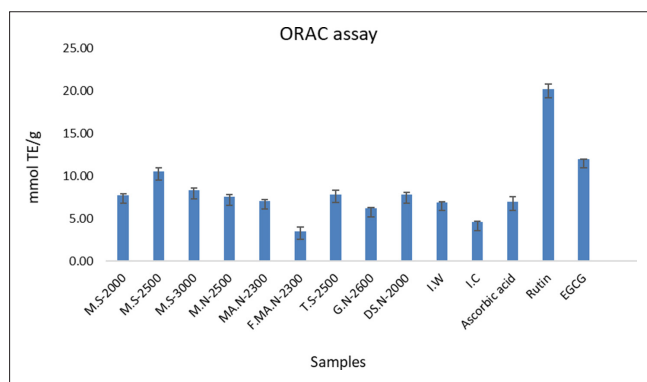


Figure 4. Antiradical activities of various plant samples of *Prangos ferulacea* evaluated by ORAC assay. Data represent the mean values of 3 experiments (\pm standard deviation).

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-C₂₄)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 μ L. To gain 200 μ L of sample, 50 μ L of DPPH reagent (100 μ M) 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 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 μ L of extracts (0.1, 0.01, and 0.005 mg/mL) were mixed with 60 μ L of AAPH (a peroxy 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 μ L 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 \pm 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 ($P < 0.05$).

Acknowledgments

We kindly acknowledge the Department of Pharmacognosy, Faculty of Pharmacy, University of Szeged, especially Prof Dr Hohmann, for cordial collaboration.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This assay was financially supported by the Department of Horticultural Science, Shahid Chamran University of Ahvaz.

References

- Gokhan Senol S, Yildirim H, Secmen O. *Prangos hulussii* sp. nov. (Apiaceae) from west. Anatolia, Turkey: Nordic Journal of Botany; 2011;29: 402-407.
- Rechinger KH, Hedge IC. *Flora Iranica*. Graz, Austria: Akademische Druke Verlagsanstalt; 1987.
- Mozaffarian V. *Flora of Iran, Umbelliferae*. Tehran: Publication of Research Institute of Forests and Rangelands; 2007.
- Ghasemi Pirbalouti A, Momeni M, Bahmani M. Ethnobotanical study of medicinal plants used by Kurd tribe in Dehloran and Abadan districts, Ilam Province, Iran. *Afr J Tradit Complement Altern Med*. 2013;10(2):368-385.
- Bahmani M, Rafeian-kopaei M, Avijgan M, et al. Ethnobotanical studies of medicinal plants used by Kurdish owner's in south range of Ilam province, west of Iran. *Am Eurasian J Agric Environ Sci*. 2012;12:1128-1133.
- Çoruh N, Celep A.G, Sağdıçođlu, Özgökçe F. Antioxidant properties of *Prangos ferulacea* (L.) Lindl., *Chaerophyllum macropodium* Boiss. and *Heracleum persicum* Desf. from Apiaceae family used as food in Eastern Anatolia and their inhibitory effects on glutathione-S-transferase. *Food Chem*. 2007;100(3):1237-1242.
- Özgen U, Kaya Y, Houghton P. Folk medicines in the villages of Ilca district (Erzurum, Turkey). *Turkish J Biol*. 2012;36:93-106.
- Baser KHC, Ermin N, Adigüzel N, Aytaç Z. Composition of the essential oil of *Prangos ferulacea* (L.) Lindl. *J Essent Oil Res*. 1996;8(3):297-298.
- Razavi SM. Chemical composition and some allelopathic aspects of essential oils of (*Prangos ferulacea* L.) Lindl at different stages of growth. *J Agr Sci Tech*. 2012;14:349-356.
- Razavi SM, Nazemiyeh H, Zarrini G, Asna-Asharii S, Dehghan G. Chemical composition and antimicrobial activity of essential oil of *Prangos ferulaceae* (L.) Lindl from Iran. *Nat Prod Res*. 2010;24(6):530-533.
- Sefidkon F, Khajavi MS, Malackpour B. Analysis of the Oil of *Prangos ferulacea* (L.) Lindl. *J Essent Oil Res*. 1998;10(1):81-82.
- Delnavazi M-R, Soleimani M, Hadjiakhoondi A, Yass N. Isolation of phenolic derivatives and essential oil analysis of *Prangos ferulacea* (L.) Lindl. aerial parts. *Iran J Pharm Res*. 2017;16(Suppl):207-215.
- Sajjadi SE, Shokoohinia Y, Gholamzadeh S. Chemical composition of essential oil of *Prangos ferulacea* (L.) Lindl. roots. *Chemija*. 2011;22:178-180.
- Mohammadhosseini M. Chemical profile and antibacterial activity in hydrodistilled oil from aerial parts of *Prangos ferulacea* (L.) Lindl. and prediction of gas chromatographic retention indices by using genetic algorithm multiple linear regressions. *Asian J Chem*. 2012;24:3814-3820.
- Akbari MT, Esmaceli A, Zare AH, Saad N, Bagheri F. Chemical composition and antibacterial activity of essential oil from leaves, stems and flowers of *Prangos ferulacea* (L.) Lindl. grown in Iran. *Bulgarian Chem Comm*. 2010;42:36-39.
- Nosrati M, Behbahani M. *In vitro* and *in silico* antibacterial activity of *PRANGOS*-*FERULACEA* (L.) Lindl and *PRANGOS* *ULOPTERA* DC, and their mutagenicity in the Ames test. *J Microbiol Biotechnol Food Sci*. 2016;6(3):930-936.
- Nosrati M, Behbahani M, Mohabatkar H, Shakeran Z. Antibacterial and antibiofilm activities of *Prangos acaulis* Bornm. extract against *Streptococcus mutans*: an *in silico* and *in vitro* study. *J herbmed pharmacol*. 2018;7(3):176-184.
- Kafash-Farkhad N, Asadi-Samani M, Rafeian-Kopaei M. A review on phytochemistry and pharmacological effects of *Prangos ferulacea* (L.) Lindl. *Life Sci J*. 2013;10:360-367.
- Mavi A, Terzi Z, Özgen U, Yildirim A, Coşkun M. Antioxidant properties of some medicinal plants: *Prangos ferulacea* (Apiaceae), *Sedum sempervivoides* (Crassulaceae), *Malva neglecta* (Malvaceae), *cruciata taurica* (Rubiaceae), *Rosa pimpinellifolia* (Rosaceae), *Galium verum* subsp. *verum* (Rubiaceae), *Urtica dioica* (Urticaceae). *Biol Pharm Bull*. 2004;27(5):702-705.
- Cesur C, Cosge-Şenkal B, Yaman C, Uskutoglu T, Koc M. Antioxidant activity of fruit extracts of *Prangos ferulacea* (L.) Lindl. from Turkey. *Iğdir Univ J Ins Sci Techno*. 2017;7(4):249-256.

21. Ahmed J, Güvenç A, Küçükboyacı N, Baldemir A, Coşkun M. Total phenolic contents and antioxidant activities of *Prangos Lindl.* (Umbelliferae) species growing in Konya province (Turkey). *Turkish J Biol.* 2011;35:353-360.
22. Movahedian A, Zolfaghari B, Mirshekari M. Antioxidant effects of hydroalcoholic and polyphenolic extracts of *Peucedanum pastinacifolium* Boiss. & Hausskn. *Res Pharm Sci.* 2016;11(5):405-411.
23. Shokoohinia Y, Sajjadi S-E, Gholamzadeh S, Fattahi A, Behbahani M. Antiviral and cytotoxic evaluation of coumarins from *Prangos ferulacea*. *Pharm Biol.* 2014;52(12):1543-1549.
24. Shokoohinia Y, Hosseinzadeh L, Alipour M, Mostafaie A, Mohammadi-Motlagh H-R. Comparative evaluation of cytotoxic and apoptogenic effects of several coumarins on human cancer cell lines: osthole induces apoptosis in p53-deficient H1299 cells. *Adv Pharmacol Sci.* 2014;2014(1):1-8.
25. Sajjadi SE, Shokoohinia Y, Gholamzadeh S, Behbahani M, Fattahi A. Antiviral evaluation of coumarins from *Prangos ferulacea* L. *Res Pharmaceut Sci.* 2012;7.
26. Shokoohinia Y, Hosseinzadeh L, Moieni-Arya M, Mostafaie A, Mohammadi-Motlagh H-R. Osthole attenuates doxorubicin-induced apoptosis in PC12 cells through inhibition of mitochondrial dysfunction and ROS production. *Biomed Res Int.* 2014;2014(2):1-7.
27. Asieh A-M, Dolatian M, Mojab F, et al. The effect of *Prangos ferulacea* vaginal cream on accelerating the recovery of bacterial vaginosis: a randomized controlled clinical trial. *J Res Pharmaceut Sci.* 2018;6:101-110.
28. Doković DD, Bulatović VM, Božić BD, Kataranovski MV, Zrakić TM, Kovacević NN. 3,5-Nonadiyne isolated from the rhizome of *Cachrys ferulacea* inhibits endogenous nitric oxide release by rat peritoneal macrophages. *Chem Pharm Bull.* 2004;52(7):853-854.
29. Farokhi F, Kaffash Farkhad N, Togmechi A, Soltani Band K, Kh S-B. Preventive effects of *Prangos ferulacea* (L.) Lindl on liver damage of diabetic rats induced by alloxan. *Avicenna J Phytomed.* 2012;2(2):63-71.
30. Kh S-B, Kafash-Farkhad N, Farokhi F, Togmechi A. Effects of hydro-alcoholic extract of *Prangos ferulacea* (L.) Lindl on histopathology of pancreas and diabetes treatment in STZ-induced diabetic rats. *Avicenna J Phytomed.* 2012;2:31-38.
31. Adams RP. *Identification of essential oil components by Gas Chromatography/Mass Spectrometry.* 4th Edition. Carol Stream, USA: Allured Publishing Corporation; 2007.
32. Gomes-Carneiro MR, Viana MES, Felzenszwalb I, Paumgartten FJR. Evaluation of beta-myrcene, alpha-terpinene and (+)- and (-)-alpha-pinene in the *Salmonella*/microsome assay. *Food Chem Toxicol.* 2005;43(2):247-252.
33. Yang H, Woo J, Pae AN, et al. α -Pinene, a major constituent of pine tree oils, enhances non-rapid eye movement sleep in mice through GABAA-benzodiazepine receptors. *Mol Pharmacol.* 2016;90(5):530-539.
34. Him A, Ozbek H, Turel I, Cihat Oner A. Antinociceptive activity of alpha-pinene and fenchone. *Pharmacologyonline.* 2008;3:363-369.
35. Orhan I, Küpeli E, Aslan M, Kartal M, Yesilada E. Bioassay-guided evaluation of anti-inflammatory and antinociceptive activities of pistachio, *Pistacia vera* L. *J Ethnopharmacol.* 2006;105(1-2):235-240.
36. Pereira Limberger R, Mendes Aleixo A, Fett-Neto AG, T. Henriques A, Limberger RP, Germano Fett-Neto A T. Bioconversion of (+)- and (-)-alpha-pinene to (+)- and (-)-verbenone by plant cell cultures of *Psychotria brachyceras* and *Rauvolfia sellowii*. *Electron J Biotechnol.* 2007;10(4):500-507.
37. Silva ACRda, Lopes PM, Azevedo MMBde, et al. Biological activities of α -Pinene and β -Pinene enantiomers. *Molecules.* 2012;17(6):6305-6316.
38. FDA. *Food and drugs-title 21. In Code of Federal Regulations.* Washington, DC; 2015.
39. Ocete MA, Risco S, Zarzuelo A, Jimenez J. Pharmacological activity of the essential oil of *Bupleurum gibraltarium*: anti-inflammatory activity and effects on isolated rat uteri. *J Ethnopharmacol.* 1989;25(3):305-313.
40. Pichette A, Larouche P-L, Lebrun M, Legault J. Composition and antibacterial activity of *Abies balsamea* essential oil. *Phytother Res.* 2006;20(5):371-373.
41. Cavaleiro C, Pinto E, Gonçalves MJ, Salgueiro L. Antifungal activity of *Juniperus* essential oils against dermatophyte, *Aspergillus* and *Candida* strains. *J Appl Microbiol.* 2006;100(6):1333-1338.
42. Miyazawa M, Yamafuji C, Ch Y. Inhibition of acetylcholinesterase activity by bicyclic monoterpenoids. *J Agric Food Chem.* 2005;53(5):1765-1768.
43. Jeong J-G, Kim YS, Min YK, Kim SH. Low concentration of 3-carene stimulates the differentiation of mouse osteoblastic MC3T3-E1 subclone 4 cells. *Phytother Res.* 2008;22(1):18-22.
44. Taherkhani M, Rustaiyan A, Sh M. Volatile constituents of the aerial parts of *Ferulago subvelutina* Rech. F., *Ferulago stellata* Boiss., leaves and flowers of *Prangos ferulacea* (L.) Lindl. and leaves of *Ferula ovina* (Boiss.) Boiss. Four Umbelliferae herbs from. *Asian J Chem.* 2012;24:1601-1606.
45. Mohebi Z, GhA H, Sefidkon F, Zare-Chahouki MA. The influence of plant growth stage, individuals of species, and extraction methods on the essential oil content and the chemical composition of *Prangos ferulacea* (L.) Lindl. *Appl Ecol Environ Res.* 2017;15(4):1765-1776.
46. Kafash-Farkhad N, Asadi-Samani M, Khaledifar B. A review on secondary metabolites and pharmacological effects of *Prangos ferulacea* (L.) Lindl. *J Shahrekord Univ Med Sci.* 2013;15:98-108.
47. Bouzenna H, Hfaiedh N, Giroux-Metges M-A, Elfeki A, Talarmin H. Potential protective effects of alpha-pinene against cytotoxicity caused by aspirin in the IEC-6 cells. *Biomed Pharmacother.* 2017;93:961-968.
48. Aydin E, Türkez H, Geyikoğlu F. Antioxidative, anticancer and genotoxic properties of α -pinene on N2a neuroblastoma cells. *Biologia.* 2013;68(5):1004-1009.
49. Dai J, Zhu L, Yang L, Qiu J. Chemical composition, antioxidant and antimicrobial activities of essential oil from *Wedelia prostrata*. *Excli J.* 2013;12:479-490.

-
50. Fukumoto LR, Mazza G. Assessing antioxidant and prooxidant activities of phenolic compounds. *J Agric Food Chem.* 2000;48(8):3597-3604.
51. Mielnik MB, Rzeszutek A, Triumf EC, Egelanddal B. Antioxidant and other quality properties of reindeer muscle from two different Norwegian regions. *Meat Sci.* 2011;89(4):526-532.