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FULL PAPER

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Variation of Chemical Constituents and Antiradical Capacity of Nine *Ferulago angulata* Populations from Iran

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The present study was designed to assess the influence of geographical factors on essential oil (EO) composition, along with antiradical potential and phytochemical contents of *Ferulago angulata* (Schltdl.) Boiss (Apiaceae) extracts for the first time. The aerial parts were hydrodistilled by Clevenger apparatus and subjected to gas chromatography coupled with flame ionization detector (GC/FID) and mass spectroscopy (GC/MS). The EO yields were significantly different from populations 'Mongar' (south-slope, 3000 m) with 1.34 $\pm$ 0.06 % and 'Male-Amiri' (north slope, 2600 m) with 0.18 $\pm$ 0.05 % of total oil. Thirty-nine compounds were identified from the EOs of nine populations. α-Pinene was the predominant component ranging from 20.84 to 49.06 % in 'Gandomkar' (north-slope, 2500 m) and 'Mongar' (3000 m), respectively. The methanolic extract of 'Mongar' (north-slope at 2500 m) possessed the highest total phenolic contents. Also, this population logically exhibited potent antiradical activity using both 1,1-diphenyl-2-picrylhydrazyl (DPPH) and oxygen radical absorbance capacity (ORAC) assays with EC_{50} of 42.07 $\pm$ 4.12 μg/mL and 8.34 $\pm$ 0.21 mmol Trolox® equivalents/g, respectively. Due to its moderate free-radical scavenging potential and high α-pinene content, the population 'Mongar' might be considered as a perspective raw material in food and phytopharmaceutical industries.

Keywords: *Ferulago angulata*, Apiaceae, ecological impacts, chemo-diversity, antiradical potential.

Introduction

The 46 members of the genus *Ferulago* W.D.J.Koch (Apiaceae) grow in Iran, Turkey, and Caucasia.[1] Seven of these species are native to Iran.[2] *F. angulata* (Schltdl.) Boiss (syn. *F. trifida* Boiss.) is famed as 'Chavil' or 'Chavir' in Iran and is a perennial endemic aromatic herb from the nine *Ferulago* species growing particularly in the western part of Iran.[2,3] Its leaves have been traditionally used as antiseptic, pain reliever, in digestive disorders, to treat intestinal worms, snake bites, hemorrhoids, chronic ulcers, and ailments of the spleen.[4] Furthermore, in Western Iran, this plant has been consumed as spice, and used as air fresher, decay preventer and flavoring oil.[5]

Monoterpene hydrocarbons were previously reported as the predominant essential oil (EO) constituents of *F. angulata*. (*Z*)-β-Ocimene was the major EO components of samples collected from Kermanshah (33.91 – 26.78 %),[6] Kurdestan (27.9 %),[7] Dena mountain (35.5 %)[8] and Kohgiluyeh va Boyer Ahmad Province (19.93 %).[9] Furthermore, the following constituents were formerly recorded as the main volatile components of *F. angulata*: (*E*)-β-ocimene (20.7 – 37.3 %),[10] *cis*-ocimene (64.8 – 76.11 %),[11] and α-pinene [(17.31 %),[12] (24.2 %),[13] (28.43 – 35.03 %),[7] (10.5 %),[14] (27.1 – 25.7 %)].[11]]

The bioactivities of the extract of aerial parts and of the EO have been previously studied. For instance, different extracts and coumarins of the plant exerted moderate antiradical scavenging,[15 – 18] antibacterial,[19] and antioxidant activities.[20] Moreover, in the literature antioxidant activity,[4,7,10,21 – 25] *in vivo* anxiolytic and antidepressant for the EO,[4] along with cytotoxicity,

Table 1. The studied *Ferulago angulata* populations and their voucher's codes.

Population Name	latitude	longitude	Slope [%]	Slope facing direction	Altitude [m]	Abbreviated Name	Voucher's Code
Mongar	31°22′45.1″N	50°12′18.2″E	10	–	3000	M-3000	KHAU_350
Mongar	31°23′01.1″N	50°11′25.2″E	50	North	2000	M.N-2000	KHAU_351
Mongar	31°22′55.6″N	50°11′50.5″E	70	North	2500	M.N-2500	KHAU_352
Mongar	31°22′44.1″N	50°12′12.2″E	70	South	2500	M.S-2500	KHAU_353
Male-Amiri	31°24′59.9″N	50°12′43.4″E	30	North	2350	MA.N-2350	KHAU_354
Male-Amiri	31°24′57.6″N	50°12′38.2″E	60	North	2600	MA.N-2600	KHAU_355
Kooh-Siah	31°15′27.6″N	50°14′37.9″E	50	North	1800	KS.N-1800	KHAU_356
Tagak	31°26′39.6″N	50°12′15.8″E	40	South	2500	T.S-2500	KHAU_357
Gandomkar	31°26′43.8″N	50°12′18.3″E	40	North	2500	G.N-2500	KHAU_358

apoptosis-inducing,[16] and *in vitro* anticancer effects[26] of the extracts were reported. Furthermore, the AcOEt and methanolic extract of *F. angulata* previously revealed the highest levels of phenolic compounds among its various extracts.[19]

Due to extensive use of *F. angulata* in Iranian cuisine and traditional medicine, the present study was aimed at quantitatively and qualitatively comparing the EO composition by GC/FID and GC/MS, along with evaluation of total phenolic, flavonoid, flavone and flavonol contents of the methanolic extracts from nine different populations. The *in vitro* antiradical activities of methanolic extracts were further assessed by DPPH and ORAC assays. To the best of our knowledge, this is the first report on comparing the EO, and phenolic contents, along with evaluation of antiradical activities of different *F. angulata* populations.

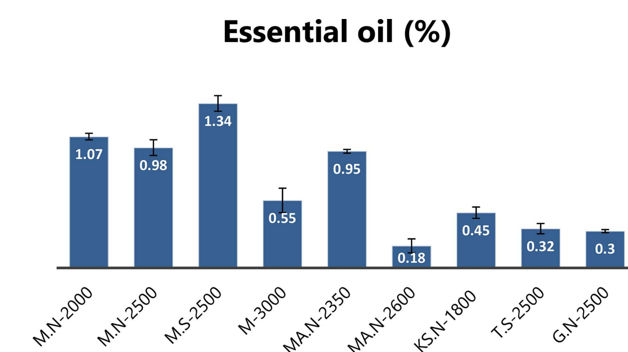

Figure 1. Volatile oil contents (w/w) of nine different *F. angulata* populations. Values are the mean ± SD of three replications ($n=3$).

Results and Discussion

Our results explicitly confirm that the quality and quantity of volatile oils and antioxidant capacities of *F. angulata* extracts were significantly influenced by ecological circumstances. The abiotic factors highly influence on the variety of secondary metabolites, and biotic components remarkably influence the terpene biosynthesis and hence chemotypes. Moreover, other ecological factors (e.g., climatic, physiographic, and edaphic) may also be considered in chemotype variations.[27] The voucher's codes, altitudes, slope facing directions and abbreviated names of the selected plant populations are listed in *Table 1*.

Volatile Oil Components

EO contents of the various samples demonstrated a significant difference according to their growth media including altitude, rainfall, temperature, soil composition, and sunlight. As shown in *Figure 1*, the plant harvested from 'Mongar' located at 3000 m (M-3000) and south-slope aspect 2500 m (M.S-2500) with 0.55 ± 0.1 % and 1.34 ± 0.06 % (w/w) (the richest sample), respectively, showed an evidence of the influence of altitude on EO contents. The EO yield of 'MA.N-2350' and 'MA.N-2600' with 0.95 ± 0.1 % and 0.18 ± 0.05 %, respectively, represented the impact of growth condition variations on EO contents, which are consistent with former reports[28] (*Figure 1*).

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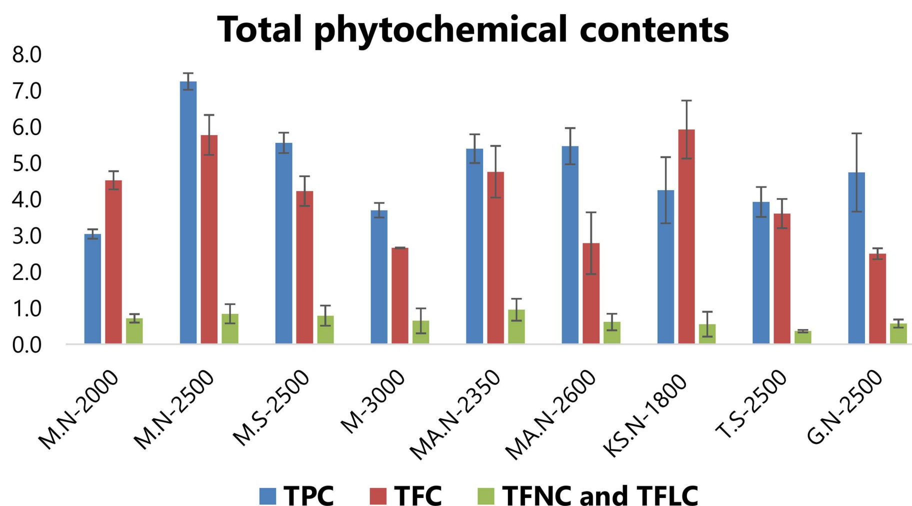

Figure 2. Total phenolic (TPC, mg GaE/g), flavonoids (TFC, mg QuE/g), flavone (TFNC, mg QuE/g), and flavonol (TFLC, mg QuE/g) contents from nine different Iranian *Ferulago angulata* populations. Values are the mean ± SD of three replications ($n=3$).

In accordance with our findings, monoterpene hydrocarbons (34.71–76.99 %), among them α-pinene (20.84–49.06 %) was characterized as the major EO terpenoid which corroborates the previous reports,[7,12–14] except 'KS.N-1800' which was notably richer in (*E*)-β-ocimene (23.65 %) (*Table 2*). Since sample 'M-3000' was harvested at the highest altitude, the EO yield was lowest and interestingly the biosynthesis pathway of volatile secondary metabolites was conducted towards α-pinene production, so this compound was identified as almost half percentage of its EO (49.06 %).

α-Pinene has been widely consumed as a food flavoring ingredient.[29] This bicyclic monoterpene is also known for its various biological and pharmacological properties; for instance, antifeedant,[30] *in vivo* anti-inflammatory,[31] cytotoxicity and antioxidant activities,[32] anti-inflammatory effects on survivability of skin flaps,[33] along with *in vitro* assessment of oxidative and cytogenetic effects[34] have been previously reported.

The acyclic monoterpene (*E*)-β-ocimene (5.95–27.7 %) was furtherly characterized as the second major component. The fragrant β-ocimene plays a relevant role in attracting several types of pollinators to a diverse range of plant flowers. This compound also exhibits an important defensive effect in vegetative plant tissues against parasites and herbivores.[35] Thus, the EO of 'M.N-2500' with the highest content of (*E*)-β-ocimene can be considered as a natural agent to protect the plants against the herbivores.

Total Phenolic Contents (TPC)

Although, the populations 'M.N-2500' and 'M.N-2000' were collected from similar regions, the highest and lowest TPC with 7.25±0.23 and 3.05±0.13 mg gallic acid equivalent (GaE)/g of the dried weight, respectively, indicated the impact of altitude and growth environmental conditions on concentrations of these compounds (*Figure 2*).

Total Flavonoid Contents (TFC)

As shown in *Figure 2*, the highest and lowest concentrations of flavonoids were analyzed in 'KS.N-1800' and 'G.N-2500' with 5.93±0.8 and 2.51±0.15 mg quercetin equivalent (QuE)/g of the dried weight, respectively.

Total Flavone and Flavonol Contents (TFNC and TFLC)

'MA.N-2350' possessed the highest TFNC and TFLC, with 0.96±0.3 mg QuE/g; whilst 'T.S-2500' was determined as the poorest sample with 0.37±0.03 mg QuE/g. However, a significant variation was not observed in the other studied populations (*Figure 2*). The plant sample 'M-3000', which was growing in highest altitude, was characterized with the lowest TPC, TFC, TFNC, and TFLC. To our knowledge, there is only one article in evaluation of TPC from *F. angulata*; which reported ethyl acetate and methanolic extracts were rich in phenolic compounds with 229.2 and 202.9 g/mg gallic acid, respectively,[18] which confirms our findings.

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Table 2. Essential oil constituents of *Ferulago angulata* populations.

No.	Compound[a]	RI[b]	RI[c]	t_R[d]	Distribution of essential oil constituents in different populations G.N-2500	KS.N-1800	T.S-2500	MA.N-2350	M-3000	M.N-2500	M.N-2000	MA.N-2600	M.S-2500
1	α-Pinene	937	939	4.12	20.84[e]	22.85[d]	35.92[b]	35.12[b]	49.06[a]	34.41[b]	32.66[b,c]	23.38[d]	31.07[c,d]
2	Camphene	951	953	4.35	0.26	0.37	1.24	1.29	1.74	1.39	1.38	0.41	1.36
3	2,4(10)-Thujadiene	956	960	4.44	1.0	0.4	0.57	0.14	0.27	0.3	0.3	0.75	0.29
4	Sabinene	975	976	4.76	0.06	0.46	0.33	0.53	0.59	0.46	0.46	ND	0.40
5	β-Pinene	980	980	4.84	1.07	1.62	1.78	2.17	2.49	1.94	1.81	1.96	1.94
6	β-Myrcene	992	991	5.05	1.24[e]	2.84[d]	2.46[d]	3.32[b]	3.71[a]	3.06[b,c]	3.18[b,c]	1.21[e]	2.94[c,d]
7	α-Phellandrene	1008	1005	5.35	0.33	0.44	0.8	1.94	ND	ND	0.18	2.07	ND
8	*d*-Limonene	1031	1031	5.86	1.49	1.98	2.59	3.06	2.82	2.5	3.17	2.5	2.26
9	(*E*)-β-Ocimene	1040	1040	6.06	5.95[f]	23.65[c]	12.21[e]	26.82[a]	14.65[d]	27.7[a]	21.07[c]	12.4[e]	16.7[b]
10	*trans*-β-Ocimene	1049	1050	6.25	2.1	2.72	1.63	1.19	1.66	1.18	1.75	2.12	0.6
11	γ-Terpinene	1061	1062	6.51	0.12	2.21	0.27	0.97	ND	0.55	0.29	ND	0.21
12	α-Terpinolene	1092	1088	7.19	0.25	0.37	0.23	ND	ND	ND	0.33	ND	ND
13	Linalool	1104	1098	7.47	5.02	0.83	2.34	1.34	0.72	1.48	1.71	3.55	1.74
14	*cis*-Verbenol	1145	1140	8.54	3.5	2.27	3.01	1.56	1.2	1.86	2.62	1.09	1.01
15	*trans*-Verbenol	1150	1144	8.67	8.31[a]	5.15[c]	7.56[a]	4.95[c]	3.01[d]	4.74[c]	6.9[a,b]	5.9[c]	4.64[b]
16	ρ-Mentha-1,5-dien-8-ol	1171	1166	9.2	3.36	0.95	1.99	1.08	0.71	1.05	1.16	1.15	1.33
17	α-Terpineol	1195	1189	9.78	0.5	0.48	0.54	0.16	0.12	0.39	0.35	0.6	0.25
18	1-Decanal	1208	1201	10.07	2.73	0.77	1.35	0.3	ND	0.17	0.35	1.58	ND
19	β-Citronellol	1234	1233	10.73	1.05	0.76	ND	ND	ND	ND	1.04	1.05	1.29
20	Carvone	1254	1250	11.15	1.02	0.27	ND	ND	ND	ND	ND	ND	ND
21	Bornyl acetate	1291	1285	12.21	5.15[g]	17.42[b]	6.42[f]	7.43[e]	5.52[g]	9.46[d]	12.59[c]	9.07[d]	20.63[a]
22	*trans*-Pinocarvyl acetate	1304	1298	12.53	0.09	0.42	ND	ND	ND	ND	0.35	ND	ND
23	Myrtenyl acetate	1334	1330	13.18	0.18	ND	ND	ND	ND	ND	0.08	ND	ND
24	*trans*-Carvyl acetate	1347	1342	13.48	0.05	ND	ND	ND	ND	ND	ND	ND	ND
25	Citronellyl acetate	1361	1356	13.84	1.63	ND	0.64	ND	ND	ND	ND	ND	ND
26	*cis*-Jasmone	1403	1394	15.1	1.19	0.51	0.49	0.14	0.66	0.33	0.30	1.43	0.15
27	Methyl eugenol	1409	1403	15.23	2.09	1.07	2.32	0.73	4.12	0.67	0.99	1.76	1.72
28	β-Funebrene	1424	1419	15.43	0.07	ND	ND	0.06	ND	0.24	0.05	ND	ND
29	α-Caryophyllene	1428	1425	15.57	0.68	0.27	0.26	0.25	0.61	0.24	0.12	0.71	0.4
30	β-Barbatene	1418	1440	16.15	1.8	1.24	0.71	0.18	1.43	0.26	0.57	2.7	0.74
31	(*E*)-β-Farnesene	1460	1458	16.42	0.89	0.14	0.64	1.43	1.18	1.13	0.63	ND	0.36
32	γ-Curcumene	1484	1481	17.0	1.29	0.17	1.35	0.6	1.00	0.58	0.36	0.54	0.15
33	α-Curcumene	1487	1483	17.09	0.57	ND	ND	0.37	ND	0.52	0.21	1.51	1.71
34	Bicyclogermacrene	1503	1500	17.46	5.18	1.65	1.12	0.37	0.73	0.43	0.64	1.65	0.58
35	(*E,E*)-α-Farnesene	1519	1513	17.7	0.07	ND	ND	ND	ND	ND	ND	ND	ND
36	δ-Cadinene	1529	1522	18.07	5.09	0.30	2.15	0.4	0.13	0.46	0.24	3.98	0.17
37	Germacrene B	1564	1559	18.89	1.85	0.22	0.27	0.10	ND	0.28	0.44	3.35	2.14
38	Spathulenol	1583	1577	19.42	3.38	2.78	1.37	0.35	0.21	0.36	0.26	4.68	0.49
39	α-Eudesmol	1660	1652	21.07	2.94	0.33	2.12	0.29	0.48	0.3	0.35	3.29	0.09
Monoterpene hydrocarbons					34.71	59.91	60.03	76.55	76.99	73.49	66.58	46.8	57.77
Oxygenated monoterpenes					35.87	30.9	26.66	17.69	16.06	20.15	28.44	27.18	32.76
Sesquiterpene hydrocarbons					17.49	3.99	6.5	3.76	5.08	4.14	3.26	14.44	6.25

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Table 2. (cont.)

No.	Compound[a]	RI[b]	RI[c]	t_R[d]	Distribution of essential oil constituents in different populations								
					G.N-2500	KS.N-1800	T.S-2500	MA.N-2350	M-3000	M.N-2500	M.N-2000	MA.N-2600	M.S-2500
	Oxygenated sesquiterpenes				6.32	3.11	3.49	0.64	0.69	0.66	0.61	7.97	0.58
	Total [%]				94.39	97.91	96.68	98.64	98.82	98.44	98.89	96.39	97.36

[a] Compounds listed in order of elution from Agilent DB-5 column; [b] Relative retention index in references (Adams); [c] Relative retention index to C_8–C_{24} *n*-alkanes; [d] Retention times; ND: not detected; Each data is an average of three replications; the means were compared using Duncan comparisons test ($p<0.05$); small letters (a, b, c, etc.) in each row show the significant difference of related component among various populations.

Antiradical Activity of the Extracts

DPPH Assay. Since the phenolic compounds are mostly responsible for scavenging of free radicals, the methanolic extracts were subjected to assessing of antiradical potential. As shown in *Figure 3*, methanolic extract of 'M.N-2500' exhibited the most powerful free-radical scavenging activity with $EC_{50}=42.07\pm4.12$ μg/mL in the DPPH assay; whereas a significant difference was observed in the case of 'T.S-2500' (184.85 ± 4.55 μg/mL) as the weakest sample. All extracts exhibited lower activity than the positive control ascorbic acid (0.30 ± 0.02 μg/mL) (*Figure 3*).

ORAC Assay. In evaluation of antiradical potential, 'M.N-2500' with 8.34 ± 0.21 mmol TE/g and 'T.S-2500' with 4.22 ± 0.19 mmol Trolox® equivalent (TE)/g represented the highest and lowest effects compared with the controls ascorbic acid (6.98 ± 0.58 mmol TE/g), epigallocatechin gallate (EGCG) (11.97 ± 0.02 mmol TE/g), and rutin (20.22 ± 0.63 mmol TE/g) (*Figure 4*).

Although several studies reported the antioxidant activities of the EO, potency of the extracts has been rarely presented. The extracts of *F. angulata* recently showed a higher potential in radical scavenging than its EO.[16] Since the antiradical potency is mainly related to phenolic compounds and these phytoconstituents are present in the extracts, it can be expected that the extracts are stronger agents than EOs.

Since 'M.N-2500' possessed the highest TPC and TFC among the studied populations, the most potent antiradical capacity of its extract is undoubtedly associated to these compounds. In a similar study, DPPH, hydroxyl radical scavenging and total antioxidant activity of methanolic extracts was determined as $IC_{50}=67.34\pm4.14$ μg/mL and 64.87 ± 4.68 μg/mL and 171.61 ± 6.05 mM ascorbic acid/g, respectively.[17]

Classification of F. angulata *Populations*

Identification of the different chemotypes of Iranian *F. angulata* populations according to their major phytochemicals and antioxidant activities was carried out by cluster analysis (CA) and principal component analysis (PCA). The dendrograms categorized the *F. angulata* populations into three major groups, each representing a distinct chemotype (*Figure 5*).

In accordance with the CA, the populations 'T.S-2500', 'G.N-2500', and 'MA.N-2600' were classified at the same category; while 'M-3000' was grouped to the individual subclass and the rest populations including 'M.N-2000', 'M.S-2500', 'MA.N-2350', 'KS.N-1800', and 'M.N-2500' were categorized in one subclass.

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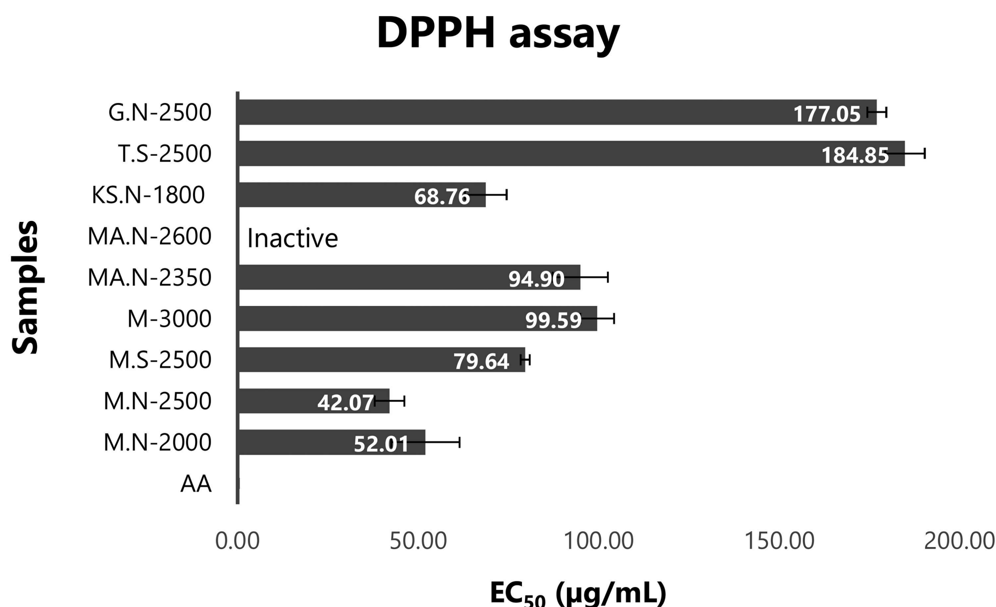

Figure 3. DPPH antiradical activities of extracts from nine studied *Ferulago angulata* populations. AA: ascorbic acid was used as positive control. Values are the mean ± SD of three replications ($n = 3$).

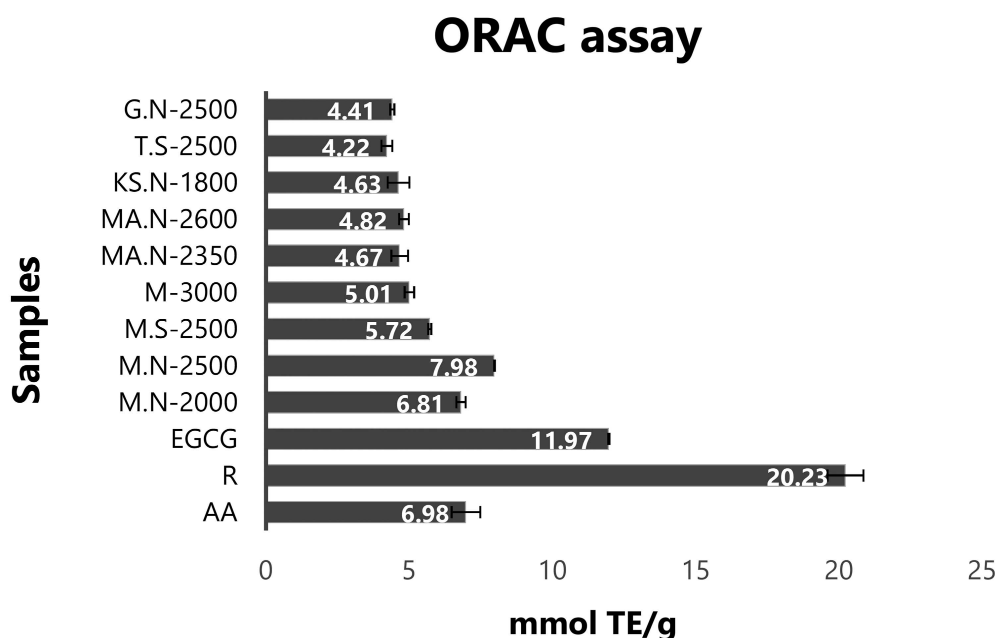

Figure 4. Antiradical potential of extracts of *Ferulago angulata* populations evaluated by ORAC assay compared with controls. R: rutin, AA: ascorbic acid, EGCG: epigallocatechin gallate. Values are the mean ± SD of three replications ($n = 3$).

According to the phytochemicals profile and anti-radical potential, PCA was classified to three major groups (PC1, PC2, and PC3). PC1 possessed 34.10% of total variation and had positive correlation with (*E*)-β-ocimene, bornyl acetate, TPC and TFC, and antioxidant activity evaluated by ORAC assay, and negative correlation with antiradical potential analyzed by DPPH. The second PC (PC2) with a 25.95% of variance had positive correlation with α-pinene, TFNC and TFLC, and negative correlation with *trans*-verbenol. In the case of PC3, positive correlation was detected for EO contents and antioxidant activity analyzed by ORAC which reported 17.69% of the total variance (*Figure 6*, *Table 3*).

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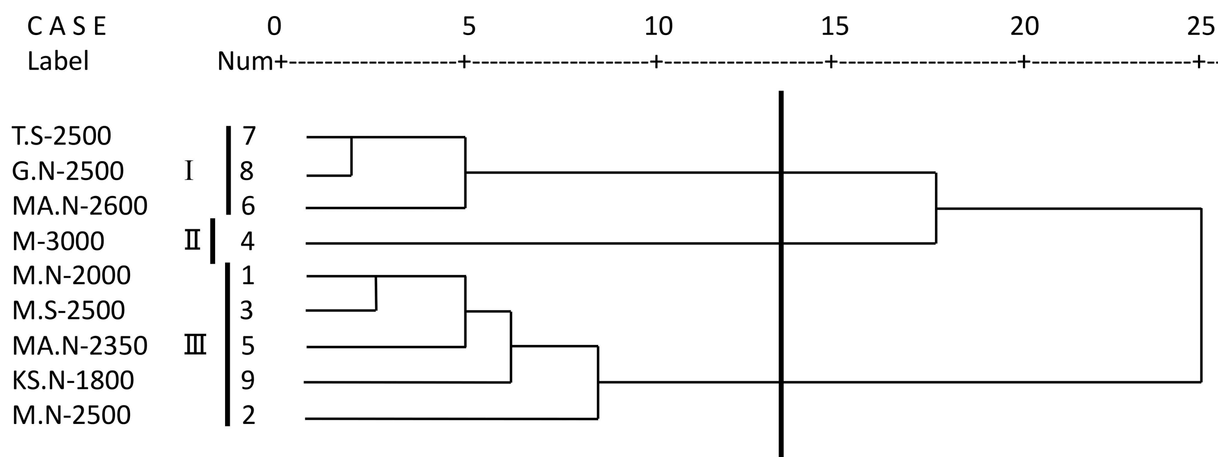

Figure 5. Dendrogram of the *Ferulago angulata* populations resulting from the cluster analysis (based on Euclidean distances) of the major phytochemicals. Chemotype I in *trans*-verbenol, chemotype II in α-pinene and total flavone and flavonol content, and chemotype III in (*E*)-β-ocimene, bornyl acetate, total phenolic compounds, total flavonoid contents, and EO content indicated the similarity.

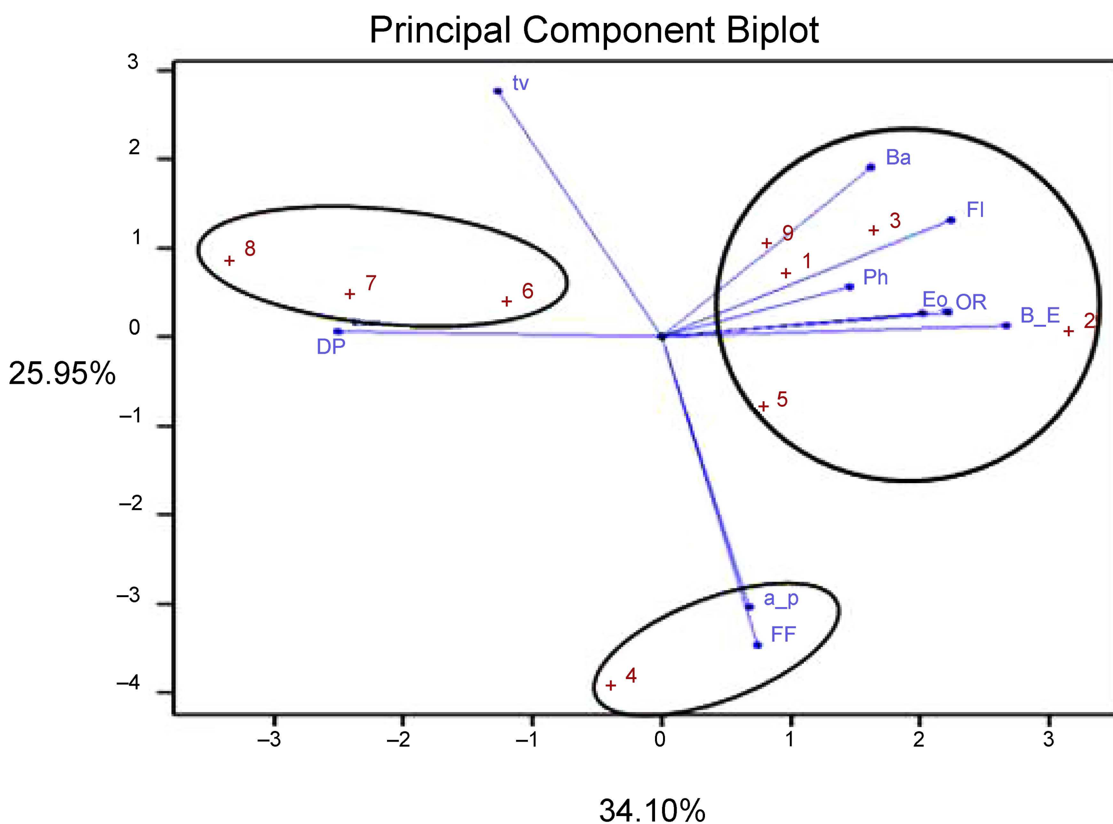

Figure 6. Principal component analysis (PCA) of the nine studied *Ferulago angulata* populations. 1: M.N-2000, 2: M.N-2500, 3: M.S-2500, 4: M-3000, 5: MA.N-2350, 6: MA.N-2600, 7: T.S-2500, 8: G.N-2500, 9: KS.N-1800, EO: essential oil contents, a_p: α-pinene, B_E: (*E*)-β-ocimene, Ba: bornyl acetate, tv: *trans*-verbenol, Ph: total phenolic compounds, Fl: total flavonoid contents, FF: total flavone and flavonol contents, DP: antiradical activity by DPPH, OR: antioxidant potential by ORAC.

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Table 3. Eigenvalues, variance and cumulative variance for three principal components.[a]

Major Factors	Principal Components		
	PC1	PC2	PC3
EO content	0.338	0.005	0.876
α-Pinene	−0.252	0.759	0.499
(*E*)-β-Ocimene	0.804	0.235	0.398
Bornyl acetate	0.730	−0.289	0.068
trans-Verbenol	−0.326	−0.916	0.179
TPC	0.520	0.02	0.135
TFC	0.857	−0.077	0.177
TFNC and TFLC	−0.057	0.971	0.078
A-DPPH	−0.821	−0.299	−0.215
A-ORAC	0.512	0.074	0.675
Eigenvalues	3.41	2.59	1.76
Variance [%]	34.10	25.95	17.69
Cumulative variance [%]	34.10	60.06	77.75

[a] TPC: total phenolic content; TFC: total flavonoid content; TFNC: total flavone content; TFLC: total flavonol content; A-DPPH: antiradical scavenging activity by DPPH assay; A-ORAC: antioxidant potential by ORAC assay.

Conclusions

Our findings on various *Ferulago angulata* populations firmly confirm that the diversity of EOs, phenolic contents and capacity of the extracts in scavenging of free radicals are highly influenced by a variety of environmental factors and ecological circumstances (such as slope aspects, altitudes, climate conditions, etc.), which were variant in growth media of the selected populations. It is indispensable to study the impact of environmental conditions to afford the most preferred chemical profile from medicinal plants for considering in phyto-cosmetic, food and phytopharmaceutical industries.

In conclusion, monoterpene hydrocarbons were the major EO compounds of the selected *F. angulata* samples. α-Pinene was characterized as the main EO constituent in almost all populations. Among the plant populations, the samples harvested from 'Mongar' demonstrated the highest α-pinene amount in EO, the highest TPC and TFC. Considering the remarkable antiradical capacity of the methanolic extracts as well, this population can be considered as a prospective raw material.

Cluster analysis demonstrated the studied populations were classified into three categories: the populations 'T.S-2500', 'G.N-2500', and 'MA.N-2600' together, 'M-3000' solely, and 'M.N-2000', 'M.S-2500', 'MA.N-2350', 'KS.N-1800', and 'M.N-2500' were sorted in the same groups. Moreover, regarding to PCA results, positive correlations with (*E*)-β-ocimene, bornyl acetate, TPC and TFC, and antioxidant activity (ORAC), and negative correlation with antiradical potential (DPPH) were grouped in PC1; positive correlations with α-pinene, TFNC and TFLC, and negative correlation with *trans*-verbenol were classified in PC2; whilst positive correlations with antioxidant activity (ORAC) and EO contents was recorded and categorized in PC3.

However, further phytochemical analyses are needed to identify the most representative phenylpropanoids and study the correlations between ecological effects, bioactivities and secondary metabolite profiles.

Experimental Section

Plant Material

About 5 kg of aerial parts of *F. angulata* were harvested for each location from five herbs in the same site at the beginning of flourish period (June 2017). The plant material was gathered from five growth locations and different altitudes (*Table 1*). The plants were identified by Dr. Mehrangiz Chehrazi at Department of Horticultural Science, Shahid Chamran University of Ahvaz, Iran, and voucher specimens are deposited with the herbarium of the department. The materials were shade dried and crushed by a grinder. Samples were powdered, homogenized, and subjected to analysis.

Chemicals and Spectrophotometric Measurements

Analytical grade 2,2′-azobis-2-methylpropionamidine dihydrochloride (AAPH), 1,1-diphenyl-2-picrylhydrazyl (DPPH), and 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox®; Sigma-Aldrich, Steinheim, Germany), epigallocatechin gallate (EGCG; Sigma-Aldrich, Germany), fluorescein (Fluka Analytical, Buchs, Germany), ascorbic acid, rutin and Na_2SO_4 (Merck, Darmstadt, Germany) were purchased from commercial suppliers. Analytical grade solvents were delivered by Merck (Germany). Spectrophotometric experiments were carried out by using a UV/VIS spectrophotometer (FLUOstar Optima BMG Labtech, Ortenberg, Germany; and Shimadzu-UV 1201, Kyoto, Japan).

Extraction of Volatile Oils

60 g of each powdered and homogenized sample were subjected to hydrodistillation using a Clevenger apparatus for 3 h. The EOs were dried with anhydrous

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sodium sulfate and were stored in refrigerator at 4 °C until analysis.

Gas Chromatographic (GC/FID) and Gas Chromatography-Mass Spectrometric (GC/MS) Analysis

GC analysis was carried out using the method published elsewhere[27] by using a Shimadzu GC-17A (Kyoto, Japan) coupled with FID detector and SGE™ BP5 capillary column (30 m×0.25 mm column, Trajan Scientific and Medical, Victoria, Australia). GC/MS analysis of the samples was carried out using a Finnigan TRACE gas chromatography (ThermoQuest Corp., Austin, TX, USA) instrument equipped with split inlet (split ratio of 100:1 mode) and using an Agilent DB-5 fused silica column (Agilent Technologies, Inc., Santa Clara, CA, USA) (30 m×0.25 mm, film thickness 0.25 μm) and 0.25 μm particle size (−60 to +320/340 °C). 1 μL of the analyte was subjected for GC/MS analysis. The oven temperature was kept at 60 °C for 1 min and next changed from 60 to 250 °C at 5 °C/min, then was kept at 250 °C for 2 min, the injection port temperature was 250 °C. Helium (99.999 %) with a flow rate of 1.1 mL/min was used as a carrier gas. The MS was operated in the electron impact mode at 70 eV and the inert ion source (HES EI) at 350 °C, with a quadrupole temperature of 150 °C, while the MS interface was set to 250 °C. The scan rate was 0.6 s (cycle time: 0.2 s), covering the mass range of 40–60 amu.

Identification of Essential Oil Compositions

EO components were identified based on their retention indexes (RI) with reference to *n*-alkanes (C_8–C_{24});[36] and considering their mass spectral data (compared to data of authentic chemicals and using the Wiley spectral library collection). If only mass spectral data was used for identification, identity was considered to be tentative. Data acquisition and analysis in GC/FID and GC/MS were carried out using Xcalibur™ software (Thermo Fisher Scientific, Waltham, MA, USA, 4.0 Quick Start) and Chrom-card™ (Scientific Analytical Solutions, Zurich, Switzerland, version DS), respectively.

Preparation of Solvent Extracts

5 g of each sample were extracted at 40 °C with MeOH (3×75 mL) using an ultrasonic bath (VWR-USC300D). The solvent was removed under reduced pressure at 50 °C (Rotavapor R-114, Büchi, Flawil, Switzerland).

Total Phenolic Content

Total phenolic content (TPC) was assessed by the Folin-Ciocalteu method, based on the optimized conditions established by Wojdyło et al.[37] Gallic acid and MeOH were applied as standard and blank, respectively. The results were recorded as mg GaE/g.

Total Flavonoid Content

Evaluation of total flavonoid content (TFC) was performed by method of Menichini et al.,[38] with a slight modification. Quercetin and MeOH were exerted as standard and blank, respectively. The mixture consisting of 0.5 mL of the sample, 0.15 mL of sodium nitrate, 0.3 mL of aluminum chloride (10 %), and 2 mL of sodium hydroxide (1 N) was diluted with distilled water to gain 5 mL volume. The absorbance was recorded at 510 nm. The results were reported as mg QuE/g.

Total Flavonol and Flavone Content

Total flavonol and flavone contents (TFLC and TFNC, respectively) were determined according to Popova et al.[39] method. 1 mL of each extract was mixed to 1 mL NH_4Cl (2 %), then MeOH was added until 2.5 mL. Each sample was subsequently subjected to UV spectrophotometer to measure the absorbance in 425 nm after 30 min.

Antiradical Capacity

DPPH Assay

Free-radical scavenging activity of the plant extracts was done by DPPH assay.[40] The absorbance was measured at 550 nm after 30 min using a microplate reader. Ascorbic acid (0.01 mg/mL) and MeOH (HPLC grade) were used as standard and blank control, respectively.

ORAC Assay

The ORAC assay was carried out on 96-well microtiter plates.[41] Activities were compared with rutin, EGCG and ascorbic acid as positive controls. Antioxidant capacities were calculated as mmol TE/g.

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Statistical Analysis

All experiments were carried out in triplicate and the results reported as means ± SD. The data were analyzed with one-way analysis of variance (ANOVA) using GraphPad Prism version 6.05 and SAS Software (version 9.2, SAS Institute Inc., Cary, NC, USA). The means were compared with Duncan comparisons test ($p < 0.05$). To categorize the different chemotypes of *F. angulata* populations according to their EO compositions, phenolic compounds and antioxidant activity, cluster analysis (CA) and principal component analysis (PCA) were used by SPSS software.

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Author Contribution Statement

S. B. harvested the plant materials and extracted the essential oils. M. M. S. designed the experiments and analyzed the essential oils. M. Z. conducted the study. Z. P. Z. carried out the bioactivity analysis. J. M. wrote and D. C. edited the manuscript.

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