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Analysis of the essential oil composition of three cultivated *Nepeta* species from Iran

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Abstract: Essential oils (EOs) of three Iranian cultivated *Nepeta* species were investigated. The oils were obtained by hydrodistillation of air-dried plant materials at full flowering stage and analyzed by gas chromatography (GC) and gas chromatography coupled to mass spectroscopy (GC/MS). In total, 89 compounds were detected. In over 2 years, a number of constituents were identified in the EO of *Nepeta binaloudensis* first and second years (26 and 37, respectively), *Nepeta cataria* (25 and 32, respectively), and *Nepeta assurgens* (45 and 50, respectively). In the oils of *N. binaloudensis*, 4a-α,7-α,7a-α-nepetalactone (NL) 59.7% and 1,8-cineole (19.6%) during the first and second years, respectively, were the main constituents. The main components of *N. cataria* were 4a-α,7-α,7a-β-NL (72.8%) and 4a-α,7-β,7a-α-NL (73.9%) during the first and second years, respectively, and 4a–α,7-α,7a-α-NL (55.5%) and 1,8-cineole (24.1%) during the first and second years, respectively, were the main constituents of *N. assurgens*. The results showed that NLs isomers and 1,8-cineole were the main components of the oils of three cultivated *Nepeta* species.

Keywords: 1,8-cineole; *Nepeta assurgens*; *Nepeta binaloudensis*; *Nepeta cataria*; nepetalactone.

1 Introduction

Nepeta is one of the largest genera of the subfamily Nepetoideae and family Lamiaceae. It comprises approximately 300 species, which are often perennial herbs. *Nepeta* species are distributed in the central and southern parts of Europe, Asia, and the Middle East. Iran is one of the main centers of diversity for the genus with about 79 species naturally growing in different regions of the country [1–3]. The genus *Nepeta* was attributed with a number of pharmacological effects. The application of essential oils (EOs) against microorganisms increased, and it is known that *Nepeta* oils also have such effects. The EOs of this genus were found to be rich in iridoid monoterpenoids, which exhibited many in vitro/in vivo biological activities including antioxidant, antimicrobial, anti-inflammatory, cytostatic, phytotoxic, and repellent properties [4–10]. In general, bioactivities of *Nepeta* EOs are attributed to the presence of nepetalactones (NLs) that have been used as the phytochemical markers.

In this study, we investigated the EO composition of three cultivated species of *Nepeta* in 2 years (*Nepeta assurgens*, *Nepeta binaloudensis* Jamzad, *Nepeta cataria* L.), two of which are endemic (Table 1). Briefly, *N. assurgens* is an herbaceous aromatic plant growing in dry areas of Kerman, Iran. Kerman province is a unique center of medicinal plants in the country. *Nepeta binaloudensis* is an endemic and rare perennial aromatic herb, which is distributed in a limited area in Binalud Mountains, Khorasan Razavi Province in North-East of Iran [11], which is used as a culinary herb, and *N. cataria*, the most intensively studied species, is commonly known as catnip or catmint because of its irresistible action on cats.

The biological and chemical diversity, species richness, as well as biological properties greatly promoted the research focusing on this genus [4]. Until now, 143 compounds were identified in the various species of this genus [12], mainly including nepetalactones, β-caryophyllene, caryophyllene oxide, 1,8-cineol, α-humulene, citronellol, linalool, geraniol, geranial, geranyl acetate, spathulenol, citronellyl acetate, germacrene-D, α-pinene, and camphor. However, in a number of *Nepeta* species, NLs are either present as minor components or are not produced at all. In general, according to EO composition, the various species of *Nepeta* may be divided into two main groups: in the first group, NLs are the dominant constituents; in the second group, compounds such as 1,8-cineole,

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Table 1: *Nepeta* species investigated in this study and their harvesting data.

Taxon	Collection locality	Collection date and elevation (m)	Herbarium voucher no.
Nepeta assurgens Hausskn. & Bornm. ex Bornm[a]	Kerman province, Darb-e Behesht, mountain slopes of Bahrasman	20.09.2017 (3323)	ANRRCIH 8636
Nepeta binaloudensis Jamzad[a]	Razavi Khorassan Province, Zoshk, Binaloud mountains	29.09.2015 (2400)	FUMH 46489
Nepeta cataria L.	Razavi Khorassan481.89 Province, Mashhad	20.08.2017 (1010)	FUMH 44565

ANRRCKH, Agricultural and Natural Resources Research Center of Kerman, Herbarium; FUMH, Ferdowsi University of Mashhad, Herbarium.
[a]Endemic of Iran.

caryophyllene, citral derivatives, and α-humulene are dominant [2, 12–18].

Furthermore, many of these species are considered of horticultural and medicinal interests in arid and semi-arid areas, due to their long flowering period, adaptation to drought conditions, as well as their high EO and phenolic compound contents [6, 19–21]. However, there are many species and constituents of EO that have not been studied yet.

In plants, the EO composition and secondary metabolite diversity are extremely dependent on environmental factors, such as light, temperature, water, soil salinity, and climatic conditions. Plant collecting from the wild could lead to further loss of genetic diversity and habitat destruction. Some previous studies highlighted the possibility of domestic cultivation of these valuable medicinal plants. Introduction into cultivation under controlled environments represents a great challenge that could modify the content of bioactive phytochemicals and lower the pressure on wild populations [22–25]. Thus, investigations of new, as well as known plant species from different geographic regions may considerably expand the existing knowledge on the EO-bearing plants [12].

Therefore, the aim of the present study was to investigate the EO composition of three *Nepeta* species and evaluate the NL isomer content in order to select the most suitable species for domestication and cultivation under the same conditions, and to meet the market and consumer needs.

2 Materials and methods

2.1 Plant material

The research was conducted in the Experimental Garden of Agricultural Faculty of Ferdowsi University of Mashhad, Iran, in 2016–2018. The seeds of three *Nepeta* species were collected from Kerman and Mashhad provinces of Iran. The origin of plant material, year of collection, and herbarium voucher are presented in Table 1.

2.2 Cultivation conditions

The seeds were placed at 4 °C for 2 weeks, then sown under greenhouse-controlled conditions with a temperature range of 20°–25 °C and relative humidity of 60 °C (latitude: 36°16′N, longitude: 59°36′E) during the last week of March. The culture bed contained peat moss, cocopeat, and perlite (40:40:20, w:w:w). After about 30 days, seedlings were transplanted into pots of 30-cm height, with the same composition of the first bed; after about 45 days, seedlings were transplanted to the field, on the base of completely randomized design including three replications (1.5×2 m^2 plot) in the Experimental Garden of Ferdowsi, University of Mashhad. Based on the climatic conditions, the plants were irrigated to maintain near-field capacity. The characteristics of the field location are reported in Table 2.

2.3 Extraction of the EOs

During the summer, aerial parts of the plants were harvested at full flowering stage in the first and second year (about 120 and 360 days after cultivation) of their establishment on the farm. Plants were harvested at 5 cm above the soil level and then air-dried in the shade.

The EOs from the dry plants (30 g) of two cuts, during the first and second years, were extracted by hydro-distillation for 4 h using a Clevenger apparatus (Ashk shishe Co., Tehran, Iran), and the EO yield percentage was

Table 2: Soil characteristics and meteorological conditions of the experimental field where *Nepeta* species were cultivated.

Physical and chemical properties	(2016–2017)	(2017–2018)
Clay %	26	26.5
Silt %	48	49.1
Sand %	26	24
Soil texture	Sandy loam	Sandy loam
FC[a]	15	–
CCE[b]	12.5	–
pH	7.28	7.8
EC[c] (dSm^{-1})	3.70	2.21
Organic matter %	0.78	–
Ca %	0.08	–
Available N (mg/kg)	16.5	11.8
Available P (mg/kg)	19.4	14.7
Available K (mg/kg)	334	278
Available Mg (ppm)	11	–
Available Cu (ppm)	1.27	–
Available Mn (ppm)	7.92	–
Available Zn (ppm)	1.93	–
Available Fe (ppm)	1.51	–
Meteorological data	(2016–2017)	(2017–2018)
Altitude (m)	985	985
Annual minimum temperature (°C)	9.1	9.9
Annual maximum temperature(°C)	22.4	23
Annual mean temperature (°C)	15.7	17.2
Absolute humidity mean (%)	46	44
Annual precipitations (mm)	308.1	185.9
Number of ice days	17	61
Number of rainy days	97	67

[a]Field capacity. [b]Calcium carbonate equivalent. [c]Electrical conductivity.

recorded as %v/w of plant dry weight. Sodium sulfate anhydrous purchased from Sigma (St Gallen, Switzerland) was added for removing water from the isolated EOs that was stored in a refrigerator in dark bottles until analyses.

2.4 Gas chromatography (GC) and gas chromatography coupled to mass spectroscopy (GC/MS) (GC/MS) analysis

The composition analysis of the essential oil samples of the first and second years was carried out using a GC–MS instrument with the following specifications: GC analysis was carried out using an Agilent-Technologies-7890A gas chromatograph equipped with an HP-5 column (30 m × 0.32 mm ID) and film thickness of 0.25 μm. The oven temperature was from 60 °C and then programmed to 210 °C at a flow rate of 3 °C/min, then from 210 °C to 240 °C at 20 °C/min and held for 8.5 min; injector and detector (FID) temperature were 280 °C and 290 °C, respectively; N_2 was used as carrier gas with a linear velocity of 1 mL/min, split ratio was 1:50.

GC/MS analyses were carried out on GC/MS (Agilent Technologies-5975C-MS, 7890A-GC) equipped with HP-5MS capillary column (30 m × 0.25 mm ID) and film thickness of 0.25 μm. The oven temperature was programmed as follows: from 60 °C to 210 °C with a rate of 3 °C/min, then increased to 240 °C with a rate of 20 °C/min, and the final temperature was kept for 8.5 min; run "time" was 60 min. The electron ionization energy was 70 eV in the electronic ionization (EI) mode, ion source 230 °C, detector MS, interface line temperature 280 °C, injector 280 °C, split ratio 1:50, carrier gas He 1 mL/min, mass range 50–480 amu.

The percentages of compounds were calculated by the area normalization method. The components of the oil were identified by comparison of retention indices (RI, HP-5) in their mass spectra with those of an Adams library and sorted in NIST and Wiley libraries or with authentic compounds reported in the literature. Retention indices were determined using retention times of n-alkane that were injected after the EO under the same chromatographic conditions [26].

2.5 Statistical analysis of EO yields

Mean values and variance analysis of EO yields were calculated according to a completely randomized design including three replications for each year. Statistical analysis of the obtained results was performed by (JMP software ve. 9.0.3). Significant differences among the samples were evaluated by Duncan test at the probability level ($p \leq 0.05$).

3 Results

3.1 EO yields

Results of the EO yields from the three *Nepeta* species for 2 years are reported in Table 3. The EOs extracted from the *N. assurgens* and *N. binaloudensis* species were yellow, whereas the *N. cataria* was whitish-yellow in color. There were significant differences in the EO yield among the species. The highest EO yields were obtained from *N. assurgens* in 2 years (0.51% and 0.75% in the first and second years, respectively). The yields of *N. cataria*

Table 3: Analysis of variances and means of essential oil yields among cultivated *N. binaloudensis*, *N. cataria*, and *N. assurgens* species during 2016/2018.

Sources	Df	Means of squares
		Essential oil yield (%)
1st year		
Species	2	0.0613[a]
Replications	2	0.0085
Error	4	0.0010
2nd year		
Species	2	0.2585[a]
Replications	2	0.0009
Error	4	0.0080
Cultivation year	**Species**	**Yield (%)**
1st year	*N. binaloudensis*	0.23[b]
	N. cataria	0.32[c]
	N. assurgens	0.51[a]
2nd year	*N. binaloudensis*	0. 18[b]
	N. cataria	0.29[c]
	N. assurgens	0.74[a]

Means having the same letter within the same column are not significantly different. [a]Significant at $p \leq 0.05$.

were 0.32% (first year) and 0.29% (second year), whereas *N. binaloudensis* yielded the lowest amounts (0.23% and 0.18% in the first and second years, respectively) (Table 3).

3.2 The composition of EOs

The constituents of the *Nepeta* EOs analyzed by GC and GC-MS are reported in Table 4. In total, 89 constituents were identified. During 2 years of cultivation, a number of constituents were identified in the EOs of *N. binaloudensis* in the first and second years (26 and 37, respectively), *N. cataria* (25 and 32, respectively) and *N. assurgens* (45 and 50, respectively) The results indicated that there were differences between the species in the first and second years in EO composition and number of compounds. The EOs were rich in monoterpenoids, as other *Nepeta* species previously reported. In the oils of *N. binaloudensis*, 4a-α,7-α,7a-α-NL (1.2–59.7%) and 1,8-cineole (14.8–19.6%) were the main components in the 2 years. Other abundant constituents included β-pinene (10.4%), p-cymene (9.7%), α-terpineol (7%), γ-terpinene (4.5%), α-pinene (4.33%), terpinen-4-ol (4.2%), δ-terpineol (3.61%), and thymol (1.7%) in the first year. Thymol, carvacrol (0.6%), α-copaene (0.2%), β-copaene (0.1%), β-bourbonene (1.0%), 4a-α,7-α,7a-β-NL (NL2) (0.9%), germacrene-D (0.2%), γ-muurolene (0.3%), and γ-terpinene were identified only in the first year, whereas 4a-α,7-β,7a-α-NL (0.9%) was detected only during the second year. The EOs of *N. cataria* were rich in NL isomers, while in the second year, the main compound was 4a-α,7-α,7a-β-NL (72.8%). In general, 4a-α,7-β,7a-α-NL (2.5–73.9%) and 4a-α,7-α,7a-α-NL (17.9–19.8%) in the first and second years, respectively, were quantified as the main components in this species. In the EOs of *N. assurgens*, 4a-α,7-α,7a-α-NL (34.7–55.5%) and 1,8-cineole (24.1–16.8%) were the main constituents in the first and second years, respectively. Other compounds included β-pinene (3.9–4.6%), trans-carveol (6.7–2.2%), NL2 (5.7% only in the first year), and 4a-α,7-β,7a-α–NL (2.7%) only in the second year.

4 Discussion

4.1 EO yields

The yields of EOs from the studied *Nepeta* species were from 0.18% (*N. binaludensi*) to 0.79% (*N. assurgens*). In previous studies, EO yields of *N. cataria* were in the range of 0.1–2.5%, in plants from different regions (Table 5), whereas the yield of *N. binaludensis* collected in Iran was 0.74% [11]. Therefore, the previously reported EO yields from *N. cataria* were remarkably higher: 2.5% [19] and 1.02% [8] from plants cultivated in Iran and Morocco, respectively. To the best of our knowledge, the yields of EO from *N. assurgens* were not reported previously. Noteworthy, the variability of EO yields during the 2 years may be attributed to environmental factors possibly modifying photosynthate allocation to secondary metabolic pathways [20–25].

4.2 The composition of EOs

Biological activities of NLs were thoroughly investigated, and some *Nepeta* species were previously introduced into cultivation due to their horticultural and medicinal values [16, 19–21]. Most EOs of the *Nepeta* species contain NLs as the main components, though different oil compositions were identified among the different species. In the present study, three types of NL isomers, 4a-α,7-α,7a-α-NL, 4a-α,7-α,7a-β-NL, and 4a-α,7-β,7a-α-NL, were identified. All these NL isomers were the dominant components of the oils of *N. cataria*, whereas in *N. assurgens* and *N. binaloudensis*, other constituents were significantly present besides 4a-α,7-α,7a-α-NL, in particular, 1,8-cineole.

Table 4: Number and percentage of the essential oil constituents from the three cultivated *Nepeta* species in 2 years.

No.	Compounds	Retention index		%					
				N. binaloudensis		*N. cataria*		*N. assurgens*	
		RI[a]	RI[b]	1st year	2nd year	1st year	2nd year	1st year	2nd year
1	(E)-2-Hexenal	948	848	–	–	–	0.1	–	–
2	Tricyclene	919	919	tr	–	–	–	0.2	0.1
3	α-Thujene	925	926	1.2	0.5	tr	tr	0.1	0.1
4	α-Pinene	932	933	4.3	0.6	0.7	0.1	1.4	3.6
5	Camphene	947	948	0.2	0.1	0.1	tr	tr	0.1
6	Thuja-2,4(10)-diene	953	953	–	–	–	–	–	tr
7	Sabinene	973	972	0.3	0.8	0.5	0.1	0.4	1.7
8	β-Pinene	978	977	10.4	1.8	2.2	0.5	3.9	4.6
9	3-Octanone	985	985	–	–	tr	tr	0.3	0.2
10	Myrcene	992	990	1.6	1.4	tr	tr	0.6	2.8
11	n-Decane	1000	1000	6.2	0.1	tr	0.1	3.2	–
12	α-Phellandrene	1006	1004	0.3	0.2	–	–	–	–
13	α-Terpinene	1016	1017	2.7	0.3	–	tr	0.1	0.2
14	p-Cymene	1024	1024	9.7	4.2	0.1	0.1	0.7	0.1
15	Limonene	1029	1028	2.2	0.3	0.1	0.1	0.3	0.2
16	1,8-Cineole	1030	1033	14.8	19.6	0.2	0.5	24.1	16.8
17	(Z)-β-Ocimene	1037	10.36	0.9	0.2	–	0.0	0.2	0.9
18	(E)-β-Ocimene	1046	1046	0.2	0.1	–	0.1	–	0.2
19	γ-Terpinene	1057	1057	4.5	–	–	tr	0.3	0.2
20	cis-Sabinene hydrate	1067	1066	0.3	0.5	–	–	–	0.2
21	trans-Linalool oxide	1071	1071	–	–	–	–	0.1	tr
22	Terpinolene	1088	1089	1.3	0.3	tr	0.1	0.3	0.2
23	Linalool	1098	1099	1.8	1.2	–	tr	0.9	0.8
24	3-Methyl butyl 2-methyl butanoate	1104	1101	0.2	0.1	–	–	–	0.2
25	n-Nonanal	1105	1105	–	–	tr	tr	–	–
26	Isopentyl isovalerate	1106	1106	tr	–	–	–	0.1	0.1
27	cis-Rose oxide	1111	1111	–	–	–	–	0.1	–
28	endo-Fenchol	1113	1113	–	–	–	–	0.1	–
29	cis-p-Menth-2-en-1-ol	1121	1121	0.6	0.1	–	–	0.2	0.1
30	α-Campholenal	1126	1126	0.2	–	–	–	0.4	0.1
31	allo-Ocimene	1128	1128	–	–	–	–	–	0.1
32	trans-Pinocarveol	1138	1136	–	0.1	–	tr	0.9	0.2
33	Nopinone	1137	1137	–	0.2	–	–	0.1	–
34	trans-p-Menth-2-en-1-ol	1138	1138	–	0.6	–	–	–	–
35	Geijerene	1141	1141	–	–	–	–	–	0.1
36	Camphor	1145	1144	–	tr	tr	0.1	–	–
37	trans-Verbenol	1145	1146	–	–	–	–	–	0.1
38	Citronellal	1154	1152	–	–	–	–	–	0.1
39	Pinocarvone	1162	1162	0.2	0.1	–	tr	0.3	0.1
40	δ-Terpineol	1166	1166	3.6	1.1	–	tr	0.2	0.9
41	Terpinen-4-ol	1178	1177	4.2	1.1	0.1	–	0.7	0.4
42	p-Cymen-8-ol	1185	1185	0.3	0.1	–	–	0.2	–
43	Cryptone	1186	1186	–	–	–	–	–	–
44	α-Terpineol	1190	1190	7.0	2.5	tr	–	1.7	1.9
45	Methyl salicylate	1194	1194	–	–	–	0.1	–	–
46	Myrtenal	1196	1196	0.7	0.1	tr	–	0.8	0.2
47	n-Dodecane	1205	1201	3.5	tr	–	–	2.6	–
48	trans-Piperitol	1207	1207	0.2	–	–	–	–	–
49	trans-Carveol	1219	1218	–	–	–	0.8	6.7	2.2
50	n-Hexyl 2-methyl butanoate	1236	1236	0.2	–	–	–	–	–
51	Neral	1238	1240	–	–	–	–	–	0.1
52	Carvone	1244	1244	–	–	–	–	0.1	tr
53	Geraniol	1254	1255	–	–	–	tr	–	0.1
54	Geranial	1268	1269	–	0.1	–	–	–	–

Table 4 (continued)

No.	Compounds	Retention index		%					
				N. binaloudensis		*N. cataria*		*N. assurgens*	
		RI[a]	RI[b]	1st year	2nd year	1st year	2nd year	1st year	2nd year
55	Bornyl acetate	1287	1287	–	–	–	–	–	–
56	p-Cymen-7-ol	1290	1290	–	–	–	–	tr	–
57	Thymol	1290	1290	1.7	–	–	–	–	–
58	Carvacrol	1297	1297	0.6	–		–	0.1	–
59	δ-Elemene	1336	1336	–	–	–	–	–	0.1
60	4a-α,7-α,7a-α-Nepetalactone	1358	1360	1.2	59.7	17.9	19.8	34.7	55.5
61	Neryl acetate	1366	1366	–	–	–	–	–	–
62	Methyl p-anisate	1374	1374	–		–		0.1	
63	α-Copaene	1376	1376	0.2	–	–	–	–	–
64	β-Bourbonene	1384	1384	1.0	–	–	–	–	–
65	Geranyl acetone	1386	1387	–	–	–	–	–	–
66	4a-α,7-α,7a-β-Nepetalactone	1388	1388	0.9	–	72.8	–	5.7	–
67	4a-α,7-β,7a-α-Nepetalactone	1391	1392	–	0.9	2.5	73.9	–	2.7
68	n-Tetradecane	1401	1402	1.4	–	–	–	1.4	–
69	Methyl eugenol	1407	1407	–	–	–	–	–	0.1
70	β-Funebrene	1413	1413	–	–	–	–	–	–
71	(E)-Caryophyllene	1419	1421	–	–	0.7	–	0.2	0.5
72	β-Copaene	1427	1427	0.1	–	–	–	–	–
73	trans-α-Bergamotene	1738	1438	–	–	–	–	–	–
74	(E)-β-Farnesene	1447	1447	–	–	0.4	–	–	–
75	α-Humulene	1545	1454	–	–	0.1	tr	–	0.1
76	allo-Aromadendrene	1460	1460	–	–	–	–	–	–
77	(E)-β-Farnesene	1458	1458	–	–	–	1	–	tr
78	γ-Muurolene	1475	1475	0.3	–	–		–	–
79	Germacrene D	1483	1483	0.2	–	–	–	–	0.1
80	Neryl isobutanoate	1486	1486	–	–	–	–	–	–
81	(E)-β-Ionone	1485	1485	0.2	–	–	–	–	–
82	Valencene	1493	1493	0.1	–	–	–	–	–
83	Bicyclogermacrene	1496	1496	–	–	–	–	0.1	0.2
84	(Z)-α-Bisabolene	1510	1510	0.3	–	–	–	–	–
85	δ-Cadinene	1524	1524	0.4	–	–	–	–	–
86	Spathulenol	1578	1578	–	0.1	–	tr	0.9	0.4
87	Caryophyllene oxide	1583	1583	–	0.1	0.6	1	0.6	0.4
88	Humulene epoxide II	1610	1610	–	–	tr	–	–	–
89	6,10,14-trimethyl-2-Pentadecanone	1846	1846	–	–	–	–	0.4	–
Total identified (%)				92.4%	95%	99%	98.3%	96.6%	99.8%
Number of compounds				26	37	25	32	45	50

tr, traces (<0.05%). [a]Retention index in the first year. [b]Retention index in the second year determined on HP-5MS capillary column.

The EO of the composition of various *Nepeta* species grown in Iran was extensively investigated [29–33]. The essential oil of *N. binaludensis*, an endemic species to Iran, was studied by Mohammadpour et al. [30] who identified 65 components including 1,8-cineol (68.31%), α-terpineol (5.24%), β-pinene (4.7%), δ-terpineol (2.57%), and α-pinene (1.54%) [30]. The most abundant constituents in *N. binaludensis* investigated by Rustaiyan and Nadji [32] were 1,8-cineole (42%), nepetalactone (25%), linalol (4%), α-terpineol (4%), and β-pinene (3%). According to the literature, the EO composition of *N. assurgens*, an Iranian endemic species was previously reported only by Moradalizadeh et al. [31] who identified 4aα,7α,7aα-NL, 4aα,7α,7aβ-NL, 1, 8-cineole, α-pinene, β-pinene, and α-terpineol as the main components.

The differences between wild and cultivated medicinal plants growing in various regions were reported in terms of EO composition. The chemical profiles shown in the present study are quite similar to the ones previously reported in other studies, except for the amounts

Table 5: Essential oil yields of *Nepeta* species reported in previous studies.

Species	Condition	Region	Oil	Reference
N. cataria	collected	Moroccan	1.02%	[8]
N. cataria	collected	Iran	0.3–0.9%	[9]
N. binaludensis	collected	Iran	0.5%	[11]
N. cataria	cultivated	Lithuania	5.94 mg/g	[12]
N. cataria	cultivated	Iran	2.5%	[19]
N. cataria	cultivated	Egypt	0.117–0.253%	[20]
N. cataria	cultivated	Egypt	0.19–2.5%	[21]
N. cataria	collected	Turkey	0.74%	[27]
N. cataria	collected	Poland	0.45–0.80%	[28]

of some compounds. For instance, the main EO constituents of *N. cataria* were 4a-α,7-α,7a-α-NL, and 4a-α,7-β,7a-α-NL, in the range of 78–91%, in plants cultivated in Iran [19], and 20.81–35.15%, in plants cultivated in Egypt [20, 21]. In the EO from *N. cataria* cultivated in Lithuania, 4aα,7α,7aβ-NL (50.16%) was the dominant constituent, in addition to 4aα,7α,7aα-NL (35.64%) and 4aα,7β,7aα-NL (1.80%), which represented 87.60% of the total EO volatiles [12]. Again, NLs were the major compounds in the EO of *N. cataria* from Turkey, Poland, and Morocco, but their percentages were different [19].

It is well known that variability in quality and quantity of secondary metabolites in plants is mainly influenced by the environmental factors possibly modifying the expression levels of the key genes/enzymes involved in the biosynthesis of the EO constituents. Therefore, introduction of *Nepeta* species into the cultivation under different growing conditions represents a promising perspective in the field of medicinal plants. Mashhad province is a region with semi-arid weather and an annual rainfall less than 200 mm; in these conditions, water deficit and high temperatures represent the limiting factors for *Nepeta* grown in the summer. Therefore, timing of sowing as well as management of nutrients and water could improve the economic *Nepeta* production.

In addition, the phytochemical differences could also be due to the existence of different *Nepeta* chemotypes [18–20]. Accordingly, on the basis of our results, *N. binaloudensis* and *N. assurgens* are characterized by high percentages of and NLs and 1,8-cineole, whereas *N. cataria* EO was composed mainly of NLs.

5 Conclusions

One of the main aims of local and global medicinal and aromatic plant market is to take a decisive step toward the modern, cost effective, and sustainable plant cultivation and production, particularly in Iran. In this context, the environmental conditions of Mashhad (Iran) are suitable to cultivate *Nepeta* species and produce EOs with peculiar aromatic traits.

Although the yields of EOs were not rather high, in general, 89 compounds were identified, which is a remarkably higher number compared with the previous reports [12]. *Nepeta assurgens* with high contents of 4a-α,7-α,7a-α-NL and 1,8-cineole was not reported previously. Evaluation of EOs of *Nepeta* species in the present study confirmed information on the existence of two main chemotypes in the *Nepeta* genus according to the previous studies. In Iran, cultivated *N. cataria* can be assigned to the NL chemotype, whereas *N. binaloudensis* and *N. assurgens* can be assigned to 1,8-cineole chemotype.

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