

Essential oil analysis of eight *Nepeta* taxa in Iran

Seyed Mehdi Talebi¹, Majid Ghorbani Nohooji² & Mahbobeh Yarmohammadi¹

Received: 5 June 2019 / Accepted: 11 October 2019 / Published online: 6 March 2020

Abstract. *Nepeta* is one of the largest and important genera of Lamiaceae that is found in many parts of the world as wild plants. These aromatic plants produce essential oil for various pharmaceutical and industrial products. The essential oil composition in eight taxa of *Nepeta* was analyzed. One natural population from each taxon was selected, and their essential oils extracted using Clevenger apparatus. Moreover, GC and GC/MS analysis methods allowed to reveal the variability in essential oil composition and profile among the studied taxa. The oxygenated monoterpenes were dominant in the oils of *N. meyeri*, *N. mirzayanii*, *N. racemosa*, *N. binaludensis*, and *N. glomerulosa*. Phytol was the major compound in the essential oil of *N. kotschyi* var. *persica* and *N. saccharata* (11.56% and 27.04%, respectively). 4 α ,7 α ,7 β -nepetalactone (73.89%) and 4 α ,7 β ,7 α -nepetalactone (83.92%) were the major constituents in essential oil of *N. mirzayanii* and *N. meyeri*, respectively. 1,8-cineol was the principal constituent in the oil of *N. glomerulosa* var. *carmanica*, *N. binaludensis*, *N. pogonosperma* and *N. racemosa* (23.34%, 43.49%, 53.94% and 70.89%, respectively). The studied taxa were classified into four distinct groups according to the UPGMA tree with high level of bootstrapping support. Each group was characterized by special trait(s) that could be used for identification of them. Therefore, four chemotypes were separated among the studied taxa: 1,8-cineol, 4 α ,7 α ,7 β -nepetalactone, 4 α ,7 β ,7 α -nepetalactone, and carvacrol. It was also noticed that the composition of essential oil was highly varied compared to previous results.

Keywords. Essential oil; *Nepeta*; chemical composition; GC/MS; chemotype.

Análisis de los aceites esenciales en ocho especies de *Nepeta* de Irán

Resumen. *Nepeta* es uno de los géneros más grandes y más importantes de la familia Lamiaceae. Está ampliamente distribuido por casi todo el mundo. Son plantas silvestres aromáticas, productoras de los aceites esenciales usados en los productos farmacéuticos e industriales. En el presente trabajo se analiza la composición de los aceites esenciales en ocho taxones diferentes de *Nepeta*. Se ha seleccionado una población natural de cada taxón. Para la extracción de los aceites esenciales se ha usado el aparato de tipo Clevenger. Además, el método de GC y GC/MS análisis se han usado para detectar la variabilidad en la composición y el perfil de los aceites entre diferentes taxones. Los monoterpenos oxigenados son el componente dominante en los aceites de *N. meyeri*, *N. mirzayanii*, *N. racemosa*, *N. binaludensis* y *N. glomerulosa*. Fitol es el componente dominante en los aceites de *N. kotschyi* var. *persica* y *N. saccharata* (11.56% y 27.04%, respectivamente). 4 α ,7 α ,7 β -nepetalactone (73.89%) y 4 α ,7 β ,7 α -nepetalactone (83.92%) son componentes predominantes para *N. mirzayanii* y *N. meyeri*, respectivamente. 1,8-cineol es el componente mayoritario en los aceites de *N. glomerulosa* var. *carmanica*, *N. binaludensis*, *N. pogonosperma* y *N. racemosa* (23.34%, 43.49%, 53.94% y 70.89%, respectivamente). Los taxones estudiados se clasificaron en cuatro grupos diferentes según el análisis UPGMA, con los altos valores de soporte. Cada grupo está caracterizado por los caracter(es) que se podrían usar para su identificación. Por lo tanto, se separaron cuatro quimiotipos entre los taxones estudiados: 1,8-cineol, 4 α ,7 α ,7 β -nepetalactona, 4 α ,7 β ,7 α -nepetalactona y carvacrol. También se observó que la composición de los aceites esenciales era muy variada en comparación con los resultados anteriores.

Palabras clave. Aceites esenciales; *Nepeta*; composición química; GC/MS, quimiotipos.

Introduction

Healing with medicinal plants is as old as mankind itself (Newman *et al.*, 2000; Paterson & Anderson, 2005), while the classification of these plants is still the most difficult research problem (Singh & Geetanjali, 2018). Over the years, taxonomists suggested many different approaches toward plant classifications such as morphological, anatomical (Talebi *et al.*, 2015), chemotaxonomic (Singh, 2016), and DNA based methods, like genetic structure (Sheidai *et al.*, 2014) and phylogeny (Molvary *et al.*, 1999). Singh (2016) considered the chemotaxonomy was the most important toward plant classification based on specific class of second-

ary metabolites and their biosynthetic pathways. Indeed, the chemotaxonomy really can resolve specific taxonomical problems.

Nepeta L. is a multiregional genus of Lamiaceae (Mint) family. The name *Nepeta* originated from the ancient Italian city Nephi (Sharma & Cannoo, 2013). It has 280 species over the world, extensively grown in many regions of Asia, Africa, North America, central and southern parts of Europe. Around 30 species are found in the plains and foothills of the Indian Himalayas (Pojarikova, 1954).

Nepeta species are mostly herbaceous perennials or annuals occurring mainly in Eurasia, while this is one

¹ Department of Biology, Faculty of Science, Arak University, Arak 38156-8-8349, Iran. Corresponding author: seyedmehdi_talebi@yahoo.com

² Medicinal Plants Research Centre, Institute of Medicinal Plants, ACECR, Karaj, Iran. Email: m.gh.nahooji@gmail.com

of the largest genera of Lamiaceae in Southwest Asia. In Iran, *Nepeta* is the largest genus of the family, with about 60% endemic species (Jamzad, 2009). Rechinger (1982) identified 63 species from Iran, classified into 13 sections according to their morphological characteristics. Jamzad *et al.* (2003), in their molecular analysis based on sequences of nuclear ITS of 34 Iranian species of *Nepeta* recognized two clades, one of them subdivided into four sub-clades. The phylogenetic relationships among the species of these groups are congruent with the distribution of some floral traits, e.g., corolla shape, bract texture, and exine ornamentation of pollen. Recently, Jamzad (2012) listed 79 species of *Nepeta* in Iran and divided them into six taxonomic groups based on morphological traits and DNA sequences.

According to Sajjadi (2005), *Nepeta* species have been divided into two groups with regard to the major compounds of their essential oils: one with different isomers of nepetalactone and the second that contained non-nepetalactone isomers like 1,8-cineole, β -caryophyllene, caryophyllene oxide, β -farnesene, α -citral and β -citronellol as their major constituents. The essential oils obtained from various organs of the plant, such as roots,

stems, leaves and inflorescence of *Nepeta* taxa, have been highly contaminated with terpenoid hydrocarbons and sesquiterpene hydrocarbons, including their oxygenated analogs (Gkinis *et al.*, 2003).

The present study aims to identify the essential oil compositions of eight *Nepeta* taxa and to compare our results with previous investigations and species classification according to the chemical oil compositions.

Material and methods

Plant materials

Eight taxa of *Nepeta* were collected from different parts of Iran (Table 1). The plants were sampled from wild populations at the initial stage of blooming in 2015–2016 and were identified according to Flora Iranica (Rechinger, 1982) and Flora of Iran (Jamzad, 2012) by authors. The voucher samples were deposited in Herbarium of Research Center of Agricultural and Natural Resources of Mashhad (MRCH) and Institute of Medicinal Plants Herbarium (IMPH).

Table 1. General information on the eight populations of *Nepeta* collected in Iran.

No.	Taxon	Locality	Voucher
1	<i>N. binaludensis</i> Jamzad	Khorasan Razavi province, Zeshk, Abdullah River, 2200 m asl, 36°30'15.20"N, 59°28'02.11"E	9022-IMPH
2	<i>N. glomerulosa</i> Boiss. var. <i>carmanica</i> Bormm.	Kerman province, Jirof, 42 km to Kerman, Deh-Bakri, 2260 m asl, 28°93'85.04"N, 57°46'27.27"E	5907-MRCH
3	<i>N. kotschyi</i> Boiss. var. <i>persica</i> (Boiss.) Jamzad	Khorasan Province, Nyshabur, 1700 m asl, 35°25'28.09"N, 58°50'45.13"E	7049-IMPH
4	<i>N. meyeri</i> Benth.	Mazandaran province, Haraz road, Polor, Lasem, 2650 m asl, 35°35'77.8"N, 52°34'12.7"E	7047-IMPH
5	<i>N. mirzayanii</i> Rech f. & Esfand.	Kerman province, Rabor, Naniz Olia, 2418 m asl, 29°19'01.9"N, 56°54'10.2"E	7052-IMPH
6	<i>N. pogonosperma</i> Jamzad & Assadi	Qazvin province, Alamout, 3000 m asl, 36°08'81.16"N, 49°85'47.34"E	7046-IMPH
7	<i>N. racemosa</i> Lam.	Qazvin province, Alamout to Moalem Kelayeh, 2100 m asl, 36°23'47.4"N, 50°45'57.1"E	7062-IMPH
8	<i>N. saccharata</i> Bunge	Zanjan province, 20km Dandi to Zanjan, 1513 m asl, 36°29'49.5"N, 47°44'54.4"E	7059-IMPH

Isolation and identification of essential oil compositions

The aerial parts of the plant samples were air-dried at room temperature in a shady place for eight days. 50 g of dried samples were hydro-distilled for 3 h using a Clevenger-type apparatus to extract the essential oil according to the European Pharmacopoeia method (Anon., 2005). Essential oil yields were calculated using the equation: $ROu = (M/B_m) \times 100$, where (M) is the mass of the extracted oil (g) and (B_m) is the initial plant biomass (g) (da Costa *et al.* 2014).

The extracted essential oils were dried over anhydrous sodium sulfate and stored in sealed vials at 4°C

before gas chromatography (GC) and gas chromatography-mass spectrometric (GC-MS) analysis. The GC analysis was performed on an Agilent 6890 N GC system equipped with a 5975 MSD and an FID, with HP-5MS column (30 m \times 0.25 mm, 0.25 mm film thickness). Injection volume was 2 μ l, the temperature of the injector was 200°C with a 1:10 split ratio. Helium was the carrier gas, and the rate of gas flow was 1.0 ml/min (constant flow mode). The column temperature was linearly programmed in the range of 60–280°C at the rate of 3°C / min and held at 280°C for 5 min. The transfer line was heated at 250°C, and the ionization energy for the MS detector was 70eV. The linear retention indices (LRI)

of essential oil compounds were calculated by linear interpolation relative to retention times of two series of n-alkanes (C8-C20 and C21-C40) under equal chromatographic conditions. The compounds were identified by comparing their linear retention indices (LRI, HP-5) with those reported in the literature (Massada, 1976; Connoll & Hill, 1991; Adams, 2007) and also by comparing their mass spectra with Wiley Registry 9th edition Library.

Statistical analyses

After the acquisition of the data by CG-MS and CG-FID, the identified volatile substances were grouped according to their presence, absence and intensities in each of the taxa studied. From these data, each volatile substance and its respective intensity were considered the variables for the analysis of Principal Coordinate Analysis (PCA) and Unweighted Paired Group Method with Arithmetic Mean (UPGMA). In order to perform it, data were standardized (Mean of 0 and standard deviation of 1) (Higgs, 1991; Podani, 2000).

The bootstrapping analysis was used to verify the correctness of the UPGMA tree. Bootstrapping calcu-

lates a support value for each node of the tree according to the fraction of samples that support the nodes. Bootstrap provides a number on each node (0-100). The highest support value is 100, while values below 70 are usually considered weak. Values below 50 are not shown and these branches are collapsed and shown as a polytomy (Tribble, 2018). We have used MVSP 2.0 (1998) and PAST 3.14 software for statistical analyses.

Results

Based on the dry weight, the yield of essential oil from eight taxa of *Nepeta* picked at the flowering stage ranged from 0.1 to 0.9%. The analysis of the chemical profile of the essential oils showed 106 compounds, including oxygenated monoterpenes (5.2–96.4%), oxygenated sesquiterpenes (0–18.8%), monoterpene hydrocarbons (0–14.1%), sesquiterpene hydrocarbons (0–6.5%) and the other compounds (0–74.3%). All the identified compounds, along with their percentages, appear in Table 2, where they have been ordered according to their Linear Retention Index (LRI).

Table 2. Percentages of chemical compositions of the essential oil among the studied taxa. Abbreviations are: LRI, Determined linear retention index against mixture of n-alkanes (C8-C31) on HP-5 column; *Nepeta* analyzed are: 1, *N. binaludensis*; 2, *N. glomerulosa* var. *carmanica*; 3, *N. kotschyi* var. *persica* (%); 4, *N. meyeri*; 5, *N. mirzayanii*; 6, *N. pogonosperma*; 7, *N. racemosa*; 8, *N. saccharata*.

Compounds	LRI	1	2	3	4	5	6	7	8
Essential oil yield (%)		0.9	0.9	0.1	0.2	0.6	0.4	0.4	0.2
2-Methylcyclopentanone	844	—	0.6	—	—	—	—	—	—
Ethyl isovalerate	846	—	—	—	—	—	0.1	—	—
3-Methylcyclopentanone	849	—	0.2	—	—	—	—	—	—
2(<i>E</i>)-Hexenal	853	—	0.2	—	0.1	—	0.1	0.2	—
(<i>O</i>)-Xylene	891	—	—	—	—	—	2.2	0.1	—
α -Thujene	927	0.4	0.2	—	—	—	0.1	—	—
α -Pinene	935	0.8	1.5	—	—	—	0.7	0.3	—
Camphene	952	—	2	—	—	—	—	—	—
Verbenene	958	—	0.2	—	—	—	—	—	—
2-Octanol	963	—	0.2	—	—	—	—	—	—
Sabinene	974	0.9	—	—	—	—	0.3	—	—
β -Pinene	979	2.8	0.2	—	0.6	—	3.0	1.2	—
1-Octen-3-ol	980	—	0.6	—	0.3	—	—	—	—
3-Octanone	988	—	—	—	0.3	—	—	—	—
Dehydro-1,8-cineole	989	0.2	—	—	—	—	—	—	—
2-Octanone	990	—	—	—	—	0.2	—	—	—
Myrcene	993	0.6	—	—	—	—	0.3	—	—
α -Phellandrene	1005	—	0.2	—	—	—	—	—	—
α -Terpinene	1017	0.7	1.1	—	—	—	1.2	—	—
(<i>O</i>)-Cymene	1026	—	—	—	—	—	1.3	—	—
(<i>P</i>)-Cymene	1027	3.4	3.9	—	—	—	—	—	—
Limonene	1033	0.6	2.3	—	—	—	0.4	—	—
1,8-Cineol	1036	43.5	23.3	—	1	—	53.9	70.9	1.8
2-acetyl-5-methyl-furane	1039	—	—	2.7	—	0.7	—	—	—
(<i>Z</i>)- β -Ocimene	1043	—	—	—	0.3	—	—	—	—
γ -Terpinene	1061	1.6	1.9	—	—	—	2.0	0.4	—
<i>cis</i> -Sabinene hydrate	1068	0.6	—	—	—	—	0.8	—	—
<i>n</i> -Octanol	1079	—	2.1	—	—	—	—	—	—
Terpinolene	1091	0.2	0.4	—	—	—	0.4	—	—

Compounds	LRI	1	2	3	4	5	6	7	8
<i>trans</i> -Sabinene hydrate	1098	—	—	—	—	—	0.3	—	—
Camphenilone	1099	—	0.3	—	—	—	—	—	—
Linalool	1102	5	0.5	1	—	—	4	0.5	1.4
Isopentyl isovalerate	1118	—	—	—	—	—	0.2	—	—
<i>cis</i> -Rose oxide	1120	—	0.4	—	—	—	—	—	—
<i>cis</i> -p-menth-2-en-1-ol	1121	—	—	—	—	—	0.3	0.2	—
<i>trans</i> -p-menth-2-en-1-ol	1124	—	—	—	—	—	0.4	—	—
α -Campholenal	1125	—	0.6	—	—	—	0.1	0.3	—
Dihydro-linalool	1132	—	0.4	—	—	—	—	—	—
<i>trans</i> -Pinocarveol	1146	—	—	—	—	—	—	1	—
<i>trans</i> -Verbenol	1151	—	1.6	—	—	—	—	0.2	—
Citronellal	1159	—	3.8	—	—	—	—	—	—
Pinocarvone	1167	0.1	—	—	—	—	0.3	0.3	—
Borneol	1169	—	4.3	—	—	—	—	—	—
γ -Terpineol	1171	2.1	—	—	—	—	1.5	1.4	—
Terpinen-4-ol	1181	3.1	5.3	—	—	—	3.8	1	0.5
(<i>P</i>)-Cymene-8-ol	1188	—	0.5	—	—	—	—	—	—
2-methyl-3-octyne	1190	—	—	0.9	—	—	—	—	0.1
α -Terpineol	1193	4.8	1.1	—	0.3	—	3.2	1.7	—
Verbenone	1212	—	0.5	—	—	—	—	—	—
2-Methoxy- <i>para</i> -cresol	1219	0.9	—	—	2.6	3.6	1.5	—	—
2(<i>E</i>),4(<i>E</i>)-nonadienal	1221	—	—	—	—	—	—	—	0.7
Nerol	1227	—	2	—	—	—	—	—	—
Isobornyl formate	1233	—	1.4	—	—	—	—	—	—
Citronellol	1234	—	1	8.4	—	—	—	4.3	—
<i>n</i> -Valeric acid <i>cis</i> -3-hexenyl	1243	—	—	—	—	—	0.3	—	—
Cumin aldehyde	1248	—	—	—	—	—	—	0.3	—
Geraniol	1256	—	5.4	—	—	—	0.2	—	—
<i>cis</i> -Carvone oxide	1263	—	0.3	—	—	—	—	—	—
Geranial	1268	—	0.4	—	—	—	—	—	—
Isobornyl acetate	1287	—	6	—	—	—	—	—	—
Thymol	1293	—	—	1.4	—	—	—	—	1.6
(<i>E</i>)-Anethole	1300	—	—	—	—	—	—	—	0.5
Terpinen-4-ol acetate	1302	—	0.6	—	—	—	—	—	—
Carvacrol	1304	—	—	9.9	—	—	—	—	22.4
4 α ,7 α ,7 α -Nepetalactone	1360	1.1	—	13.4	1.3	13	6.2	5.3	—
Geranyl acetate	1379	—	0.3	4.8	—	—	1.7	—	—
4 α ,7 α ,7 β -Nepetalactone	1383	23.5	—	—	7.4	73.9	—	3	—
β -Bourbonene	1387	0.2	—	—	—	0.7	0.3	0.6	0.4
7- <i>epi</i> -Sesquithujene	1388	—	0.3	—	—	—	—	—	—
4 α ,7 β ,7 α -Nepetalactone	1394	0.6	—	—	83.9	1.6	—	—	—
(<i>E</i>)-Caryophyllene	1413	—	—	—	—	0.8	0.6	—	0.9
Neryl acetate	1437	—	—	—	—	—	—	—	0.3
(<i>Z</i>)- β -Farnesene	1443	—	—	—	—	3	0.3	0.3	—
α -Humulene	1463	—	0.3	—	—	—	0.1	—	—
(<i>E</i>)- β -Ionone	1485	—	—	2.7	—	—	—	—	1.5
<i>ar</i> -Curcumene	1487	—	0.2	—	—	—	—	—	—
Germacrene D	1488	0.3	—	—	0.5	0.3	0.2	0.5	—
Cubenol	1515	—	—	1.2	—	—	—	—	—
1-endo-Bourbonanol	1520	—	—	1.5	—	—	—	—	—
δ -Cadinene	1523	—	—	—	—	—	—	—	0.4
(<i>Z</i>)- α -Bisabolene	1530	—	—	—	—	—	5	2.4	—
1-nor-Bourbonan-1-one	1574	—	—	2.9	—	—	—	—	2.4
3-(<i>Z</i>)-Hexenyl benzoate	1577	—	—	—	—	0.6	—	—	—
Spathulenol	1585	—	—	1.5	1.1	—	—	1	1.3
Caryophyllene oxide	1591	—	0.4	2.7	0.1	0.6	0.6	0.4	2.8

Compounds	LRI	1	2	3	4	5	6	7	8
Hexadecane	1599	—	—	1.5	—	—	—	—	1.4
(<i>E</i>)-Isoeugenol acetate	1607	—	—	3.3	—	—	—	—	—
Citronellyl pentanoate	1631	—	0.8	—	—	—	—	—	—
β -Sesquiphellandrene	1634	—	0.3	—	—	—	—	—	—
Widdrol	1671	—	—	3	—	—	—	—	1.9
<i>ar</i> -Tumerone	1679	—	—	—	—	—	—	—	0.9
14-Hydroxy-9- <i>epi</i> -(<i>E</i>)-caryophyllene	1688	—	—	—	—	—	—	—	1.9
Heptadecane	1700	—	—	0.9	—	—	—	—	—
Germacrone	1703	—	—	2	—	—	—	—	—
Benzyl benzoate	1793	—	—	—	—	—	—	—	0.5
Octadecane	1800	—	—	2.1	—	—	—	—	0.3
Hexahydrofarnesyl acetone	1843	—	—	—	—	0.3	—	—	—
6,10,14-trimethyl-2-pentadecanone	1847	—	—	2.9	—	—	—	—	2.7
Isobutyl phthalate	1873	—	—	—	—	—	—	—	2.9
Farensyl acetone	1922	—	—	1.7	—	—	—	—	—
<i>n</i> -Hexadecanoic acid	1964	—	—	—	—	—	—	—	9.3
Dibutyl phthalate	1968	—	—	2.2	—	—	—	—	3.3
Manoyl oxide	2004	—	—	—	—	—	—	0.3	—
<i>epi</i> -13-Manoyl oxide	2009	—	—	—	—	—	0.3	—	—
Phytol	2125	—	—	15.6	—	—	0.1	—	31.2
Abietol	2395	—	—	2.2	—	—	—	—	—
Monoterpene Hydrocarbons		11.8	14.1	—	0.9	—	—	1.9	—
Oxygenated Monoterpene		85.3	60.8	31.8	96.4	92	78.1	89.6	5.2
Sesquiterpene Hydrocarbons		0.5	1.1	—	0.5	4.8	6.5	3.8	1.8
Oxygenated Sesquiterpene		—	0.9	18.8	1.2	0.6	0.6	1.4	13.2
Other		—	7.0	42.7	0.7	1.8	3.6	1.2	74.3

The oxygenated monoterpenes comprised to 96.4% of the oils of *N. meyeri* Benth., 92 % of *N. mirzayanii* Rech f. & Esfand., 89.6% of *N. racemosa* Lam., 85.3% of *N. binaludensis* Jamzad, and 60.8% of *N. glomerulosa* Boiss. var. *carmanica* Bornm. The oils of *N. saccharata* Bunge (74.3%) and *N. kotschyi* Boiss. var. *persica* (Boiss.) Jamzad (40.7%) were mainly composed of other compounds. Except for the sesqui- and monoterpenes, some of the studied samples had high amounts of aliphatic compounds of diterpenes, non-terpenic origin, and also contained low amounts of ester, aldehyde and ketones.

The essential oil of *N. binaludensis* was dominated by 1,8-cineol (43.5%), 4 α ,7 α ,7 β -nepetalactone (23.5%), linalool (5%), α -terpineol (4.8%), and terpinen-4-ol (3.1%). Moreover, the oil of *N. glomerulosa* var. *carmanica* contained 1,8-cineol (23.3%), isobornyl acetate (6%), geraniol (5.4%), terpinen-4-ol (5.3%) and borneol (4.3%). *N. kotschyi* var. *persica* oil consisted of phytol (15.6%), 4 α ,7 α ,7 α -nepetalactone (13.4%), carvacrol (9.9%), citronellol (8.4%) and geranyl acetate (4.8%).

Phytol (31.2%), carvacrol (22.4%), *n*-hexadecanoic acid (9.3%) and dibutyl phthalate (3.3%) were the dominant compounds in the essential oil of *N. saccharata*; while the 4 α ,7 α ,7 β -nepetalactone (73.9%), 4 α ,7 α ,7 α -nepetalactone (13%), 2-methoxy-para-cresol (3.6%), and *Z*- β -farnesene (3 %) were the main compositions in the essential oil of *N. mirzayanii*.

In the essential oil composition of *N. pogonosperma* Jamzad & Assadi, we found 1,8-cineol (53.9%),

4 α ,7 α ,7 α -nepetalactone (6.2%), *Z*- α -bisabolene (5%), linalool (4.1%) and terpinen-4-ol (3.8%) as the main compounds. The oil of *N. racemosa* showed the presence of 1,8-cineol (70.9%), 4 α ,7 α ,7 α -nepetalactone (5.3%), citronellol (4.3%) and 4 α ,7 α ,7 β -nepetalactone (3%). Finally, the presence of 4 α ,7 β ,7 α -nepetalactone (83.9%), 4 α ,7 α ,7 β -nepetalactone (7.4%) and 2-methoxy-para-cresol (2.6%) in the oil of *N. meyeri* made it chemotaxonomically distinct from the other *Nepeta* taxa were examined in this study.

The studied taxa were clustered separately in the UPGMA tree (Figure 1). The tree had two branches, *N. meyeri* was placed in the small branch (with 100% bootstrap support), while the big branch was composed of two sub-branches. *N. mirzayanii* was placed separately (by 87% bootstrap support), and other species were clustered in two groups. One group was presented by *N. binaludensis*, *N. racemosa* with *N. pogonosperma* (bootstrapping value of 96%), and another group was composed of *N. saccharata*, *N. kotschyi* var. *persica* and *N. glomerulosa* var. *carmanica* (by 95% bootstrap support). In this group *N. saccharata* and *N. kotschyi* var. *persica* were clustered as a pair and *N. glomerulosa* var. *carmanica* was joined them with a relatively long distance.

Furthermore, the PCA plot produced similar results (Figure 2). The X axis (Axis 2) divided the taxa into two main groups. *N. meyeri* and *N. mirzayanii* were placed far from the other taxa. These species constituents the first and second clades in the UPGMA tree. The highest amount of 4 α ,7 β ,7 α -nepetalactone identified *N.*

meyeri; furthermore, this species had 3-octanone and (Z)- β -ocimene in its essential oil, while these compounds were not reported from the other studied species. *N. mirzayanii* that made the second clade in the UPGMA tree had the highest amount of 4 α ,7 α ,7 β -nepetalactone; furthermore, its oil contained some minor compounds such as 3-(Z)-hexenyl benzoate, hexahydrofarnesyl acetone, and 2-octanone which were absent in the other

species. In the plot, there was a clear division of two large groups by the Y-axis (Axis 1). It was the cut factor to leave *N. binaludensis*, *N. racemosa*, *N. glomerulosa* var. *carmanica* and *N. pogonosperma* that grouped in the third cluster of the UPGMA tree. All species were identified according to the highest amounts of 1,8-cineol and also the presence of α -pinene and γ -terpinene, which were not detected in other taxa.

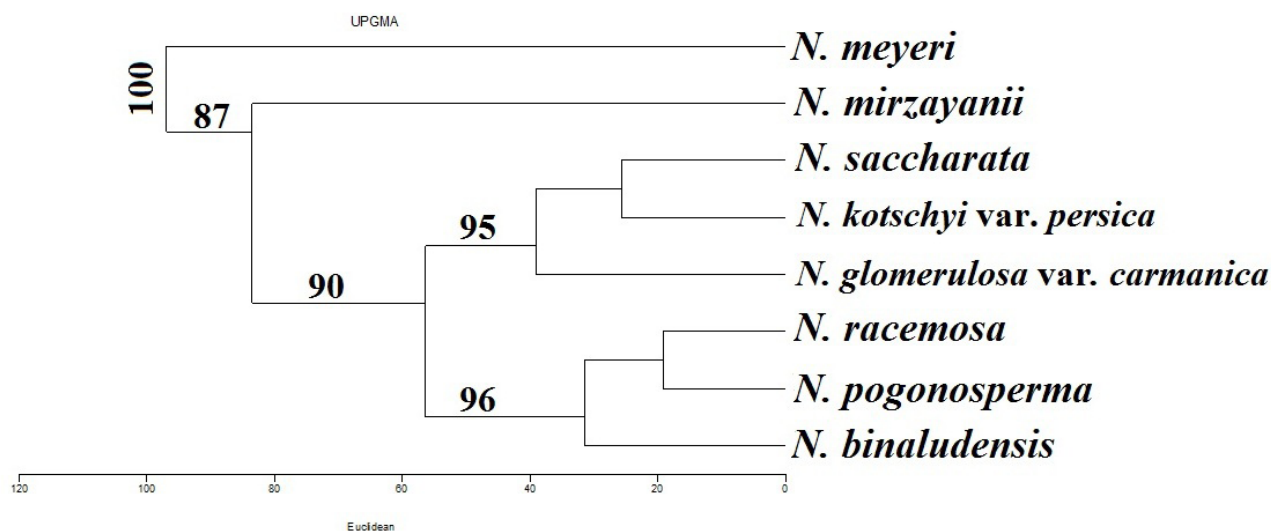


Figure 1. UPGMA tree with Bootstrapping support of the studied *Nepeta* taxa based on essential oil compositions.

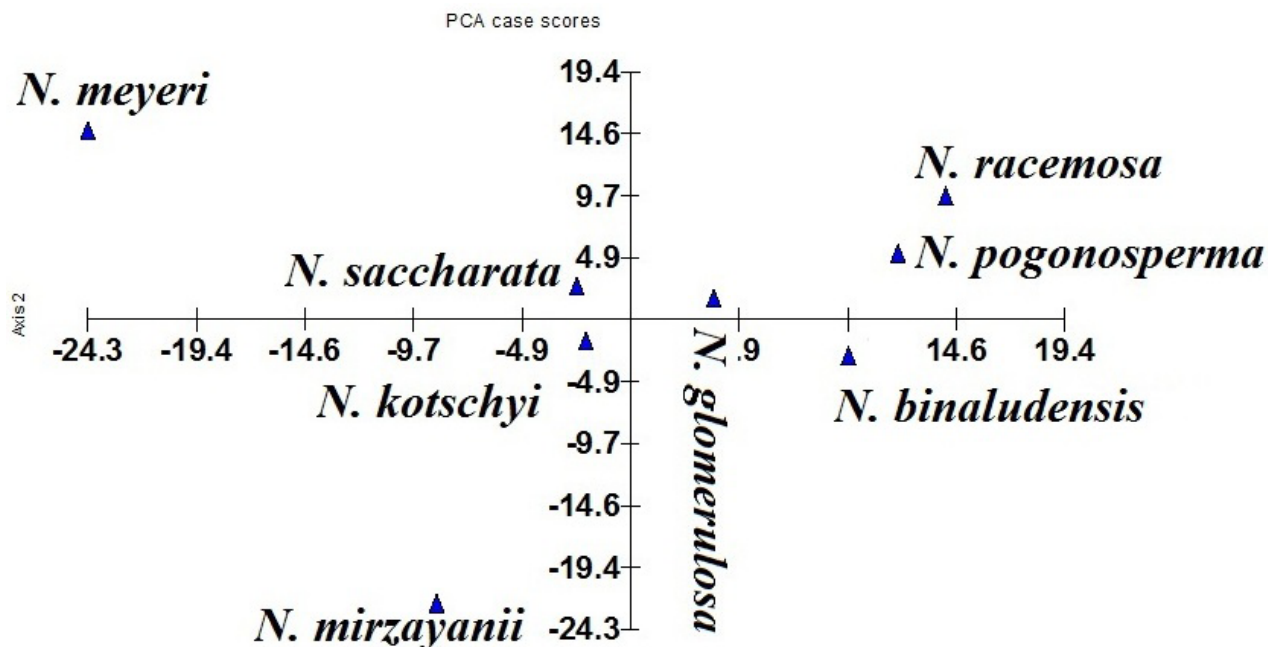


Figure 2. PCA plot of the studied *Nepeta* taxa based on essential oil compositions.

Furthermore, this axis separated *N. saccharata* and *N. kotschy* that grouped in the fourth cluster of the UPGMA tree. Both taxa had the highest amounts of phytol and carvacrol among the studied taxa. Besides, some compounds such as (E)- β -Ionone, 1-nor-Bourbonan-1-one, widdrol, octadecane and dibutyl

phthalate were found in *N. saccharata* and *N. kotschy*, while these compounds were absent in the other studied taxa.

Therefore, we have divided the studied taxa into four primary groups. *N. mirzayanii* and *N. meyeri* were characterized by high amounts of 4 α ,7 α ,7 β -

nepetalactone and $4\alpha,7\beta,7\alpha$ -nepetalactone and formed the first and second groups, respectively. The characteristic of *N. binaludensis*, *N. racemosa*, *N. glomerulosa* var. *carmanica* and *N. pogonosperma* was high concentrations of

1,8-cineol, and they formed the third group. *N. saccharata* and *N. kotschy* var. *persica* were characterized by high percentages of phytol and carvacrol and formed the fourth group (Figure 3).

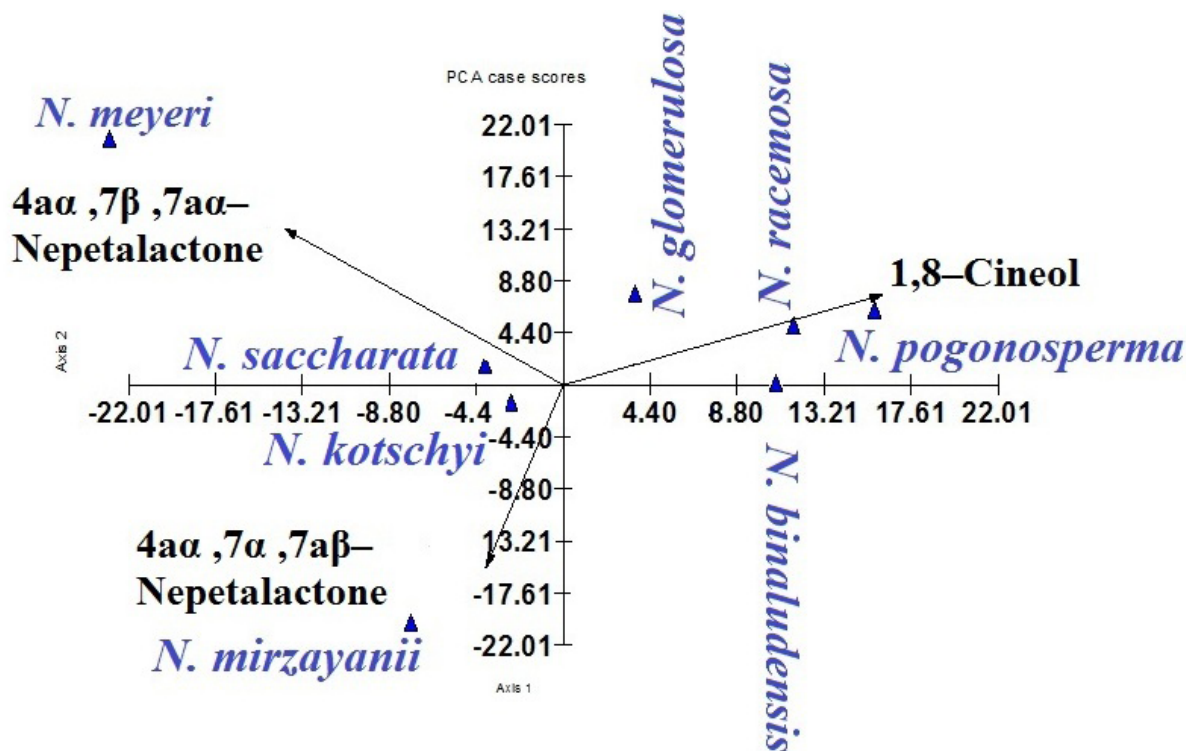


Figure 3. PCA bi-plot of the studied taxa and essential oil compositions.

Discussion

Our results of essential oil composition suggested higher variability in comparison with previous studies of these taxa. Some authors (Nejad Ebrahimi *et al.*, 2008; Saharkhiz & Mohammadi, 2011) showed some differences in the essential oil compositions even among the populations of the same species.

We have shown that phytol (15.6%), $4\alpha,7\alpha,7\alpha$ -nepetalactone (13.4%), carvacrol (9.9%), citronellol (8.4%) and geranyl acetate (4.8%) were the main essential oil compounds in *N. kotschy* var. *persica*. Nevertheless, Hadi *et al.* (2016) investigated essential oil compositions of 18 populations of this species in two consecutive years. In the first and second years, the oil extraction was performed in summer and spring, respectively. Their results revealed annual variation in the amount of $4\alpha,7\alpha,7\alpha$ -nepetalactone, the major compound of most populations. The maximum and minimum annual changes were reported for Khorasan and Taft1 populations by 17% and 1.3%, respectively.

The annual differences in essential oil compositions may relate to harvest time. Marques *et al.* (2012) have suggested the seasonal fluctuations in the essential oil compositions of aromatic plants. The seasonality has a direct influence on the vegetative development and foliar biomass, which are the key points for oil production.

Moreover, amounts of $4\alpha,7\alpha,7\alpha$ -nepetalactone differed among the studied populations of each year.

In the first year, highest amount of this compound was registered in Khorasan population (85.9%), while Taft 5 population had its lowest amount (0.5%). In the second year, the maximum and minimum percentages of this compound were 82.4 % (in Yazd population) and 0.3% (in Taft 5 populations), respectively.

Abdelmajeed *et al.* (2013) believed that different physical and chemical environmental factors (e.g. temperature, hydric and osmotic stress, relative humidity, photoperiod, nutrition, and soil properties) and genetic structure could affect the quality and quantity of the essential oils. It seems that the variations in essential oil compositions among the populations each year related to the mentioned above environmental factors.

For the first year, in the oils of populations (Taft5, Semirum, and Taft4) with very low percentages of $4\alpha,7\alpha,7\alpha$ -nepetalactone, the main compound of oils was cubenol by 57.1%, 42.5%, and 53.9%, respectively. However, in the second year, the main compounds of these populations oil were cubenol (34.2%), $4\alpha,7\alpha,7\beta$ -nepetalactone (44.7%), and geranyl acetate (46.3%), respectively.

We registered that two isomers of nepetalactone, $4\alpha,7\beta,7\alpha$ - and $4\alpha,7\alpha,7\beta$ -nepetalactone, accounted for more than 90% of essential oil of *N. meyeri*, while 2-methoxy-para-cresol made about of 2.59%. However, Sefidkon & Shaabani (2004) analyzed essential oil of *N. meyeri* (Ghom population) and considered $4\alpha,7\alpha,7\beta$ -nepetalactone (53.2%) and 1,8-cineole (29.3%) as the

main oil constituents. In both studies, two isomers of nepetalactone were the major compounds of the oil, which constituent more than 53% of oil composition. The Ghom population was harvested in semi-dried habitat in the central part of Iran at the altitude of about 1000 m asl, while our studied population was collected from the slopes of Albourz mountains at an altitude of 2650 m asl. Not only these populations were harvested from different habitats, but also from different altitudes, more than 1600 m in some cases.

According to Kofidis & Bosabalidis (2008), altitude is one of the most important environmental factors. Several environmental factors such as wind exposure, partial CO₂ pressure, light intensity, and ozone concentration may also differ among different altitudes. Moreover, several investigations (Giuliani *et al.*, 2013; Sadeghi *et al.*, 2015; Talebi *et al.*, 2019) have revealed that altitude significantly affected the chemical composition of essential oil in Lamiaceae taxa.

In this study phytol (31.2%), carvacrol (22.4%) and n-hexadecanoic acid (9.3%) were the main compounds in *N. saccharata* oil, but, Rustaiyan *et al.* (2013) reported that the major compounds of this species were neo-isomenthol (18.6%) and n-hexadecanoic acid (12.1%). The habitat characteristics widely differed between these populations. Our studied population belongs to the Atropatenian district (northwest of Iran) that is largely influenced by the Mediterranean climate. Its thermal climate is similar to that of the eastern Mediterranean; its climate is continental. In addition, spring arrives in late March and its precipitation tends to be moderate in amount. The studied population by Rustaiyan *et al.* (2013) was harvested from central Iran district (Isfahan) with continental desert climate, whose climate pattern is similar to that of central Asia desert. This region is characterized by a distinct seasonal climate, with strong, long-lasting frost in winter and extremely hot summer, in combination with very low annual precipitation of less than 100 mm. Moreover, spring arrives as soon as early March (Zohary, 1973). Although, both populations were harvested in May, because of the environmental conditions, they were in different growth phases. Esfahan population was at the end of the annual growth, while Zanjan population reaches its maximum growth rate in Mey. These conditions highly influence essential oil compositions of these populations. Dudai *et al.* (2001) have suggested that when growth rates are in maximal, pulegone constituted up to 80% of the essential oil in *Micromeria fruticosa*, in a period of growth-rest, pulegone levels dropped dramatically to a few percents, while isomenthol constituted up to 80% of the essential oil.

It seems that the enhancement of n-hexadecanoic acid percentage in Isfahan population might be related to environmental temperatures. Habitat of Isfahan population is warmer than Zanjan population. Shamloo *et al.* (2017) have reported that a significant increase in n-hexadecanoic acid amount was recorded at an increase in growth temperature for all wheat genotypes.

A relatively high amount of dibutyl phthalate (3.3%) was found in the essential oil composition of this species. Manayi *et al.* (2014) have suggested that phthalate are chiefly used as plasticizers and cause several human

health and also environmental hazards. Due to the problems regarding waste disposal in developing countries such as Iran, phthalate derivatives can easily release from waste disposal to the water and soil, resulting in probable absorption and accumulation by medicinal and dietary plants. Therefore, it seems that a relatively high concentration of phthalate in *N. saccharata* and *N. kotschyi* var. *persica* comes from environmental pollutions.

Based on our results 1,8-cineol (70.9%), 4 α ,7 α ,7 α -nepetalactone (5.3%), citronellol (4.3%) and 4 α ,7 α ,7 β -nepetalactone (3%) were the major compounds of oil in *N. racemosa*. Nevertheless, according to Rustaiyan *et al.* (2000) the main constituents of *N. racemosa* oil were 4 α ,7 α ,7 α -nepetalactone (64.9%), (Z)- β -cymene (9.5%), (E)-nerolidol (8.8%) and 4 α ,7 α ,7 β -nepetalactone (7.4%). Moreover, Dabiri & Sefidkon (2003) considered 4 β ,7 α ,7 β -nepetalactone (33.6%), 4 α ,7 α ,7 β -nepetalactone (25.6%), 4 α ,7 α ,7 α -nepetalactone (24.4%), and 1,8-cineole (9.0%) as major components of oil in this species.

Our studied population was harvested in June at an altitude of 2100 m asl. The samples of Rustaiyan *et al.* (2000) and Dabiri & Sefidkon (2003) studies were harvested in June at the altitude of 3100 m asl and in September at the altitude of more than 3000 m asl, respectively. Although our study and Rustaiyan *et al.* (2000) populations were selected in the same month of the year, a 1000 m difference exists between their altitudes. Moreover, these populations were harvested in different years (at least 15 years difference). Nepetalactones are oxygenated monoterpenes compounds, and several reports (Niinemets *et al.*, 2004; Figueiredo *et al.*, 2008; Lakusic *et al.*, 2012) have revealed that biosynthesis of terpene is influenced by different factors, primarily the genotype of species, but also by several biotic and abiotic environmental factors. Therefore, it is not unusual for populations of the same species to have various essential oil compositions.

Setzer (2016) has suggested there are eight possible stereoisomers of nepetalactone, of those six stereoisomers have been found in nature as we can see, the authors registered different isomers of nepetalactone as the main parts of essential oil of this species except for 1,8-cineol.

According to our study the main essential oil composition of *N. pogonosperma* were 1,8-cineol (53.9%) and 4 α ,7 α ,7 α -nepetalactone (6.2%), while Sefidkon & Akbari-Nia (2003) considered 4 α ,7 α ,7 β -nepetalactone (57.6%) and 1,8-cineole (26.4%) as the major components. The percentages of 1,8-cineol and 4 α ,7 α ,7 β -nepetalactone differed nearly by two and eight times between these researches, respectively. Both populations were harvested from the same region (Alamout-Qazvin). It seems that variations in nepetalactone isomers related to annual difference, which was reported from different taxa such as two varieties of *N. kotschyi* (Hadi *et al.*, 2016). Moreover, other major compounds found in the study of Sefidkon & Akbari-Nia (2003) are present in our study but in different amounts.

Sefidkon & Jamzad (2007) analyzed the essential oil composition of *N. mirzayanii* and reported 4 α ,7 α ,7 α -

nepetalactone (61.0%) and caryophyllene oxide (7.8%) as the main constituents. In our study we registered 4 α ,7 α ,7 β -nepetalactone (73.9%), 4 α ,7 α ,7 α -nepetalactone (13%), 2-methoxy-para-cresol (3.6%), and Z- β -farnesene (3%) as the principal oil components. The percentage of 4 α ,7 α ,7 α -nepetalactone varied up to 4.7 times between these studies. Moreover, in the study of Sefidkon & Jamzad (2007), the amount of 4 α ,7 α ,7 β -nepetalactone was trace (0.6%), therefore we had more than 123 times variations in the amount of the compound compared to the results of this investigation.

These populations were harvested from habitats with more than 900m variation in the altitude. According to Hadi *et al.* (2016), percentages of nepetalactone isomers have annually and geographically variations. Moreover, altitude has a strong effect on environmental conditions and the synthesis of secondary metabolites (Talebi *et al.*, 2019). It is important to know that cresols are released via high traffic areas, automobile exhaust, and gas stations are likely to have high levels of cresols. Moreover, these compounds are also the product of combustion of wood, municipal solid waste, and coal. So, residents near coal and petroleum-fueled facilities, as well as residents near municipal waste incinerators, may have amplified exposure to cresols. Several studies revealed that cresols are highly corrosive and toxic compounds (Tisserand & Young, 2014). It seems that a high amount of 2-methoxy-para-cresol in oil of this plant comes from environmental pollutions.

We have shown 1,8-cineol (43.5%), and 4 α ,7 α ,7 β -nepetalactone (23.5%) were the major components in essential oils of *N. binaludensis*. Mohammadpour *et al.* (2013) reported that 1,8-cineol (68.3%) and α -terpineol (5.2%) were the major components, whereas Rustaiyan & Nadji (1999) suggested the main parts of the oil were 1,8-cineole (42.3%) and 4 α ,7 α ,7 α -nepetalactone (25.2%).

The main compound of all studies was 1,8-cineol, but with various percentages. Tounekti *et al.* (2011) have reported that some environmental factors such as salinity stress affect some secondary plant metabolite such 1,8-cineol. It was found that soil salinity significantly increased the relative abundance of 1,8-cineol. Based on the author's field observations, the habitat of populations in the study of Mohammadpour *et al.* (2013) is more saline than other populations, which could lead to a higher percentage of 1,8-cineol. However, our findings of *N. binaludensis* oil composition were more similar to Rustaiyan & Nadji (1999) rather than Mohammadpour *et al.* (2013). Three main compounds that constitute more than 71% of the oil were the same with a nearly equal amount. The populations in our study, as well as in the study of Rustaiyan & Nadji (1999), were harvested in the same month (July), while Mohammadpour *et al.* (2013) harvested the plant samples in August. Moreover, according to Hadi *et al.* (2016), the percentages of nepetalactone isomers were strongly affected by harvest time, ecological, and genetic parameters. Therefore, the similarity of essential oil compositions of these populations seems reasonable.

We have recorded that 1,8-cineol (23.3%), isobornyl acetate (6%), geraniol (5.4%), terpinen-4-ol (5.27%), and borneol (4.29%) were the main compounds in the oil of *Nepeta glomerulosa* subsp. *carmanica*. Furthermore, Sajja-

di & Ghassemi (1999) analyzed the essential oil composition of Isfahan population and reported α -pinene (18.3%), 1,8-cineole (13.9%), limonene (9.7%) and linalool (4.8%) as the major compounds. We have found more than 59% variation in 1,8-cineol amounts between these populations. Against, other main compounds of Isfahan populations were found in our population in very low amounts (less than 3%). Both populations were harvested in the same month of the year, while they were selected from habitats with more than 500 m difference in altitude. Meanwhile, Zohary (1973) has suggested that the soil salinity in Kerman province (the habitat sampled in our study) is higher than Isfahan province. It seems that the observed variations in the amount of 1,8-cineol could be mainly related to soil salinity. According to Tounekti *et al.* (2011), salinity stress has a strong effect on 1,8-cineol amount.

Diefendorf *et al.* (2011) have stated that chemotaxonomy of plant species is the interpretation of proxy records based on n-alkanes and the compositions of their associated carbon isotope. We calculate bootstrap percentages for all groups in UPGMA tree. All bootstrapping values were more than 87% for all groups in the tree. Burleigh *et al.* (2006) have suggested that the nonparametric bootstrapping methods are useful for assessing confidence in a super-tree inference. Moreover, non-parametric bootstrapping may be an over-conservative estimator of node reliability (Hillis & Bull, 1993), and values below 70 are usually considered weak. Therefore, all identified groups in the UPGMA tree of essential oil data have reliable support. As the chemical composition of essential oil varied significantly in the studied taxa, and according to UPGMA and PCA analysis, we have clustered these taxa into four groups. We also identified four chemotypes among these species: *N. racemosa*, *N. pogonosperma*, *N. binaludensis* and *N. glomerulosa* var. *carmanica* (with a high concentration of 1,8-cineole), *N. mirzayanii* and *N. meyeri* (due to high percentages of 4 α ,7 α ,7 β -nepetalactone and 4 α ,7 β ,7 α -nepetalactone, respectively). According to the high phytol and carvacrol amounts, the separate group has been formed by *N. kotschyi* var. *persica* and *N. saccharata*.

Conclusions

The obtained results suggested significant variations in essential oil compositions among eight *Nepeta* taxa in Iran, which could be the diagnostic characteristics for their identification. The UPGMA hierarchical clustering method formed the similar five groups as the PCA plot. Furthermore, the bootstrapping analysis showed strong support for these groups. Therefore, we divided these taxa into four chemotypes based on the essential oil compositions. Each of these chemotypes had a major compound with the highest percentage with the existence of some specific minor compounds. The chemotypes were 1,8-cineol; 4 α ,7 α ,7 β -nepetalactone; 4 α ,7 β ,7 α -nepetalactone; and carvacrol. We also considered that the essential oil compositions of the studied species significantly differed with results from previous similar investigations.

References

- Abdelmajeed, N.A., Danial, E.N. & Ayad, H.S. 2013. The effect of environmental stress on qualitative and quantitative essential oil of aromatic and medicinal plants. *Arch. Sci.* 66(4): 100–120.
- Adams, R.P. 2007. Identification of Essential Oil Components by Gas Chromatography/ Mass Spectroscopy. Allured Publishing Corporation, Illinois.
- Anonymous. 2005. European Pharmacopoeia. European directorate for the quality of medicines (EDQM) (5th Ed), Strasbourg.
- Burleigh, J.G., Driskell, A.C. & Sanderson, M.J. 2006. Supertree bootstrapping methods for assessing phylogenetic variation among genes in genome-scale data sets. *Syst. Biol.* 55(3): 426–444.
- Connoll, J.D. & Hill, R.A. 1991. Dictionary of Terpenoids. Chapman & Hall, London.
- Dabiri, M. & Sefidkon, F. 2003. Chemical composition of the essential oil of *Nepeta racemosa* Lam. from Iran. *Flavour Fragr. J.* 18(2): 157–158.
- da Costa, O.B., Del Menezzi, C.H.S., Benedito, L.E.C., Resck, I.S., Vieira, R.F. & Bizzo, H.R. 2014. Essential oil constituents and yields from leaves of *Blepharocalyx salicifolius* (Kunt) O. Berg and *Myracrodruon urundeuva* (Allemão) collected during daytime. *Int. J. For. Res.* 982576, 6 p. doi:10.1155/2014/982576.
- Diefendorf, A.F., Freeman, K.H., Wing, S.L. & Graham, H.V. 2011. Production of n-alkyl lipids in living plants and implications for the geologic past. *Geochim. Cosmochim. Acta* 75(23): 7472–7485.
- Dudai, N., Larkov, O., Ravid, U., Putievsky, E. & Lewinsohn, E. 2001. Developmental control of monoterpene content and composition in *Micromeria fruticosa* (L.) Druce. *Ann. Bot.* 88(3): 349–354.
- European Pharmacopoeia. 2005. European directorate for the quality of medicines (EDQM) (5th Ed). Strasbourg, Council of Europe.
- Figueiredo, A.C., Barroso, J.G., Pedro, L.G. & Scheffer, J.J.C. 2008. Factors affecting secondary metabolite production in plants: volatile components and essential oils. *Flavour Fragr. J.* 23: 213–226.
- Giuliani, C., Maggi, F., Papa, F., and Maleci Bini, L. 2013. Congruence of phytochemical and morphological profiles along an altitudinal gradient in *Origanum vulgare* ssp. *vulgare* from Venetian region (NE Italy). *Chem. Biodivers.* 10: 569–583.
- Gkinis, G., Tzakou, O., Iliopoulou, D. & Roussis, V. 2003. Chemical composition and biological activity of *Nepeta parnassica* oils and isolated Nepetalactones. *Z. Naturforsch. C* 58(9–10): 681–686.
- Hadi, N., Sefidkon, F., Shojaeiyan A. & Jafari A.A. 2016. Essential oil diversity of 21 populations from Iranian endemic species *Nepeta kotschyi* Boiss. *Iran. J. Med. Arom. Plants* 32(2): 189–202.
- Higgs, N.T. 1991. Practical and innovative uses of correspondence analysis. *Statistician* 40(2): 183–94.
- Hillis, D.M. & Bull J.J. 1993. An empirical-test of bootstrapping as a method for assessing confidence in phylogenetic analysis. *Syst. Biol.* 42: 182–192.
- Jamzad, Z. 2012. Flora of Iran no. 76, Lamiaceae. Research Institute of Forest and Rangelands, Tehran.
- Jamzad, Z. 2009. Notes on the genus *Nepeta* L. (Lamiaceae, Nepetoideae). *Iranian Journal of Botany* 15(2): 141–145.
- Jamzad, Z., Chase, M.W., Ingrouille, M., Simmond, M.S.J. & Jalili, A. 2003. Phylogenetic Relationship in *Nepeta* L. (Lamiaceae) and related Genera Based on ITS Sequence Data. *Taxon* 52(1): 21–32.
- Kofidis, G. & Bosabalidis, A.M. 2008. Effects of altitude and season on glandular hairs and leaf structural traits of *Nepeta nuda* L. *Bot. Stud.* 49: 363–372.
- Lakusic, D., Ristic, M., Slavkovska, V., Sinzar-Sekulic, J. & Lakusic, B. 2012. Environmental-related variations of the composition of the essential oils of rosemary (*Rosmarinus officinalis* L.) from the Balkan Peninsula. *Chem. Biodivers.* 9: 286–1302.
- Manayi, A., Kurepaz-Mahmoodabadi, M., Gohari, A.R., Ajani, Y. & Saeidnia, S. 2014. Presence of phthalate derivatives in the essential oils of a medicinal plant *Achillea tenuifolia*. *DARU* 22(1): 78. doi:10.1186/s40199-014-0078-1.
- Marques, M.O., Facanali, R., Haber, L.L. & Vieira, M.A.R. 2012. Essential oils: history, biosynthesis, and agronomic aspects. In: Sousa, D.P.(Ed). *Medicinal essential oils: Chemical, Pharmacological and Therapeutic Aspects*. New York, Nova Science Publisher, Inc.
- Massada, Y. 1976. Analysis of Essential Oils by Gas Chromatography and Mass Spectrometry. Wiley & Sons, New York.
- Mohammadpour, N., Emami, S.A. & Asili, J. 2013. Identification of volatile oil components of *Nepeta binaludensis* Jamzad by GC-MS and ¹³C-NMR methods and evaluation of its anti-microbial activity. *J. Essent. Oil Bear Pl.* 16(1): 102–107.
- Molvary, M., Kores, P.J. & Chase, M.W. 1999. Phylogenetic relationships within *Korthalsella* (Viscaceae) based on nuclear ITS and plastid trnL-F sequence data. *Am. J. Bot.* 86: 249–260.
- Nejad Ebrahimi, S., Hadian, J., Mirjalili, M.H., Sonboli, A. & Yousefzadi, M. 2008. Essential oil composition and anti-bacterial activity of *Thymus caramanicus* at different phenological stages. *Food Chem.* 110(4): 927–931.
- Newman D.J., Cragg G.M. & Snader K.M. 2000. The influence of natural products upon drug discovery. *Nat. Prod. Rep.* 17: 215–234.
- Niinemets, Ü., Loreto, F. & Reichstein, M. 2004. Physiological and physicochemical controls on foliar volatile organic compound emissions. *Trends Plant Sci.* 9: 180–186.
- Paterson, I. & Anderson, E.A. 2005. The renaissance of natural products as drug candidates. *Science* 310: 451–453.
- Podani, J. 2000. Introduction to the Exploration of Multivariate Biological Data. Leiden.

- Pojarkova, A.I. 1954. Flora of the U.S.S.R. Moscow, Vol. 20, Izdatel'stvo Akademii Nauk, SSSR.
- Rechinger, K.H. 1982. Flora Iranica Vol. 150, Lamiaceae. Graz.
- Rustaiyan, A., Khosravi, M., Larijany, K. & Masoudi, S. 2000. Composition of the essential oil of *Nepeta racemosa* Lam. from Iran. *J. Essent. Oil Res.* 12: 151–152.
- Rustaiyan, A. & Nadjji, K. 1999. Composition of the essential oils of *Nepeta isphanica* Boiss. and *Nepeta binaludensis* Jamzad from Iran. *Flavour Fragr. J.* 14: 35–37.
- Rustaiyan, A., Masoudi, S., Vahedi, M. & Fathollahi, R. 2013. Composition of the essential oils of four *Nepeta* species from Iran. *J. Essent. Oil Res.* 16(6): 699–704.
- Sadeghi, H., Robati, Z., and Saharkhiz, M.J. (2015). Variability in *Zataria multiflora* Bioss. Essential oil of twelve populations from Fars province. Iran. *Ind. Crops Prod.* 67: 221–226.
- Saharkhiz, M.J. & Mohammadi, S.H. 2011. Changes in essential oil content and composition of catnip (*Nepeta cataria* L.) during different developmental stages. *J. Essent Oil. Bear Pl.* 14 (4): 396–400.
- Sajjadi, S.E. 2005. Analysis of the essential oil of *Nepeta sintenisii* Bornm. from Iran. *DARU* (2): 61–64.
- Sajjadi, S.E. & Ghassemi N. 1999. Volatile constituents of *Nepeta glomerulosa* Boiss. subsp. *carmanica*. *Flavour Fragr. J.* 14: 265–267.
- Sefidkon, F. & Akbari-Nia, A. 2003. Essential oil composition of *Nepeta pogonosperma* Jamzad et Assadi from Iran. *J. Essent. Oil Res.* 15(5): 327–328.
- Sefidkon F. & Jamzad, Z. 2007. Essential oil composition of four Iranian *Nepeta* species (*N. cephalotes*, *N. bornmuelleri*, *N. mirzayanii* and *N. bracteata*). *J. Essent. Oil Res.* 19, 262–265.
- Sefidkon, F. & Shaabani, A. 2004. Essential oil composition of *Nepeta meyeri* Benth. from Iran. *Flavour Fragr. J.* 19(3): 236–238.
- Setzer, W.N. 2016. Catnip essential oil: There is more to it than making your cat go crazy. *Am. J. Essent. Oil. Nat. Prod.* 4(4): 12–15.
- Shamloo, M., Babawale, E.A., Furtado, A., Henry R.J., Eck P.K. & Jones P.J.H. 2017. Effects of genotype and temperature on accumulation of plant secondary metabolites in Canadian and Australian wheat grown under controlled environments. *Sci. Rep.* 7: 9133. doi:10.1038/s41598-017-09681-5.
- Sharma, A. & Cannoo, D.S. 2013. Phytochemical composition of essential oils isolated from different species of genus *Nepeta* of Labiatae family: A review. *Pharmacophore* 4 (6): 181–211.
- Sheidai, M., Ziaee, S., Farahani, F., Talebi, S.M., Noormohammadi, Z. & Hasheminejad-Ahangarani- Farahani, Y. 2014. Infra-specific genetic and morphological diversity in *Linum album* (Linaceae). *Biologia* 69: 32–39.
- Singh, R. 2016. Chemotaxonomy: a tool for plant classification. *J. Med. Plants. Stud.* 4(2): 90–93.
- Singh, R. & Geetanjali, B. 2018. Chemotaxonomy of medicinal plants: possibilities and limitations, In: Banerjee, S. et al. (Eds.). *Natural Products and Drug Discovery. An Integrated Approach*. Pp. 119-136. Elsevier, Amsterdam.
- Talebi S.M., Ghorbani Nohooji M., Yarmohammadi M., Khani M. & Matsyura A. 2019. Effect of altitude on essential oil composition and on glandular trichome density in three *Nepeta* species (*N. sessilifolia*, *N. heliotropifolia* and *N. fissa*). *Mediterr. Bot.* 40(1): 81–93.
- Talebi, S.M., Rashnou-Taei, M., Sheidai, M. & Noormohammadi, Z. 2015. Use of anatomical characteristics for taxonomical study of some Iranian *Linum* taxa. *Environ. Exp. Biol.* 13: 123–131.
- Tisserand, R. & Young, R. 2014. *A Guide for Health Care Professionals*. Churchill, Livingstone.
- Tribble, C. 2018. Distance and parsimony inference using PAUP; UPGMA, neighbor-joining, bootstrap, and jackknife. Univ. California, Berkeley.
- Tounekti, T., Vadel, A.M., Ennajeh, M., Khemira, H. & Munné-Bosch, S. 2011. Ionic interactions and salinity affect monoterpene and phenolic diterpene composition in rosemary (*Rosmarinus officinalis*). *J. Plant Nutr. Soil Sci.* 174: 504–514.
- Zohary, M. 1973. *Geobotanical foundation of the Middle East*. Fisher Verlag, Stuttgart.