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Essential Oils of *Nepeta kurdica* and *Nepeta Chionophila* from Iran as two Sources of Nepetalactone Isomers

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ABSTRACT

This research aimed to identify the volatile compounds of essential oils found in the aerial parts of two *Nepeta species (N. kurdica* Hausskn. & Bornm, and N. *chionophila* Boiss. & Hausskn.). This is the first phytochemical investigation of these two *Nepeta* species. The essential oils were obtained by hydro-distillation and analyzed by a combination of capillary gas chromatography (GC-FID) and gas chromatography-mass spectrometry (GC/MS). Nineteen compounds accounting for 90.8% of *N. kurdica* oil were identified, while there were fifteen volatile components corresponding to 95.1% in *N. chionophila* oil. Nepetalactone isomers make up 64.2% of *N. kurdica* and 90.5% of *N. chionophila* oils. The main component of *N. kurdica* oil was 4aα-7α-7aα-nepeta lactone (57.7%), while 4aα-7α-7aβ-nepeta lactone (71.8%) and 4aα-7α-7aα-nepeta lactone (15.9%) were the major constituents of *N. chionophila* oil. So the essential oils of *N. chionophila* (endemic) and *N. kurdica* (native) can be used the same as catnip (*N. cataria*) oil that is used for antimicrobial, repellent and insecticide effect, as a fortifier, a disinfectant and a cure against cold.

Keyword: *Nepeta kurdica*, *Nepeta chionophila*, Essential oil, Nepetalactone

INTRODUCTION

The genus Nepeta L. (Lamiaceae) with about 300 species in the Near East, and central and southern Europe and Asia [1], has 67 species in Iran (more than 50% endemics) with the common Persian name of ‘Pune-sa’ [2].

The aerial parts of some Nepeta species as well as their herbal tea are used as medicinal herbs in Iran, traditionally, for example *N. ispahanica*, *N. binaloudensis*, *N. bracteata* and *N. pogonosperma* [3]. The extracts of some species are also used because of their diuretic property and slight bacteriostatic activity, and in ointments to heal skin disorders of eczema type [4]. Some Nepeta species are used in folk medicine as diuretic, diaphoretic, antitussive, antispasmodic, antiasthmatic, febrifuge, emmenagogue and sedative agents [5].

The most intensively studied and well known species is *N. cataria* that is found in the Eastern Mediterranean, Southern Asia, Iran and China, and is commonly known as ‘Catnip’ because of its irresistible action on cats [5]. The essential oil of *N. cataria* is a rich source of nepetalactone isomers. It is estimated that other Nepeta species with the same essential oil composition (containing high amounts of nepetalactone isomers) can have similar medicinal properties with *N. cataria* that is used as a fortifier, a disinfectant and a cure against cold [4]. Antimicrobial, Repellent and insecticide, Larvicidal, Immunomodulatory, Anticonvulsant and myorelaxant, Analgesic and anti-inflammatory, effects on sexual behavior and other medicinal effects were reported for *N. cataria* and other Nepeta species containing nepetalactone isomers in their essential oils [6-8].

So we were interested to study the essential oil content and composition of Iranian Nepeta species.

Previous studies showed some Iranian *Nepeta* species contain nepetalactones as the main components of their essential oils, such as *N. eremophila* [9], *N. pogonosperma* [10], *N. meyeri* [11], *N. crassifolia* [12-13], *N. racemosa* [14] and *N. persica* [4].

The major components of *N. pogonosperma* were 4aα-7α-7aβ-nepetalactone (57.6%) and 1,8-cineole (26.4%). The main constituents of the essential oil of *N. meyeri* were 4aα-7α-7aβ-nepetalactone (53.2%) and 1,8-cineole (29.3%). The main constituent of *N. crassifolia* oil was 4aα-7α-7aα-nepetalactone (92.6%). The main components of *N. racemosa* oil were 4aβ-7α-7aβ-nepetalactone (33.6%), 4aα-7α-7aβ-nepetalactone (25.6%) and 4aα-7α-7a α –nepetalactone (24.4%). Nepetalactone (up to 80%) was also the main compound in the oil of

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N. kotschy var. *persica* [15]. Of course there is no nepetalactone isomer in the oils of some other Nepeta species such as *N. rivularis* [9], *N. macrosiphon* [16], *N. fissa* [17], *N. satureioides* [18], *N. prostrata* [19], *N. ucrainica* [20] and *N. glomerulosa* [21].

In this research we studied the essential oil content and composition of two native *Nepeta* species, named *N. kurdica* Hausskn. & Bornm, and *N. chionophila* Boiss. & Hausskn. from Iran for the first time.

Nepeta kurdica that is synonym with *N. wettsteinii* H. Braun [2] is an annual plant that grows in mountainous areas, steep rocky slopes in Irano-Turanian region of Iran. It is distributed in Azarbaijan and Kurdestan provinces [22]. *Nepeta chionophila* is an endemic herbaceous perennial plant that grows in mountainous areas, in snow patch vegetation at an altitude of 2700-3200m in Irano-Turanian region of Iran. From protection position point of view, both of these species are vulnerable [22].

Experimental

Plant Material

The aerial parts of *N. kurdica* and *N. chionophila* were collected at full flowering stage, from East Azarbaijan province (Tabriz to Urmia, Islamic Island, altitude 1301m) and Chaharmahal and Bakhtiari province (Zard kuh, altitude 2710m), respectively. The voucher specimens have been deposited in the national herbarium of Iran (TARI) with herbarium number of 431 and 96910, respectively. Images of collected plants can be seen in Figures 1 and 2.

Fig. 1 Image of *Nepeta kurdica* Hausskn. & Bornm

Fig. 2 Image of *Nepeta chionophila* Boiss. & Hausskn.

Isolation Produce

Air-dried aerial parts of the plants (60-80g) were subjected to hydro-distillation for 3h using a Clevenger-type apparatus. The oils separated from water and dried over anhydrous sodium sulfate and stored in sealed vials at low temperature before analysis.

GC and GC/MS Analysis

GC analyses were performed using a Shimadzu GC-9A gas chromatograph equipped with a DB-5 fused silica column (30 m x 0.25 mm i.d., film thickness 0.25 μm). Oven temperature was held at 50°C for 5 min. and then programmed to 240°C at a rate of 3°C/min. Detector (FID) temperature was 265°C and injector temperature was 250°C. Helium was used as carrier gas with a linear velocity of 32 cm/s.

Gas chromatography–mass spectrometry (GC/MS) analysis was conducted with a Varian 3400 GC connected to a mass spectrometer Saturn model, equipped with a DB-5-fused silica capillary column (30 m length, 0.25 mm internal diameter and 0.25 μm film thickness); the injection chamber temperature and the transfer line were set at 260 and 270°C, respectively. The carrier gas was helium with a linear velocity of 31.5 cm/s, split ratio of 1 to 60, 1 s of scan time, and mass range of 40–300 a.m.u. The oven temperature adjustment program was the same as the one mentioned for GC.

The percentages of compounds were calculated by the area normalization method, without considering response factors. The retention indices were calculated for all volatile constituents using a homologous series of n-alkanes. The components of the oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and confirmed by comparison of their retention indices either with those of authentic compounds or with data published in the literature [23-25].

RESULTS AND DISCUSSION

Plant Materials and Essential Oil Extraction and Analysis

The aerial parts of two Iranian *Nepeta species* named *N. kurdica* Hausskn. & Bornm, and *N. chionophila* Boiss. & Hausskn. were collected from their natural habitats (Azarbaijan and Chaharmahal and Bakhtiari Provinces, respectively) and their essential oils were obtained by hydro-distillation. Essential oil yields were calculated based on dry weights of plant materials. The oils were analyzed by a combination of capillary gas chromatography, using flame ionization detector (GC-FID) and gas chromatography-mass spectromety (GC/MS).

Essential Oil Content and Composition

The essential oil yields of *N. kurdica* and *N. chionophila* were 0.18% and 0.68% (w/w based on dried plants), respectively.

Table 1 Essential oil composition of *Nepeta kurdica* and *Nepeta chinophyla*

Compound	RI (cal.)	RI (Lit.)	*Nepeta kurdica* (%)	*Nepeta chinophyla* (%)
1,8-cineole	1032	1026	-	0.4
(Z)-β-ocimene	1040	1032	-	0.1
(E)-β-ocimene	1052	1044	-	0.2
γ-terpinene	1062	1054	-	0.1
cis sabinene hydrate	1072	1065	-	0.1
linalool	1100	1095	-	0.1
trans-sabinol	1142	1137	-	0.1
δ-terpineol	1170	1162	0.2	-
terpinen-4-ol	1180	1174	0.8	-
α-terpineol	1192	1186	0.4	-
trans-carveol	1219	1215	0.7	-
pulegone	1239	1233	2.3	-
thymol	1294	1289	2.5	-
4aα-7α-7aα-nepetalactone	1363	1357	57.7	15.9
α-copaene	1380	1374	0.7	-
4aα-7α-7aβ-nepetalactone	1390	1386	4.9	71.8
β-bourbonene	1392	1387	7.1	-

4aα-7β-7aα-nepetalactone	1395	1391	1.6	2.8
E-β-farnesene	1460	1454	2.0	2.4
γ-muurolene	1483	1478	0.4	-
γ-himachalene	1486	1481	2.4	-
β-bisabolene	1510	1505	0.6	0.7
trans-calamenene	1525	1521	tr	-
δ-cadinene	1527	1522	-	0.2
elemol	1553	1548	-	0.1
1-nor-bourbonene	1567	1563	0.2	-
spathulenol	1581	1577	4.2	0.1
caryophyllene oxide	1586	1582	2.1	-
total			90.8	95.1

RI = Retention indices in elution order from DB-1 column
RI (cal.) = Calculated retention indices on the DB-5 column by co-injection of a homologous series of n-alkanes C_8–C_{24}
RI (Lit.) = Literature retention indices (23-25)

tr = less than 0.05%

Nineteen components were identified in the oil of *N. kurdica* (representing 90.8% of the oil) with 4aα-7α-7aα-nepeta lactone (57.7%), β-bourbonene (7.1%), 4aα-7α-7aβ-nepetalactone (4.9%) and spathulenol (4.2%) as main constituents.

Fifteen compounds were identified in the oil of *N. chionophila* (representing 95.1% of the oil) with 4aα-7α-7aβ-nepetalactone (71.8%) and 4aα-7α-7aα-nepetalactone (15.9%) as major components. Another nepetalactone isomer, 4aα-7β-7aα-nepeta lactone, was found at 1.6% and 2.8% in the oils of *N. kurdica* and *N. chionophila,* respectively.

Chemical composition of the oils can be seen in Table 1. The components are listed in order of their elution on the DB-5 column.

The results showed the oils of *N. kurdica* and *N. chionophila* contained high amount of nepetalactone isomers, but in different percentage of each isomer.

The composition of *Nepeta* phytochemicals as well essential oil can define the medicinal value of it. Nepetalactone isomers as mentioned are group of the important essential oil compounds in *Nepeta* species to which most of the biological properties of the genus attributed.

Catnip (*N. cataria*) and some other *Nepeta* species produced nepetalactone isomers. Nepetalactones are iridoid monoterpenes with a broad range of biological activities that act as defensive compounds in some flowering plants. Nepetalactones protect these plants from herbivorous insects by functioning as insect repellents. They are also produced by many aphids, in which they are sex pheromones [26].

As observed in the present research, 4a*α*-7*α*-7a*α*-nepetalactone (57.7%) in *N. kurdica* oil, was higher than *N. chionophila* (15.9%), but 4aα-7α-7aβ-nepetalactone (4.9%) was lower than *N. chionophila* oil (71.8%). Totally, 64.2% of *N. kurdica* essential oil and 90.5% of *N. chionophila* essential oil were nepetalactone isomers.

Also nepetalactone isomers were reported in the essential oils of *N. eremophila* [9], *N. pogonosperma* [10], *N. meyeri* [11], *N. crassifolia* [12-13], *N. racemosa* [14] and *N. persica* [4], but they were not found in *N. schiraziana* [39], *N. glomerulosa* subsp. *carmanica* [32], *N. depauperata*, *N. oxyodonta* [31], *N. ucrainica* ssp. *kopetdaghensis* [35], *N. pungens* [40], *N. macrosiphon* [33], *N. involucrate* [36], *N. bracteata* [37], and *N. crispa* [38].

Previous research on repellent activity of the *N. cataria* oil and the main iridoid compounds (nepetalactone isomers), against the malaria mosquito, the brown ear tick, *Rhipicephalus appendiculatus*, and the red poultry mite, *Dermanyssus gallinae* showed the nepetalactone isomers have the potential to be used in human and livestock protection against major pathogen vectors [27].

There are many other references about medicinal properties of nepetalactone isomers [6-8, 28-31]. So it can be suggested that the essential oils of *N. kurdica* and *N. chionophila* as two Iranian Nepeta species have the same properties and can be used for those purposes.

CONCLUSION

In this study, for the first time, the essential oil content and composition of two Iranian *Nepeta* species were investigated and compared. Ninteen and fifteen compounds were identified in the oils of *Nepeta kurdica* Hausskn. & Bornm, and *N. chionophila* Boiss. & Hausskn. that approximately constitute 90.8% and 95.1% of the oils, respectively.

Nepetalactone isomers, as the main components of some *Nepeta* species oils, were found in both studied essential oils in high amount, but in different percentage. 4aα-7α-7aβ-nepetalactone that found in the essential oil of *N. chionophila* at 71.8%, was observed less than 5% in the oil of *N. kurdica*. Another nepetalactone isomer, 4aα-7α-7aα-nepetalactone was found at 57.7% in the oil of *N. kurdica*, but it was observed at 15.9% in the oil of *N. chionophila*. Totally, more than 90% of the essential oil of *N. chionophila* consisted of three nepetalactone isomers that is remarkable amount. So the essential oils of *N. chionophila* (endemic) and *N. kurdica* (native) can be used the same as catnip (*N. cataria*) oil that is used for antimicrobial, repellent and insecticide effect, as a fortifier, a disinfectant and a cure against cold.

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