Chemical composition and antibacterial activity of the essential oil the leaf of *Nepeta persica*

Soraya AKHSHI, Ali SHAFAGHAT and Jaber SALEHZADEH*

Department of Chemistry, Islamic Azad University, Ardabil Branch, Ardabil, Iran
E-mails: chem.salehzadeh@yahoo.com; soraya.akhshi@yahoo.com; ali.shafaghat@gmail.com

* Corresponding author, Phone: +98 9144524649; Fax: +98 4512239088

Abstract

The essential oil from the leaf of *Nepeta persica* Boiss, analyzed by gas chromatography (GC) and gas chromatography (GC)/mass spectrometry (MS), were shown to contain 4aα, 7α, 7aβ-nepetalactone (49.46%) and 4aα, 7α, 7aα-nepetalactone (14.18%). The other main constituents were n-octane (13.10%), n-decane (3.67%) and germacrene-D (2.04%). Antibacterial activities of the leaf oil were evaluated using the micro-dilution broth method. Inhibitory effects on *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Salmonella typhi* and *Enterococcus faecalis* were recorded. The leaf oil has difference activities against the test microorganisms. The antibacterial property of the essential oil might be ascribed to their high content of nepetalactone isomers.

Keywords

Nepeta Persica; Lamiaceae; Essential oil composition; Nepetalactone; antibacterial activity

Introduction

Several *Nepeta* species (Lamiaceae/Labiatae) are reported to contain nepetalactone in their essential oils. The genus *Nepeta,* with almost 280 species, is widespread in Europe, Asia and a few parts of Africa; 67 species of this genus are found in Iran [1], one of which, *N. persica* Boiss, is endemic [2]. *Nepeta* species are used in folk medicine [3,4] for the treatment of various disorders, such as nervous, respiratory and gastrointestinal diseases [5].

Nepeta species are also used in the traditional medicine of many countries as a diuretic, diaphoretic, antitussive, anti-asthmatic, vulnerary, antispasmodic, febrifuge, tonic, emmenagogue, and sedative agent [6].The bacteriostatic, fungistatic [7] and antiviral activities have been attributed to nepetalactones [8]. Antibacterial and cytotoxic activity of *N. cataria* and *N. cataria* var. *Citriodora* essential oils [9], essential oil composition of two subspecies of *N. glomerulosa* [10] and antimicrobial activity of *N. isolates* has been reported. The main component of the essential oil of *N. cephalotes* from Iran was 4aα, 7α, 7aα-nepetalactone [11], whereas 1, 8-cineol was the major component of *N. denudata*, *N. ispahanica* and *N. binaludensis* [12], terpinen-4-ol of *N. asterotrichus*, and spathulenol of *N. depauperata* [13]. The hydrodistilled oils of *N. sintenesii* from Iran were reported to have, as their major components, 4aβ, 7α, 7aβ-nepetalactone (60.3%), germacrene-D (12.7%) and 1, 8-cineol (8.2%) in the flower oil, 4aβ, 7α, 7aβ-nepetalactone (34.6%), germacrene D (14.1%), 1, 8-cineol (7.9%), α-cadinol (6.8%) and δ-cadinene (5.8%) in the leaf oil, 4aβ, 7α, 7aβ-nepetalactone (64.2%), α-cadinol (8.9%) and α-pinene (6.7%) in the stem oil, and 4aβ, 7α, 7aβ-nepetalactone (61.2%), germacrene-D (12.0%), 4aα, 7α, 7aβ-nepetalactone (8.5%), and 1, 8-cineol (5.7%) in the root oil [14]. The major compound found in the essential oils of *N. racemosa* collected from different localities in Turkey was 4aα, 7α, 7aβ-nepetalactone (31.5-91.5%) [15]. Relatively high concentrations of nepetalactones in many *Nepeta* species have been reported [16].

Material and method

Plant material

The plant material was collected on June 29, 2013 in the Khalkhal (Ardabil province) area in northwest Iran at an altitude of 1955 m. A voucher specimen (N-324) is kept at the

Herbarium of Agriculture Research in Ardabil Center, Iran.

Distillation

Plant material was air-dried in the shade prior to isolation of their oil. Leaf (120 g) was subjected to 3 h of hydro distillation in a Clevenger-type apparatus. The resulting oil (yield: 1.1%, v/w) was dried over anhydrous sodium sulfate and immediately placed into a dark glass tube and sealed. The sample was stored at 2°C until chemical analysis.

GC and GC/MS analysis

GC analysis was performed on a Shimadzu 15A Gas Chromatograph equipped with a split/split less injector (250°C) and a flame ionization detector (250°C). N_2 was used as carrier gas (1 mL/min) and the capillary column used was a DB-5 (50 m × 0.2 mm, film thickness 0.32 μm). The column temperature was kept at 60°C for 3 min and then raised to 220°C at a 5°C/min rate and kept constant at 220°C for 5 min. Alkanes (C_8—C_{22}) were used as reference points in the calculation of relative retention indices (RRI). The relative percentages of the characterized components are given in Table 1. GC/MS analysis was performed using a Hewlett Packard 5973 with an HP-5MS column (30 m × 0.25 mm, film thickness 0.25 μm). The column temperature was kept at 60°C for 3 min and programmed to 220°C at a rate of 5°C/min and kept constant at 220°C for 5 min. The flow rate of helium as carrier gas was 1 mL/min. MS were taken at 70 eV. Identification of the constituents of the oils was made by comparison of their MS and RIs with those given in the literature and with those of authentic samples [17]. Relative percentage amounts were calculated from peak area using a Shimadzu C-R4A chromatopac, without the use of correction factors.

Antibacterial screening

Screening of the essential oil for activity by the agar diffusion disc impregnated method was adopted. The oil was prepared as 50% v/w solution. A Whatman paper disc, 4mm in diameter, was impregnated and oven dried at 37°C for 1 h to remove the presence of solvent. 1×10^6 CFU/mL of the test bacteria was prepared and seeded into the solid agar medium. The impregnated paper discs were placed at intervals and incubated for 24 h at 37°C. After 24 h the plate reading was taken and the zone of inhibition was measured and recorded (Table 2). The microorganisms used were: *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Salmonella typhi* and *Enterococcus faecalis.*

Results and discussion

The compositions of the leaf oil of *N. persica* are listed in Table 1. In dried plant oil, 19 components, which represented about 93.46% of the total composition, were identified. In addition, as can be seen in Table 1, the leaf oil consisted of five sesquiterpenes (4.82%), eight monoterpene hydrocarbons (8.79%), two oxygenated nonoterpenes (63.64%) and four other components (16.21%). 4aα, 7α, 7aβ-nepetalactone (49.46%) and 4aα, 7α, 7aα-nepetalactone (14.18%), n-octane(13.10%) and n-decane (3.67%) were the major components of this oil. Oxygenated monoterpenes (total nepetalactones, 63.64%) constituted the major fraction of the oil, while monoterpene, sesquiterpene and the other hydrocarbons accounted for only 29.82%.

Table 1. Chemical composition (%) of the oil of *Nepeta persica* leaf

Compound	RI	Percentage
Octene(1-)	792	1.56
Octane(n-)	**800**	**13.10**
Citronellene	937	0.30
Decane(n-)	**1000**	**3.67**
1, 8-Cineole(Eucalyptol)	1031	0.68
Cis-β-ocimene	1037	1.06
Trans-β-ocimene	1050	1.05
γ-Terpinene	1060	0.39
α-Terpinolene	1088	0.48
Dodecane(n-)	1200	1.16
4aα, 7α, 7aα-nepetalactone	**1360**	**14.18**
4aα, 7α, 7aβ-nepetalactone	**1387**	**49.46**
β-Caryophyllene	1419	0.81
Trans-β-farnesene	1457	1.01
Germacrene-D	1485	2.04
Spathulenol	1578	0.50
α-Cedrene epoxide	1575	0.46
Methyl hexadecanoate	1922	0.34
Methyl linoleate	2095	1.21
% Identification	----	**93.46**

In a previous study, the essential oil of *N. persica* was investigated by GLC and GC-MS. Forty-one components, representing 86.4% of the oil, were characterized, the major ones being 1, 4-hexadiene-2, 3, 4, 5-tetramethyl and 4aβ, 7α, 7aα-nepetalactone [18]. The oil of the aerial parts of *N. sintenisii* contained 4aβ, 7α, 7aβ-nepetalactone (23.4%), elemol (16.1%), (E)-β-famesene (9.5%) and 1, 8-cineole (8.2%) as the major constituents among the forty characterized, comprising (96.5%) of the total components detected [19].

In the *N. sintenisii Bornm*. 4aβ, 7α, 7aβ-nepetalactone was characterized in the flower,

leaf, stem and root oils (60.3%, 34.6%, 64.2% and 61.2%, respectively) as the main constituent [11].

Essential oils from the aerial parts of *N. crispa* Willd, *N. mahanensis* Jamzad & Simmonds, *N. ispahanica* Boiss and *N. eremophila* Hausskn. & Bornm. were analyzed. 1, 8-Cineole (62.8%), 4aα, 7α, 7aα-nepetalactone (10.3%) and 4aβ, 7α, 7aβ-nepetalactone (9.2%) were reported as the main constituents of *N. crispa* oil. Eighteen compounds were identified in the oil of *N. mahanensis* with nepetalactone (37.6%), 1, 8-cineole (27.2%) and germacrene-D (6.5%) as the main components. Twenty-seven compounds were characterized in the oil of *N. ispahanica* with 1, 8-cineole (71.7%) as the main constituent. Twenty-six compounds were recorded for the oil of *N. eremophila* with 4aβ, 7α, 7aβ-nepetalactone (73.3%) and 1, 8-cineole (13.1%) as the main constituents. The results of this study showed that, although the nepetalactone isomers are the main components of the essential oils of *N. sintenisii*, *N. mahanensis* and *N. eremophila*, the oil of *N. crispa* consists of about 20% nepetalactone and the oils of other species have different compositions [20].

Nepetalactone isomers also present in the leaf oil (63.64%) were constituted. Our survey showed that *Nepeta* species can be divided into two groups of nepetalactone-containing and nepetalactone-free species. The essential oils of *N. racemosa* collected from different localities in Turkey had as their major compound 4aα, 7α, 7aβ-nepetalactone (31.5-91.5%) [21]. In the Iranian *N. raceme*, 4aα, 7α, 7aα-nepetalactone (25.6%) and 4aβ, 7α, 7aβ-nepetalactone (33.6%) were reported [22]. Also, nepetalactone isomers have been recorded for *N. cratssifolia* [23], *N. nuda* ssp. *albiflora* [24], *N. italica* [25], *N. cadmea* [26] and *N. persica* [18]. However, in the oils of *N. depauperata* [27], *N. cilicia* [28], *N. nuda* ssp. *nuda* [29], *N. glomerulosa* ssp. *carmanica* [30] and *N. macrostphone* [31], no nepetalactor es were found. Spathulenol (31.8%), β-caryophyllene (12.9%) and caryophyllene oxide (10.3%) were the major components of the oil of *N. depauperata* Benth [27].

Results obtained in the antibacterial study of the essential oil from leaf of *N. persica* are shown in Table 2. With the agar disc diffusion assay the oil of leaf was found to be active against all the test microorganisms. Comparison of the composition of *N. persica* leaf oil revealed some differences, especially in the major components. It is conceivable that the antibacterial property of the essential oil might be ascribed to the high content of nepetalactone isomers in the oil.

Table 2. Antibacterial activity of the essential oil from leaf of *Nepeta persica*

Tested bacteria	Zone of inhibition (mm)
Escherichia colt	11
Pseudomonas aeruginosa	9
Staphylococcus aureus	12
Salmonella typhi	10
Entemcoccus faecalis	12

The most sensitive microorganisms were *Staphylococcus aureus* and *Entemcoccus faecalis*, with inhibition zones of 12 mm.

Conclusion

The finding of the present study and previously published one suggest that two chemotypes of oil: neptalactone and n-octane may be present in *Nepeta persica*.

References

1. Mozaffarian V., *A dichoticy of Iranian plant names,* Farhang Moaser Publishers, Tehran, Iran, 1996, p. 360-364.
2. Rechinger K. H., *Flora Iran,* Akademische Druck and Verlagsanstalt, Graz, Austria, 1982, p. 150-158.
3. Poote H. L., Nicolai B. M., Laet L., Buyck L. F., Schamp N., Getghebeur R., *The essential oils of five Nepeta species*, *A preliminary evaluation of their use in chemotaxonomy by cluster analysis*, Flavour and Fragrance Journal, 1988, 3, p. 155-159.
4. Zargari A., *Medicinal plant,* Tehran University Publications, Tehran, Iran, 1990, 4, p. 106-112.
5. Amin G. R., *Popular Medicinal plants of Iran,* Ministry of Health Publications, Tehran, 1991, 1, p. 40-41.
6. McGuffin I. L., Hobbs C., Upton R., Goldberg A., *Botanical safety handbook,* (Edits), CRC Press: New York, 1997, p. 79-81.
7. Bourrel C., Perineau F., Michel G., Bessiere L. M., Catnip *(Nepeta cataria* L.) *essential*

oil analysis of chemical constituents, bacteriostatic and fungistatic prope-ties, Journal of Essential Oil Research, 5, 1993, p. 159-167.

8. Sattar A., Bankova V., Kujumgiev A., Galabov A., Ignatova A., Todorova C., Popov S., *Chemical composition and biological activity of leaf exudates from some Lamiaceae plants*, Pharmazie, 1995, 50, p. 62-65.
9. Javidnia K., Miri R., Rezazadeh S. R., Soltani M., Khosravi A. R., *Essential oil* composition *of two subspecies of Nepeta glomerulosa Boiss from Iran*, Natural Product Communications, 2008, 3, p. 833-836.
10. Mathela C. S., Joshi N., *Antimicrobial activity of Nepeta isolates*, Natural Product Communications, 2008, 3, p. 945-950.
11. Rustaiyan A., Komeilizadeh H., Monfared, A., Nadji K., Masoudi S., Yari M., *Volatile* constituents *of Nepeta denudata Benth and N. cephalotes Boiss, from Iran,* Journal of Essential Oil Research, 2000, 11, p. 459-461.
12. Rustaiyan A., Nadji K., *Composition of the essential oils of Nepeta ispahanica Boiss and* Nepeta *binaludensis Jamzad from Iran,* Flavour and Fragrance Journal, 1999, 71, 14, p. 35-37.
13. Rustaiyan A., Monfared A., Masoudi S., *Composition of the essential oil of Nepeta asterotrichus Rech. F et Aell from Iran*, Journal of Essential Oil Research, 1999, 1, p. 229-230.
14. Shafaghat A., Salimi F., Akhlaghi H., *Chemical composition of the essential oil isolated from flower, leaf, stem and root of Nepeta sintenisii Bornm from Iran Journal of* Essential Oil Bearing Plants, 2008, 11 , p. 391-396.
15. Baser K. H. C., Ozek T., Akgiil A., Tumen G., *Composition of the essential oil of Nepeta racemosa Lam,* Journal of Essential Oil Research, 1993, 5, p. 215-217.
16. Sefidkon F., Shaabani A., *Essential oil composition of Nepeta meyeri Benth from Iran*, Flavour and Fragrance Journal, 2004, 19, p. 236-238.
17. Suschke U., Sporer F., Schneele J., Geiss H. K., Reichling Journal, *Antibacterial and cytotoxic activity of Nepeta cataria L., N. cataria var. citriodora (Beck) Ball and Melissa officinalis L. essential oils,* Natural Product Communications, 2007, 2, p. 1277-1286.
18. Javidnia K., Miri R., Safavi F., Azarpira A., Shafiee A., *Composition of the essential oil of Nepeta persica Boiss from Iran*, Flavour and Fragrance Journal, 2001, 1, p. 20-22.
19. Sajjadi S. E., *Analysis of the essential oil of Nepeta sintenisii Bortun from Iran*, Daru,

2005, 13, p. 61-64.

20. Sefidkon F., Jamzad Z., Mirza M., *Chemical composition of the essential oil of five* Iranian *Nepeta species (N. crispa, N. mahanensis, N. ispahanica, N. eremophila and N. rivularis)*, Flavour and Fragrance Journal, 2006, 21, p. 764-767.
21. Dabiri M., Sefidkon F., *Chemical composition of essential oil of Nepeta racemosa from Iran*, Flavour and Fragrance Journal, 2003, 18, p. 157-158.
22. Dabiri M., Sefidkon F., *Chemical composition of Nepeta crassifolia Boiss. & Buhse. oil from Iran*, Flavour and Fragrance Journal, 2003, 18, p. 225-227.
23. Kokdil G., Kurucu S., Topcu G., *Composition of the essential oil of Nepeta nuda L. ssp. albiflora (Bolas.) Gams*, Flavour and Fragrance Journal, 1991, 11, p. 167-169.
24. Kokdil G., Kurucu S., Topcu G., *Chemical constituents of the essential oils of Nepeta italica L. and Nepeta sulfuriflora RH. Daws*, Flavour and Fragrance Journal, 1997, 12, p. 33-35.
25. Baser K. H. C., Demircakmak B., Altinas A., Duman H., *Composition of the essential* oils *of Nepeta cadmea Boiss*, Journal of Essential Oil Research, 1998, 10, p. 327-328.
26. Mehrabani M., Asadipour A., Saber Amoli S., *Chemical constituents of the essential oil of Nepeta depauperata Benth, from Iran*, Daru, 2004, 12, p. 98-100.
27. Kokdil G., Tanker M., Kurucu S., Topcu G., *Essential oil analysis of Nepeta cilicia Boiss*, Flavour and Fragrance Journal, 1997, 12, p. 99-101.
28. Kokdil G., Kurucu S., Yildiz A., E*ssential oil composition of Nepeta nuda L ssp. nuda,* Flavour and Fragrance Journal, 1998, 13, p. 233-234.
29. Sajjadi S. E., Ghassemi N., *Volatile constituents of Nepeta glomerulosa Boiss. subsp. carmanica,* Flavour and Fragrance Journal, 1999, 14, p. 265-267.
30. Javidnia K., Miri R., Jafari A., Rezai I., *Analysis of the volatile oil of Nepeta macrosiphone Boiss grown in Iran,* Flavour and Fragrance Journal, 2004, 19, p. 156-158.
31. Adams R. P., *Identification of essential oil components by gas chromatography/mass spectroscopy,* Allured Publishing Corp., Carol Stream, IL. USA, 1995, p. 14-698.