Antimicrobial Activity and Chemical Composition of the Essential Oil of *Nepeta crispa* Willd. from Iran

Ali Sonboli[a,*], Peyman Salehi[b], and Morteza Yousefzadi[c]

[a] Department of Biology, Medicinal Plants Research Institute, Shahid Beheshti University, Evin, P. O. Box 19835-389, Tehran, Iran. Fax: (+9821)24186779. E-mail: a-sonboli@sbu.ac.ir
[b] Department of Phytochemistry, Medicinal Plants Research Institute, Shahid Beheshti University, Evin, Tehran, Iran
[c] Department of Biology, Faculty of Sciences, Shahid Beheshti University, Evin, Tehran, Iran

* Author for correspondence and reprint requests

Z. Naturforsch. **59 c**, 653–656 (2004); received May 28, 2004

The composition and antimicrobial activity of the essential oil of *Nepeta crispa* Willd., an endemic species from Iran, was studied. The oil was obtained from the aerial parts of the plant and analyzed by GC and GC/MS. Twenty-three compounds, accounting for 99.8% of the total oil, were identified. The main constituents were 1,8-cineol (47.9%) and 4aα,7α,7aβ-nepetalactone (20.3%). The antimicrobial activity of essential oil of *N. crispa* was tested against seven gram-negative or gram-positive bacteria and four fungi. The results of the bioassays showed the interesting antimicrobial activity, in which the gram-positive bacteria, *Bacillus subtilis* and *Staphylococcus aureus*, were the most sensitive to the oil. Also, the oil exhibited a remarkable antifungal activity against all the tested fungi.

Key words: Nepeta, Essential Oil, Antimicrobial Activity

Introduction

The genus *Nepeta* belongs to the family Lamiaceae, which comprises 69 species to date growing in Iran, 38 and 31 of which are endemic and native, respectively (Rechinger, 1987; Jamzad, 1990, 1991, 1992; Delghandi, 1993; Jamzad *et al.*, 2003b). *Nepeta crispa* Willd. is one of the most aromatic endemic plants of Iran. This plant with the common local name *Mofarrah* (because of its sweet odor) has been of great interest to Iranian traditional medicine, especially in Hamadan province (Mozaffarian, 1996). Infusion and beverage obtained from the aerial parts of *N. crispa* were used traditionally as sedative, relaxant, carminative, restorative tonic for nervous and respiratory disorders.

The identification of leaf surface flavonoids of *N. crispa* was recently reported (Jamzad *et al.*, 2003a). The literature survey revealed that the essential oil composition of different *Nepeta* species from Iran have been reported earlier (Rustaiyan *et al.*, 1999; Rustaiyan and Nadji, 1999; Sajjadi and Ghassemi, 1999; Rustaiyan *et al.*, 2000a, b; Sajjadi and Khatamsaz, 2001; Sefidkon, 2001; Sefidkon *et al.*, 2002; Sefidkon and Akbari-nia, 2003; Dabiri and Sefidkon, 2003a, 2003b; Sefidkon and Shaabani 2004), but the essential oil composition and antimicrobial activity of *N. crispa* have not been investigated to date, and hence we focused our attention on the study of chemical composition and antimicrobial activity of *N. crispa* for possible uses of its oil in aromatherapy, pharmacy and foods.

Material and Methods

Plant material

The aerial parts of *N. crispa* were collected from Alvand mountain (Hamadan, Iran) in July 2003 at flowering stage. A voucher specimen was deposited in the Medicinal Plants Research Institute Herbarium (MPRIH), Shahid Beheshti University,Tehran, Iran.

Isolation procedure

Air-dried aerial parts (75 g) were subjected to hydrodistillation for 3 h, using a Clevenger-type apparatus. The oil was dried over anhydrous sodium sulphate and stored in a sealed vial. The yield of the oil was found to be 1.02% (w/w) and it was stored at 4 °C until analysis.

0939–5075/2004/0900–0653 $ 06.00

Gas chromatography (GC)

GC analysis of the essential oil was performed using a Varian CP-3800 instrument equipped with a DB-1 capillary fused silica column (25 m × 0.25 mm i.d., film thickness 0.25 μ). Nitrogen was used as the carrier gas at the constant flow of 1.1 ml/min. The oven temperature was held at 60 °C for 1 min, then programmed to 250 °C at a rate of 4 °C/min, then held for 10 min. The injector and detector (FID) temperatures were kept at 250 °C and 280 °C, respectively.

Gas chromatography/mass spectrometry (GC/MS)

GC/MS analysis was performed on a Thermoquest-Finnigan Trace GC-MS equipped with a DB-1 fused silica column (60 m × 0.25 mm i.d., film thickness 0.25 μ). The oven temperature was raised from 60 °C to 250 °C at a rate of 5 °C/min, then held at 250 °C for 10 min; transfer line temperature was 250 °C. Helium was used as the carrier gas at a flow rate of 1.1 ml/min; split ratio was 1/50. The quadrupole mass spectrometer was scanned over the 45–465 amu with an ionizing voltage of 70 eV and an ionization current of 150 μA.

Identification of components

The constituents of the obtained oil were identified by calculation of retention indices under temperature programmed conditions for *n*-alkanes (C_6–C_{24}) and the essential oil on a DB-1 column under the same chromatographic conditions and by comparison of their mass spectra with authentic compounds or with those of the internal reference mass spectra library, and finally confirmed by comparison of their retention indices and mass spectra with data published in the literature (Shibamoto, 1987).

Preparation of oil dilutions

The solvent showing no antimicrobial activity, *i.e.* methanol, was selected as a diluting medium for the oil. Undiluted oil was taken as dilution 1 and 1/2, 1/4, 1/8, and 1/16 dilutions of the oil were made with methanol. For antibacterial activity 15 μl and for antifungal property 30 μl of each dilution was used.

Antimicrobial activity

The antibacterial activity of the essential oil was evaluated by disc diffusion method using Mueller-Hinton agar (Baron and Finegold, 1995) and determination of inhibition zones at different oil dilutions. The microorganisms used were as follows: *Bacillus subtilis* ATCC 9372, *Enterococcus faecalis* ATCC 15753, *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 12228, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27852, *Klebsiella pneumoniae* ATCC 3583, *Candida albicans* ATCC 5027, *Saccharomyces cerevisiae* ATCC 9763, *Microsporium gypsium* ATCC 5070 and *Aspergillus niger* ATCC 16404.

The antifungal property of the oil was tested by agar-well diffusion method using Sabouraud Dextrose Agar. Standard reference antibiotics were used in order to control the sensitivity of the tested bacteria (ampicillin and tetracycline) and fungi (nystatine). The incubation conditions used were 24 h at 37 °C for bacteria and 48–72 h at 24 °C for fungi. All the experiments were carried out in triplicate and averages were calculated for the inhibition zone diameters.

Results and Discussion

Essential oil analysis

The hydrodistillation of aerial parts of *N. crispa* gave a yellow oil in 1.02% (w/w) yield, based on the dry weight of the plant. Twenty-three components were identified representing 99.8% of the total oil. The qualitative and quantitative essential oil compositions are presented in Table I, where compounds are listed in order of their elution on the DB-1 column. The major constituents of the oil were 1,8-cineol (47.9%) and 4aα,7α,7aβ-nepetalactone (20.3%) followed by β-pinene (6.9%), α-terpineol (4.8%), 4-terpineol (2.8%), α-pinene (2.5%), δ-terpineol (2.1%) and 4aβ,7α,7aβ-nepetalactone (1.9%). The sesquiterpene fraction comprised 2.2% of the total oil.

1,8-Cineol has been reported as the major compound of the following species: *N. ispahanica* and *N. binaludensis* (65.2% and 42.3%, respectively; Rustaiyan and Nadji, 1999); *N. denudata* (48%; Rustaiyan *et al.*, 2000a); *N. meyeri* (29.3%; Sefidkon and Shaabani, 2004) and *N. heliotropifolia* (19%; Sajjadi and Khatamsaz, 2001). 4aα,7α,7aβ-Nepetalactone has been reported as the main constituent of *N. meyeri* (53.2%; Sefidkon and Shaabani, 2004) and *N. pogonosperma* (57.6%; Sefidkon and Akbari-nia, 2003).

Table I. Essential oil composition of *Nepeta crispa*.

Compound[a]	RI	Percentage
α-Thujene	0926	0.4
α-Pinene	0936	2.5
Sabinene	0970	1.3
β-Pinene	0977	6.9
Myrcene	0982	0.9
Dehydro-1,8-cineol	0989	0.4
α-Terpinene	1013	0.6
p-Cymene	1016	0.2
1,8-Cineol	1028	47.9
γ-Terpinene	1053	0.9
trans-Sabinene hydrate	1059	1.2
Terpinolene	1084	0.1
cis-Sabinene hydrate	1086	1.9
Linalool	1091	0.4
δ-Terpineol	1155	2.1
4-Terpineol	1169	2.8
α-Terpineol	1180	4.8
4aα,7α,7aα-Nepetalactone	1332	0.1
4aα,7α,7aβ-Nepetalactone	1342	20.3
4aβ,7α,7aβ-Nepetalactone	1372	1.9
β-Caryophyllene	1430	0.4
(*Z*)-β-Farnesene	1449	0.6
Germacrene-B	1497	1.2
Monoterpene hydrocarbons		13.7
Oxygenated monoterpens		83.9
Sesquiterpene hydrocarbons		2.2
Total		99.8

[a] Compounds listed in order of their elution from a DB-1 column.

Antimicrobial activity

The essential oil was tested against seven gram-positive and gram-negative bacteria and four fungi. The results of the bioassays (Table II) showed that the oil exhibited moderate to strong antibacterial activity against all the tested bacteria and strong activity against the fungi. The 1 (absolute essential oil) and ½ oil dilutions showed inhibitory activity against all the tested bacteria, especially *Bacillus subtilis*, and four fungi tested. The ¼ oil dilution was active against all tested microorganisms, except two gram-negative strains, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae.* No activity was observed against two gram-negative (*Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) and a gram-positive (*Enterococcus faecalis*) bacteria at ⅛ and 1/16 oil dilutions. The present study revealed that the essential oil of *N. crispa* at different dilutions also showed a similar inhibitory type of activity to that of standard antibiotics (Table II).

Our results support the ethnopharmacological uses of this plant in folk medicine and could provide useful data for the utilization of this essential oil in pharmaceutical, cosmetic and food industries. Further studies are necessary to explore the efficacy and palatability of suitable concentrations of this essential oil in foods.

Table II. Antimicrobial activity of *N. crispa* essential oil.

Microorganism	Inhibition zone [mm][a]							
	Oil dilutions					Standard antibiotics		
	1	½	¼	⅛	1/16	Ampicillin[b]	Tetracycline[c]	Nystatine[c]
Bacillus subtilis	26.5	22*	18	14*	10.5	14	21	nt
Enterococcus faecalis	10.5*	9.5*	7	–	–	11	9	nt
Staphylococcus aureus	19.5*	16	12.5*	9	7	13	20	nt
Staphylococcus epidermidis	15	12	9.5	8	7	19	34	nt
Escherichia coli	16*	12.5*	9	8	7	12	16	nt
Klebsiella pneumoniae	10.5	7	–	–	–	–	–	nt
Pseudomonas aeruginosa	9.5*	7	–	–	–	9.7	–	nt
Candida albicans	28	23	16*	12	9	nt	nt	18
Saccharomyces cerevisiae	24	20	16*	12	–	nt	nt	18
Aspergillus niger	26	22	14	10	–	nt	nt	16
Microsporium gypsium	26	21	16*	10	–	nt	nt	17

[a] Includes diameter of disc (6 mm).
[b] Tested at 10 μg/disc.
[c] Tested at 30 μg/disc.
* A similar inhibitory type of activity of the oil to that of standard antibiotics.
(–) Inactive; (7–13) moderately active; (> 14) highly active; nt: not tested.

Acknowledgements

We are grateful to Shahid Beheshti University Research Council for financial support of this work. We are also thankful to Mr. Najafi and Mr. Kalvandi for their generous helps in collecting plant material.

Baron E.-J. and Finegold S.-M. (1995), Bailey and Scott's Diagnostic Microbiology, 8th ed. Mosby, St. Louis, MO, USA, pp. 171–193.

Baser K.-H.-C., Ozek T., Bemirci B., and Tumen G. (2001), Composition of the essential oil of *Nepeta betonicifolia* C. A. Meyer from Turkey. J. Essent. Oil Res. **13**, 35–36.

Dabiri M. and Sefidkon F. (2003a), Chemical composition of the essential oil of *Nepeta racemosa* Lam. from Iran. Flavour Fragr. J. **18**, 157–158.

Dabiri M. and Sefidkon F. (2003b), Chemical composition of *Nepeta crassifolia* Boiss. & Buhse. oil from Iran. Flavour Fragr. J. **18**, 225–227.

Delghandi M. (1993), *Nepeta leucostegia* (Labiatae), a new record for the flora of Iran. Iran J. Botany **6**, 149–151.

Jamzad Z. (1990), Two new species of *Nepeta* L. (Labiatae) from Iran. Iran J. Botany **8**, 43–48.

Jamzad Z. (1991), *Nepeta menthoids* and species allied to it in Iran. Iran J. Botany. **5**, 17–27.

Jamzad Z. (1992), Two new species from Labiatae in Iran. Iran J. Botany. **5**, 69–74.

Jamzad Z., Grayer R.-J., Kite G.-C., Simmonds M.-S.-J., Ingrouille M., and Jalili A. (2003a), Leaf surface flavonoids in Iranian species of *Nepeta* (Lamiaceae) and some related genera. Bioch. Sys. Ecol. **31**, 587–600.

Jamzad Z., Ingrouille M., and Simmonds M.-S.-J. (2003b), Three new species of *Nepeta* (Lamiaceae) from Iran. Taxon **52**, 93–98.

Mozaffarian V. (1996), A Dictionary of Iranian Plant Names. Farhang Moaser, Tehran, Iran.

Rechinger K.-H. (1987), *Nepeta.* In: Flora Iranica, No. 150. Graz, Austria.

Rustaiyan A. and Nadji K. (1999), Composition of the essential oils of *Nepeta ispahanica* Boiss., and *Nepeta binaludensis* Jamzad from Iran. Flavour Fragr. J. **14**, 35–37.

Rustaiyan A., Monfared A., and Masoudi Sh. (1999), Composition of the essential oil of *Nepeta asterotrichus* Rech. f. from Iran. J. Essent. Oil Res. **11**, 229–230.

Rustaiyan A., Komeilizadeh H., Monfared A., Nadji K., Masoudi Sh., and Yari M. (2000a), Volatile constituents of *Nepeta denudata* Benth. and *N. cephalotes* Boiss. from Iran. J. Essent. Oil Res. **11**, 459–461.

Rustaiyan A., Khosravi M., Larijani K., and Masoudi Sh. (2000b), Composition of the essential oil of *Nepeta racemosa* Lam. from Iran. J. Essent. Oil Res. **12**, 151–152.

Sajjadi S.-E. and Ghassemi N. (1999), Volatile constituent of *Nepeta glomerulosa* Boiss. subsp. *carmanica.* Flavour Fragr. J. **14**, 265–267.

Sajjadi S.-E. and Khatamsaz M. (2001), Volatile constituent of *Nepeta heliotropifolia* Lam. J. Essent. Oil Res. **13**, 204–205.

Sefidkon F. (2001), Essential oil of *Nepeta glomerulosa* Boiss. from Iran. J. Essent. Oil Res. **13**, 422–423.

Sefidkon F. and Akbari-nia A. (2003), Essential oil composition of *Nepeta pogonosperma* Jamzad et Assadi from Iran. J. Essent. Oil Res. **15**, 327–328.

Sefidkon F. and Shaabani A. (2004), Essential oil composition of *Nepeta meyeri* Benth. from Iran. Flavour Fragr. J. **19**, 236–238.

Sefidkon F., Dabiri M., and Alamshahi A. (2002), Analysis of the essential oil of *Nepeta fissa* C. A. Mey. from Iran. Flavour Fragr. J. **17**, 89–90.

Shibamoto T. (1987), Retention indices in essential oil analysis. In: Capillary Gas Chromatography in Essential Oil Analysis (Sandra P. and Bicchi C. (eds.). Huethig Verlag, New York.