Journal of Essential Oil Research

ISSN: 1041-2905 (Print) 2163-8152 (Online) Journal homepage: http://www.tandfonline.com/loi/tjeo20

Composition and Antimicrobial Activity of the Essential Oil from Mentha spicata L. subsp. Spicata

E. Şarer , S. Yağmur Toprak , B. Otlu & R. Durmaz

To cite this article: E. Şarer , S. Yağmur Toprak , B. Otlu & R. Durmaz (2011) Composition and Antimicrobial Activity of the Essential Oil from Mentha spicata L. subsp. Spicata , Journal of Essential Oil Research, 23:1, 105-108, DOI: 10.1080/10412905.2011.9700435

To link to this article: http://dx.doi.org/10.1080/10412905.2011.9700435

Composition and Antimicrobial Activity of the Essential Oil from *Mentha spicata* L. subsp. *spicata*

E. Şarer*
Department of Pharmacognosy, Faculty of Pharmacy, Ankara University, Ankara, Turkey
S. Yağmur Toprak
Department of Pharmacognosy, Faculty of Pharmacy İnönü University, Malatya, Turkey
B. Otlu and R. Durmaz
Department of Medicinal Microbiology, Faculty of Medicine, İnönü University, Malatya, Turkey

Abstract

The air-dried aerial parts of *M.spicata L. subsp. spicata*, which were collected from eastern Turkey, were subjected to hydrodistillation and the essential oil was obtained in a yield of 3.24% (v/w). The oil was analyzed by GC and GC/MS. Thirty-seven constituents, accounting for more than 95.3% of the total oil composition, were identified. The main compounds of the essential oil were carvone (48.4%), 1,8-cineole (21.3%), β-pinene (3.5%), β-caryophyllene (3.3%) and *trans*-dihydrocarvone (2.9%). The antimicrobial activity of the oil was studied. It was evaluated against six microorganisms using the disc diffusion and broth microdilution methods. The oil showed great potential for its antimicrobial activities against *Escherichia coli, Candida albicans, Candida tropicalis* and moderate activities against *Staphylococcus aureus*.

Key Word Index

Mentha spicata subsp.*spicata*, Lamiaceae, essential oil composition, carvone, 1,8-cineole, antimicrobial activity.

Introduction

The genus *Mentha* belonging to the family *Lamiaceae* is found in temperate regions of Eurasia, South Africa, the north, west and east portions of Europe, as well as Turkey and Russia. Mint has been used as a medicinal and aromatic plant since ancient times, in both western and eastern cultures (1,2). Many studies on the therapeutic values of mint and mint oils have been reported; these are stomachic, carminative, antispasmodic, stimulant, local anesthetic, anti-inflammatory, diuretic, anthelmentic, antibacterial, antifungal and antioxidant (1-4).

The mint plants produce a number of commercially valuable essential oils viz. spearmint oil (*M. spicata* oil), peppermint oil (*M. piperita* oil), cornmint oil (*M. canadensis* oil) and pennyroyal oil (*M. pulegium* oil) (5). The oil yields from mint plants vary greatly with the seasons, cultural conditions, geographical location and genetic composition (species, subspecies, cultivar) (6).

The genus *Mentha* L. is represented in Turkey by 15 taxa belonging to 8 species including hybrids (7). Among of these, *M. spicata* has two subspecies: *M. spicata* subsp. *spicata* and *M. spicata* subsp. *tomentosa* (Briq) Harley.

There is a lack of information concerning native *M. spicata* plants since almost all references concern cultivated or naturalized ones (8). And there is limited study on the essential oil of *M. spicata* subsp. *spicata*. This work has constituted the most elaborate study on the oil of wild growing *M. spicata* subsp. *spicata* from eastern Turkey (Malatya).

Commercially exploited *M. spicata* plants are always rich in carvone and related compounds; on the other hand, wild populations are very variable. While some samples had carvone or related compounds as major constituents, others were rich in menthone, isomenthone, piperitone, piperitone oxide, piperitenone oxide, linalool, *trans*-sabinene hydrate and 1,8-cineole (9-14). Four different chemotypes were previously distinguished within the species (8).

Lawrence reported three chemotypes with several subgroups of wild *M. spicata*. These are: carvone-type, dihydrocarvone-type and dihydrocarveol-type (chemotype I); piperitenone/piperitone, pulegone/menthone and/or isomenthone, isomenthone/menthone (chemotype II); and piperitone oxide and piperitenone oxide (chemotype III) (15). In another report, Kokkini et al. indicated four chemotypes of wild growing *M. spicata* plants in Greece. These were linalool (chemotype I); piperitone oxide or piperitenone oxide (chemotype II); carvone-dihydrocarvone (chemotype III); and pulegone-menthone-isomenthone (chemotype IV) (8).

*Address for correspondence

Rec: Aug 2008
Rev: Jan 2009
Acc: March 2009

Table I. Percentage Composition of the oil of *Mentha spicata* subsp. *spicata*

Compound	RI	%(v/v)
α-thujene	929	0.1
α-pinene	937	1.2
β-pinene	978	3.5
myrcene	990	1.5
3-octanol	993	0.1
α-terpinene	1019	0.1
sabinene	1021	0.1
1,8-cineole	1031	21.3
(Z)-β-ocimene	1038	0.4
(E)-β-ocimene	1049	0.2
γ-terpinene	1059	0.1
linalool	1097	0.7
p-mentha-2,8-dien-1-ol	1136	0.2
terpinen-4-ol	1175	0.6
α-terpineol	1188	0.4
trans-dihydrocarvone	1199	2.9
carvone	1240	48.4
carvone oxide*	1260	0.4
bornyl acetate	1283	0.1
dihydroedulan II	1285	0.2
dihydroedulan I	1290	0.1
dihydrocarvyl acetate	1303	1.3
piperitenone	1339	0.7
cis-carvyl acetate	1365	0.4
β-bourbonene	1382	1.8
β-elemene	1390	0.9
β-caryophyllene	1418	3.3
aromadendrene	1434	0.3
epi-bicyclosesquiphellandrene	1482	0.3
α-farnesene *	1506	0.3
γ-cadinene	1513	0.9
δ-cadinene	1525	0.3
cis-calamenene	1539	0.3
caryophyllene oxide	1582	1.0
viridiflorol	1589	0.4
α-muurolol	1644	0.2
α-cadinol	1656	0.3
Total identified		95.3

Retention indices (RI) relative to C_9-C_{24} n-alkanes on the HP 5 column. * Correct isomer not identified.

Table II. MICs (Minimum Inhibitory Concentrations) of the essential oil of *Mentha spicata* subsp. *spicata*

Test Microorganisms	MIC Values (µg/mL)
Staphylococcus aureus	15.6
Enterococcus faecalis	125
Pseudomonas aeruginosa	125
Escherichia coli	< 3.19
Candida albicans	< 3.19
Candida tropicalis	< 3.19

Başer et al. indicated the occurrence of at least three chemotypes of wild growing *M. spicata* subsp. *spicata* collected from northern Turkey (16).

The present study focuses on the qualitative and quantitative analysis of the essential oil of *M. spicata* subsp. *spicata* from eastern Turkey, as it has not been the subject of previous study. The authors have also investigated the antimicrobial activities of the sample, as there is only one study in the literature on the antimicrobial activity of wild *M. spicata* subsp. *spicata* from southern Turkey (17).

Experimental

Essential oils: The aerial parts of fully flowered *M. spicata* subsp. *spicata* plants were collected from eastern Turkey (Malatya 964 m, July 2005). The aerial parts of flowering plants, after air-drying at room temperature, were hydrodistilled using a Clevenger-type apparatus for 3.5 h and their essential oil content was expressed in mL/100 g d.w. The obtained oil was dried over anhydrous sodium sulphate and then stored at 4°C until tested.

In GC analysis, the oil was chromatographed using a Agilent Technologies 6890 N gas chromatograph equipped with flame ionization detector (FID) and a HP-5 fused silica capillary column (30 m x 0.32 mm, 0.25 µm). The carrier gas was He with a flow rate of 1.0 mL/min. The injector and detector temperatures were 275°C and 280°C, respectively. The column temperature program adopted was initially 45°C (3 min), increasing at a rate of 3°C/min up to 150°C and increasing at a rate of 5°C/min up to 260°C. Split ratio was 1/50.

GC/MS analysis was conducted using a GC-MS/QP 5000 Shimadzu system equipped with a TC-5 fused silica capillary column (30 m x 0.32 mm, 0.25 µm). The carrier gas was He with a flow rate of 0.9 mL/min. Temperatures were: injector 275°C, detector 300°C, and split ratio was 1/20. The temperature program started at 40°C (3 min), increasing at a range of 4°C/min up to 50°C and held at this temperature for 10 min, then increased at a rate of 4°C/min up to 250°C and held at this temperature for 2 min. The detector was a quadrupolar system with ionisation energy of 70 eV; a scan time of 1s ; mass range of 20–350 amu. The oil components were identified by comparing their RI and MS with those of the authentic samples, literature and a computerized MS-data bank.

Test organisms: The essential oil was tested against *Staphylococcus aureus (ATCC 25923), Enterecoccus faecalis(ATCC 29212), Pseudomonas aeruginosa (ATCC 27928), Escherichia coli (ATCC 22922), Candida albicans and Candida tropicalis* (standard strains).

Determination of antibacterial activity by the disc diffusion method: The antimicrobial activity of the oil was carried out by a disc diffusion test (18). A suspension of the test microorganism (2 x 10^8 cfu/mL of bacteria and 2 x 10^6 cfu/mL of yeast) was spread on the agar media plates (Mueller- Hinton agar for bacterial strains and Sabouraud dextrose agar for yeast strains). Sterile filters paper (Whatman No. 1, diameter 6 mm) were impregnated with 10 µL of oil placed on the inoculated agar. Siprofloxacin, cefaperazone + sulbactam and netilmicine were used as positive reference standards to determine the sensitivity of one strain/isolate in each microbial

species tested. The inoculated plates were incubated at 37ºC for 24 h for clinical bacterial strains, and at 30ºC 48 h for yeast. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms.

Determinations of minimum inhibitory concentration (MIC): A broth microdilution method was used to determine the minimum inhibitory concentration (MIC) according to the National Committee for Clinical Laboratory Standards (19,20). The oil of *M. spicata* subsp. *spicata* dissolved in 10% dimethyl sulfoxide (DMSO) were first diluted to the highest concentration (500 µg/mL) to be tested, and then serial twofold dilutions were prepared in a 96-well microtiter plate in order to obtain a concentration range from 7.8–500 µg/mL in 10 mL sterile test tubes containing nutrient broth. Ceftriaksone and fluconazole were used as reference antibiotics for bacteria and yeast, respectively. Inoculated plates were incubated at 37ºC for 24 h for bacteria and at 37ºC for 48 h for yeasts. After the incubation period, minimum inhibitory concentration (MIC) values were defined as the lowest concentration of the oil that inhibited the visible growth of microorganisms.

Results and Discussion

Air-dried aerial parts of the plant were subjected to hydrodistillation using a Clevenger-type apparatus and the essential oil was obtained (yield 3.24%). In previous studies, a noticeable variation has been found in the oil yield of *M. spicata*, it ranged 0.16–3.35% (6, 21-23). The yields of *M. spicata* subsp. *spicata* oils varied between 0.5–2.0 % (16, 24).

GC and GC/MS analysis resulted in the identification of 37 compounds representing 95.3% of the oil. The oil was found to be rich in oxygenated monoterpenes (77.5%). Carvone (48.4%), 1,8-cineole (21.3 %), β-pinene (3.5 %), β-caryophyllene (3.3%) and *trans*-dihydrocarvone (2.9 %) were found to be main components of the oil (Table I).

As previously mentioned, essential oil yields and chemical composition of *M. spicata* essential oil vary greatly with geographical location and the genetic composition.

The chemical composition of *M. spicata* essential oils from the different populations studied revealed the existence of different chemotypes within the species. According to studies by Lawrence and Kokkini, the present study's essential oil sample from Malatya region may belong to carvone/dihydrocarvone-chemotype. However, it contained a high amount of 1,8-cineole.

There is only one study about the chemotypes of *M. spicata* subsp. *spicata* in Turkey. Başer et al. indicated the occurrence of at least three chemotypes of wild growing *M. spicata* subsp. *spicata* collected from northern Turkey (16). These were: chemotype I (menthone/isomenthone), chemotype II (*trans*-sabinene hydrate/carvone/terpinen-4-ol) and chemotype III (1,8-cineole/linalool/carvone). Second and third chemotypes contain carvone at 18.6% and 14.0%, respectively; however, the present sample contained 48.4% carvone. The present oil sample resembled chemotype III (1,8-cineole, 18.8%) with its high amount of 1,8-cineole (21.3 %), while it differed from the same chemotype (linalool, 17.4%) with its low amount of linalool (0.7%). The oil with this composition also differed from the three chemotypes reported by Başer et al. As a result the present sample can be a new chemotype, therefore it must be examined with more samples.

In the literature, there are some studies concerning the antimicrobial activity of the essential oil of *M.spicata*. In these studies, different results were found. These might be due to different composition of the oil used, different types of strain used, and different assay methods (13-14, 21, 25-29).

Özgüven et al. determined the antimicrobial activity of five wild *M. spicata* subsp. *spicata* samples from southern Turkey against *S. aureus*, *E. coli*,and *E. faecalis*. The authors stated that all oils at concentrations > 10 µL exhibited strong activity against the bacteria tested (17).

In vitro antimicrobial activities of *M. spicata* subsp. *spicata* oil investigated against four bacteria and two yeasts were employed, and their activity potentials were qualitatively and quantitatively assessed by presence or absence of inhibition zones, zone diameters and MIC values. In a disc diffusion assay, the results showed that the oil of *M. spicata* subsp. *spicata* had strong activity against *S. aureus, E. coli, Candida albicans* and *C. tropicalis*. On the other hand, the oil showed no antimicrobial activity against *Pseudomonas aeruginosa* and *E. faecalis*. According to the MIC results given in Table II, the oil showed great potential of antibacterial and antifungal activities against *E. coli, C. albicans, C. tropicalis* and moderate activities against *S. aureus*. The oil had no activities on *E. faecalis* and *P. aeruginosa*. The results of the antibacterial activity of the oil were in contrast to the findings of Özgüven et al.(17), with the exception of *E.coli*. The antimicrobial activity of the studied oil could be due mainly to carvone and 1,8-cineole.

Acknowledgements

The authors would like to thank Hayri Duman (Gazi University, Faculty of Art and Science, Department of Biology) for confirming the identification of the plant material.

References

1. M.S. Ali, M. Saleem, W. Ahmad, M. Parvez and R. Yamdagni, A chlorinated monoterpene ketone, acylated β-sitosterol glycosides and a flavanone glycosides from *Mentha longifolia* (Lamiaceae). *Phytochemistry,* **59**, 889-895 (2002).
2. K.J. Park, Z. Vohnikova and F.P.R Brod, Evaluation of drying parameters and desorption isotherms of garden mint leaves (*Mentha crispa* L.). *J. Food Engineer.,* **51**, 193-199 (2002).
3. K. Adam, A. Sivropoulou, S. Kokkini, T. Lanaras and M. Arsenakis, Antifungal activities of *Origanum vulgare* subsp. h*irtum, Mentha spicata, Lavandula angustifolia,* and *Salvia fruticosa* essential oils against human pathogenic fungi. *J. Agric. Food Chem.*, **46**, 1739-1745 (1998).
4. I. Stefanini , R. Piccaglia, M. Marotti and B. Biavati, Characterization and biological activity of essential oils from fourteen *Labiatae* species. *Acta Hort.,* **723**, 221-226 (2006).
5. S. Kokkini, R. Karousou and T. Lanaras, Essential oils of spearmint (carvone-rich) plants from the island of Crete (Greece). *Biochem. Syst. Ecol.*, **23**, 425-430 (1995).
6. H. MacTavish and D. Harris, *An economic study of essential oil production in the UK,* ADAS Consulting Ltd., London, UK (2002).
7. P.H. Davis, *Flora of Turkey and The East Aegean Islands.* V.7, p.384, University Press, Edinburgh, UK (1982).
8. S. Kokkini and D. Vokou, *Mentha spicata* (Lamiaceae) chemotypes growing wild in Greece. *Econ.Bot.,* **43**, 192-202 (1989).
9. A.O. Tucker, H. Hendriks, R. Bos and D.E. Fairbrothers, The origin

of *Mentha x gracilis* (Lamiaceae) II. Essential oils. *Econ.Bot.,* **45**, 200-215 (1991).
10. A. Sivropoulou, S. Kokkini, T. Lanaras and M. Arsenakis, Antimicrobial activity of mint essential oils. *J. Agric. Food Chem.,* **43**, 2384-2388 (1995).
11. T. Stoeva and L. Iliev, Influence of some phenylurea cytokinins on spearmint essential oil composition. *Bulg. J. Plant Physiol.,* **23**, 66-71 (1997).
12. A. Hadjiakhoondi, N. Aghel, N. Zamanizadeh-Nadgar and H. Vatandoost, Chemical and biological study of *Mentha spicata* L. essential oil from Iran. *DARU*, **8**, 19-21 (2000).
13. A. Sartoratto, A.L.M. Machado, C. Delarmelina, G.M. Figueira, M.C.T. Duarte and V.L.G. Rehder, Composition and antimicrobial activity of essential oils from aromatic plants used in Brazil. *Braz. J. Microbiol.*, **35**, 275-280 (2004).
14. B.M. Lawrence, *Mint: The Genus Mentha,* CRC Press, London, UK (2006).
15. B.M. Lawrence, *A Study of the Monoterpene Interrelationship in the Genus Mentha with Special Reference to the Origin of Pulegone and Menthofuran.* Ph.D. Dissertation, Groningen State University, Groningen, Netherlands (1978).
16. K.H.C. Başer and M. Kürkçüoğlu, Essential oils of *Mentha* species from northern Turkey. *J. Essent. Oil Res.*, **11**, 579-588 (1999).
17. M. Özgüven, S. Kırıcı, A. Yaman, P. Aksungur and A. Gür, Antimicrobial activity of essential oil of wild *Mentha* species growing in Southern Turkey. *Pharm. Pharmacol. Lett.* **8**, 164-167, 1998.
18. NCCLS (National Committee for Clinical Laboratory Standards), Performance Standards for Antimicrobial Disk Susceptibility Test, 6th Edn. Approved Standard. M2-A6. NCCLS: Wayne, PA (1997).
19. NCCLS (National Committee for Clinical Laboratory Standards), Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically IV. Ed., Approved Standart M7-A4, NCCLS: Wayne, PA (1997).
20. NCCLS (National Committee for Clinical Laboratory Standards), Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standart M27-A, NCCLS: Wayne, PA (1997).
21. K.K. Aggarwal, S.P.S Khanuja, A. Ahmad, T.R.S Kumar, V.K. Gupta and S. Kumar, Antimicrobial activity profiles of the two enantiomers of limonene and carvone isolated from the oils of *Mentha spicata* and *Anethum sowa. Flav. Frag. J.,* **17**, 59-63 (2002).
22. D. Karasawa, A. Ujihara and S. Shimizu, The essential oil of *Mentha spicata* L. var. *crispa* Benth. from Nepal. *J. Essent. Oil Res.,* **3**, 447-448 (1991).
23. S. Kokkini, R. Karousou and T. Lanaras, Essential oils with 1,2-epoxy-p-menthane derivatives from *Mentha spicata* plants growing across the island of Crete. *Bot. Acta,* **110**, 184-189 (1997).
24. T. Stoeva, S. Ivancheva and M. Behar, Flavonoids, phenolic and triterpenic acids in the *Mentha spicata* (L.) Huds. group from Bulgaria. *Phytol. Balcanica,* **3/1**, 89-93 (1997).
25. G. Singh, I.P.S. Kapoor, S.K. Panday, U.K. Singh and R.K. Singh, Studies on essential oils Part II: Antibacterial investigations of volatile oils of some higher plants. *Ind. Drugs,* **35**, 591-593 (1998).
26. K.A. Hammer, C.F. Carson and T.V. Riley, Antimicrobial activity of essential oils and other plant extracts. *J. Appl. Bact.*, **86**, 985-990 (1999).
27. H. Imai, K. Osawa, H. Yasuda, H. Hamashima, T. Arai and M. Sasatsu, Inhibition by the essential oils of peppermint and spearmint of the growth of pathogenic bacteria. *Microbios*, **106**, 31-39 (2001).
28. M. Özcan and O. Erkmen, Antimicrobial activity of the essential oils of Turkish plant species. *Eur. Food Res. Technol.*, **212**, 658-660 (2001).
29. K.P.Pandey, S.K. Shahi, R. Singh, S.Dutta and A.Dikshit, Antifungal efficacy of *Taxodium* and *Mentha oils* against some human pathogenic fungi. *Flav. Frag. J.,* **17**, 443-444 (2002).

Chemical Composition of the Fruit Essential Oil of *Phellodendron chinense* (Rutaceae) from China and Its Antifungal Activity against Plant Pathogenic Fungi

Y. Gong, L. Zhou*, X. Shi, Z. Ma and Z. Guo
College of Agronomy and Biotechnology, China Agricultural University, Beijing 100193, China
M. Wang
College of Science, China Agricultural University, Beijing 100193, China
J. Wang and X. Li
College of Agricultural Resources and Environmental Sciences, China Agricultural University, Beijing 100193, China

Abstract

Essential oil of *Phellodendron chinense* fruit was obtained by hydrodistillation and analyzed by GC-FID and GC-MS. Myrcene (70.7%), β-elemene (4.7%) and p-cymene (4.4%) were the major compounds of the 20 identified components. The antifungal activity of the oil, evaluated against fifteen plant pathogenic fungi, was determined by mycelial radial growth inhibition method. The values of the mean inhibitory concentration (IC_{50}) against the tested fungi were at a range of 0.24–1.46 mg/mL. Furthermore, IC_{50} of the oil against spore germination of *Magnaporthe*

*Address for correspondence: lgzhou@cau.edu.cn

Rec: July 2008
Rev: Oct 2008
Acc: March 2009

1041-2905/11/0001-0108$14.00/0—