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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Chemical composition and antibacterial activity of the essential oils from the medicinal plant *Mentha cervina* L. grown in Portugal

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Abstract *Mentha cervina* is a medicinal plant traditionally used in Portugal in folk medicine, in different gastric disorders and inflammations of the respiratory tract. In order to validate those traditional uses, *M. cervina* essential oils (EOs) were characterized by GC and GC–MS and their antimicrobial activity was tested against 23 bacterial strains (including multiresistant strains). The EOs were dominated by the monoterpenes pulegone (52–75%), isomenthone (8–24%), limonene (4–6%), and menthone (1–2%). The antibacterial activity of these EOs was compared to that of the main components standards. The most effective antibacterial activity was expressed by the EOs against the Gram-negative bacteria, *Escherichia coli* and *Acinetobacter baumanni*, with MIC values of 1 mg/ml. The EOs complex mixtures were more active than the individual aromatic components supporting the hypothesis that the EOs antibacterial activity is a function of the synergistic effect of their different aromatic components. These results show the potential role of *M. cervina* EOs as antibacterial agents and validate the traditional use of this plant.

Keywords Lamiaceae · GC · GC–MS · Essential oils · Monoterpenes · Antimicrobial activity · MIC

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Introduction

Essential oils (EOs) and their components are gaining increasing interest in the food, cosmetic, and pharmaceutical industries, because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use (Ormancey *et al*., 2001). In this view, there is an ongoing effort to screen plants medicinally used in different regions of the world, which is the case of plants from the Lamiaceae family that are long know as important sources of EOs bearing plants used in food, perfume, cosmetic, and pharmaceutical industries because of their culinary, fragrance, and antimicrobial properties (Lis-Balchin and Deans, 1997; Ohloff, 1994;). The EOs from *Mentha* species have been in use since ancient times for the treatment of many digestive tract diseases and in culinary (İşcan *et al*., 2002), and they are known to have antimicrobial properties (Flamini *et al*., 1999; Naigre *et al*., 1996). As such, mints are valuable crops with a substantial importance in the botanical economy and to the pharmaceutical industry. Concerning the antimicrobial properties of mint EOs, several species of *Mentha* have been studied, in particular *Mentha x piperita* L. (peppermint) (İşcan *et al*., 2002; Yadegarinia *et al*.,

2006), *M. suaveolens* Ehrh. (Oumzil *et al*., 2002), *M. rotundifolia* (L.) Hudson (Derwich *et al*., 2010), *M. pulegium* L. (Mahboubi and Haghi, 2008 and citations there in), *M. aquatica* L. and *M. longifolia* (L.) Hudson (Gulluce *et al*., 2007; Mimica-Dukic *et al*., 2003). These studies yielded results that are difficult to compare, mainly due to the great variation found in the chemical composition of mint EOs and to a lesser extent to differences in the experimental techniques applied. Different *Mentha* species show differences in their pattern of oil composition, which are the result of their specific metabolic pathways (McConkey *et al*., 2000). Also, the same taxon growing in different areas may have widely differing chemical components resulting in the existence of intraspecific chemical differences (chemotypes), which is very common in the *Mentha* genus (Kokkini, 1991). Biological activity, which is dependent on the chemical composition, is similarly subject to variation, explaining the conflicting results concerning their biological properties (Oumzil *et al*., 2002).

Mentha cervina L., commonly known as hart's pennyroyal, is an aromatic plant traditionally used in Portugal to flavor recipes and in folk medicine, where it is used as an infusion, preventing different gastric disorders and inflammations of the respiratory tract (Monteiro *et al*., 2007; Póvoa *et al*., 2006; Rodrigues *et al*., 2008). This plant is native of the Iberian Peninsula and North Africa, and in Portugal it can be found in streams, bogs and humid places, that are representative of the priority habitat Natura 3170 "temporary Mediterranean ponds" (Silva *et al*., 2009). The unfavorable conservation status of this habitat, the excessive harvesting for consumption and overgrazing are leading to the disappearance of this species from natural settings (Póvoa *et al*., 2006).

In a previous study, the *M. cervina* EOs extracted from cultivated populations, were characterized as belonging to the same chemotype—the pulegone chemotype (Rodrigues *et al*., 2008). Considering the bioactivity of *M. cervina* EOs, there is only one study reporting the antifungal activity against *Candida*, *Aspergillus*, and dermatophyte strains (Gonçalves *et al*., 2007). These authors suggest that *M. cervina* EOs can be used as alternative antifungal agents in the treatment of dermatophytosis. Nevertheless, studies on the antibacterial activities of these EOs are missing.

Given the lack of knowledge on the antibacterial activity of the EOs from *M. cervina* grown in Portugal, the antibacterial capacity of three *M. cervina* EOs was tested against 23 bacterial strains, some of them responsible for digestive and respiratory human diseases and including multiresistant strains. The antibacterial activity of standards from the three main oxygen-containing monoterpenes from the EOs was evaluated. To our knowledge, this is the first report on the antibacterial activity of *M. cervina* EOs from Portuguese populations.

Methods and materials

Plant material

This study was based on three populations of *M. cervina* collected from natural habitats and kept under culture in the essay field of the Instituto Superior de Agronomia (Lisbon). Voucher specimens from the 3 populations have been deposited in the LISI herbarium under the voucher numbers 532/2005 (MC1), 523/2005 (MC2), and 520/2005 (MC3).

Essential oil isolation procedure

For each EO sample, 20 g of full flowering aerial parts were subjected to hydro distillation for 1 h in a Clevenger-type apparatus according to the European Pharmacopoeia method (Council of Europe, 2007). The EOs were stored at −20°C in the dark until analysis.

Gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS)

GC and GC–MS analysis were performed according to Rodrigues *et al*. (2008). The identity of the components was assigned by comparison of their retention indices, relative to a C_9–C_{16} hydrocarbon standard mixture, and with GC–MS spectra from a home-made library, constructed based on the analyses of reference oils, laboratory-synthesised components and commercial available standards.

Bacterial strains

Twenty-three bacterial strains were tested most of them pathogenic for humans and showed multiresistance to antibiotics. The Gram-positive strains are: *Staphylococcus aureus* (ATCC and MRSA—Meticillin resistant *S. aureus*), *S. caprae*, *Enterococcus faecalis* (VRE—Vancomycin resistant *Enterococcus*), *E. faecium*, *E. hirae*, and four *Listeria monocytogenes* strains. The Gram-negative strains are: *Escherichia coli* (ATCC and β-lactamase CTX-M-15 producers), *Salmonella braenderup*; *S. typhimurium*; *S. choleraesuis*, *Klebsiella pneumoniae* (CIP and extended-spectrum β-lactamases-ESBL producer), *Acinetobacter baumannii* (ATCC and multiresistant European clone II strain and the metallo-β-lactamase IMP-5 producer), *Pseudomonas aeruginosa* (ATCC and multiresistant strain by efflux pump). The microorganisms were derived from reference cultures (ATCC and CIP) and stock cultures from CBLBFF (Colecção de Bactérias do Laboratório de Bacteriologia da Faculdade de Farmácia) and CBISA (Colecção de Bactérias do Instituto Superior de Agronomia).

Antimicrobial activity assay

The bacterial strains were challenged with the three different *M. cervina* EOs and also with pure standards of the three main components of these EOs, menthone, isomenthone, and pulegone (Fluka) in order to evaluate their antimicrobial activities. Since a preliminary control test with the solvent DMSO, in a range of 125–250 mg/ml yielded no effect on microbial growth, the EOs and the standards were solubilized in this solvent (ratio of 1:1) and then diluted in culture media for use. The minimum inhibitory concentration (MIC) values were determined by the microdilution broth method, as reported in NCCLS (2006). Microdilution broth test was performed in Mueller–Hinton broth medium, in 96-well micro plates, as follows: 100 µl of Mueller–Hinton broth was added into each well of the micro plate and 100 µl of each EO or pure standards diluted in DMSO (1:1) were added to the first row of the micro plate and then serially twofold diluted in a final volume of 100 µl, respectively, with concentrations ranging from 250 to 0.25 mg/ml. The wells were then inoculated with 10 µl of each bacterial suspension, adjusted to 0.5 McFarland (about 10^7–10^8 CFU/ml). The last row containing the bacterium in Mueller–Hinton broth without the test sample was used as a control for strain viability. Ampicillin and Riphampicin were used as a reference compound for antibacterial activities of Gram-negative and Gram-positive bacteria, respectively. The microplates were covered and incubated for 24 h at 37°C. Each experiment was performed in triplicate. The MIC was determined as the lowest concentration of the product inhibiting the growth of the microorganisms.

Interpretation of the results

The interpretation of microbial growth was based on the following criterion: the result was considered positive, thus, presenting microbial growth, when at least two of three replicates presented visible growth. When visible growth was detected only in one or in none of the three replicates, the result was considered negative, thus, indicating absence of microbial growth.

Results and discussion

Essential oil chemical composition

Thirty-three components were identified in the *M. cervina* evaluated EOs, covering 88–97% of the total (Table 1). Oxygen-containing monoterpenes constituted the main fraction in all EOs (80–88%), pulegone (52–75%), isomenthone (8–24%), limonene (4–6%), and menthone (1–2%) being the main components (Table 1). These results are in accordance with previous studies, which have shown high chemical correlation for Portuguese *M. cervina* EOs

Table 1 Composition of the essential oils isolated by hydrodistillation from the flowering aerial parts of three *Mentha cervina* cultivated populations

Components	RI[a]	*Mentha cervina* populations		
		MC1	MC2	MC3
3-Methyl cyclohexanone	914	t	t	t
α-Thujene	924	t	t	t
α-Pinene	930	0.5	0.6	0.1
Camphene	938	t	t	0.1
Sabinene	958	0.3	0.1	t
1-Octen-3-ol	961	t	t	t
β-Pinene	963	0.2	0.8	0.2
2.5-Dimethyl-1-hexene	970	t	t	t
3-Octanol	974	1.0	0.6	2.4
β-Myrcene	975	0.6	0.4	t
p-Cymene	1003	t	0.1	t
1.8-Cineole	1005	0.3	0.3	0.1
Limonene	1009	6.1	5.6	4.0
cis-*β*-Ocimene	1017	0.1	t	t
trans-*β*-Ocimene	1027	t	t	0.1
γ-Terpinene	1035	t	t	t
n-Octanol	1045	t	t	0.1
cis-Linalool oxide	1045	0.1	t	t
trans-Limonene oxide	1112	0.1	t	0.1
Menthone	1120	0.9	2.2	1.8
Isomenthone	1126	8.3	21.2	24.4
Menthofuran	1134	t	t	t
cis-Isopulegone	1134	1.0	0.9	1.2
Terpinen-4-ol	1148	t	t	t
Verbenone	1164	t	t	t
Myrtenol	1168	t	t	t
Pulegone	1210	74.9	62.7	52.2
Piperitone	1211	t	0.2	t
Carvotanacetone	1222	t	t	0.6
Piperitenone	1289	1.5	0.6	t
β-Caryophyllene	1414	0.3	0.2	t
β-Caryophyllene oxide	1561	t	0.8	1.0
Humulene epoxide	1580	0.1	t	t
% Identification		96.3	97.3	88.4
Grouped components				
Monoterpene hydrocarbons		7.8	7.6	4.5
Oxygen-containing monoterpenes		87.1	88.1	80.4
Sesquiterpene hydrocarbons		0.3	0.2	t
Oxygen-containing sesquiterpenes		0.1	0.8	1.0
Others		1.0	0.6	2.5

[a] Retention Index relative to C_9–C_{16} *n*–alkanes on the DB–1 column; *t* trace (<0.05)

(Gonçalves *et al*., 2007; Rodrigues *et al*., 2008), although some variability was shown in the EOs isolated from this species grown in other countries (Lawrence, 2007). Despite the chemical homogeneity between the evaluated oils, three different profiles were considered, a pulegone-rich with the lowest level of isomenthone (MC1 in Table 1) and two pulegone-rich with similar high levels of isomenthone (MC2 and MC3 in Table 1) but that showed slight differences in the relative amount of the minor EOs components.

Antibacterial activity of the *M. cervina* EOs and of the pure aromatic compounds

The antibacterial capacity of the three different EOs profiles was compared among each other and to standards from the EOs oxygen-containing monoterpene main components, pulegone, isomenthone, and menthone. The results showed that the antibacterial activity of the *M. cervina* EOs and the standard compounds is dependent of the type of microorganisms and in different degrees on the EO profile and the pure aromatic compound used (Table 2).

In general, Gram-negative bacteria were more sensitive than Gram-positive bacteria (Table 2). Gram-negative bacteria showed MIC values of 1 mg/ml, using the complex mixture of EOs. The OEs activity against Gram-positive bacteria was less noteworthy with the lowest MIC values equal or more than 7.8 mg/ml, with exception for *S. aureus* ATCC 6533 that was more susceptible (2 mg/ml with MC3). With the pure compounds, we had the same behavior, the Gram-positive showed MIC values equal or more than 62.5 mg/ml (also with exception for *S. aureus*), and in the Gram-negative bacteria we could find MIC values of 2 mg/ml.

Although it has been established that Gram-positive bacteria are much more sensitive to drug action than Gram-negative bacteria (Cos *et al*., 2006), because of their less complex membrane structure (Cosentino *et al*., 1999; Karaman *et al*., 2003; Sahin *et al*., 2002), the results presented in this study with *M. cervina* EOs are not in accordance with this. The same results were obtained in other studies using *M. pulegium* and *M. longifolia* EOs (Gulluce *et al*., 2007; Hajlaoui *et al*., 2009; Hafedh *et al*., 2010; Mahboubi and Haghi, 2008). The results obtained in our study are promising because the *M. cervina* EOs could be important in future formulations for treatment of multiresistant Gram-negative pathogens, including *Acinetobacter* spp., *P. aeruginosa* and, because of their production of extended-spectrum β-lactamase, Enterobacteriaceae, responsible for serious infections in community and hospital patients (Slama, 2008).

The most considerable antibacterial activity was obtained against *E. coli* and *A. baumannii*, using the EOs complex mixtures. The EO MC2 showed the lowest MIC value of 1 mg/ml for both *E. coli* strains (ATCC and the multiresistant) and the *A. baumannii*. Although we can find in the literature studies using mint EOs, the results are difficult to compare because the methodologies and the bacterial strains used are different among studies. Moreover, the same species may also present different chemotypes. For *E. coli* strains, we could find MIC values of 0.78 and 2.25 mg/ml using *M. longifolia* EO (Hafedh *et al*., 2010 and Hajlaoui *et al*., 2009, respectively), 2.25 and 4 mg/ml with *M. pulegium* EO (Hajlaoui *et al*., 2009 and Mahboubi and Haghi, 2008, respectively), and 250 mg/mL with *M. rotundifolia* EOs (Derwich *et al*., 2010). Interestingly the plant species with more pronounced antibacterial activity were the ones presenting EOs with high content in the monoterpenes pulegone, menthone and isomenthone (*M. pulegium*, *M. longifolia*, and *M. cervina* in this study).

The antibacterial activity of the EOs was higher (Table 2) when compared with the pure standard compounds. Considering the bioactivity of the pure standards alone, in general, isomenthone and menthone, were less active than pulegone (its precursor), with the exceptions for *S. thyphimurium* and *S. aureus* ATCC (Table 2). Similar results were obtained by other authors that reported pulegone as showing a more potent bioactivity (Flamini *et al*., 1999; Gulluce *et al*., 2007; Hajlaoui *et al*., 2008; Naigre *et al*., 1996; Oumzil *et al*., 2002; Mimica-Dukic *et al*., 2003; Oyedeji and Afolayan, 2005). Considering the results with the three EOs profiles, MC3 (the EO with less content in pulegone), was the one who exhibited higher antibacterial activity against all Gram-positive and Gram-negative bacteria (Table 2). In general, the order of efficacy was MC1 $<$ MC2 $<$ MC3, which appears to be related to the decrease in pulegone content. So, although pulegone showed the higher antimicrobial activity (considering the pure standard compounds alone), these results do not agree with the data obtained with the EOs complex mixtures, where the EO with less content in pulegone exhibited the best results. Using *M. cervina* EOs for antifungal activities against *Candida*, *Aspergillus*, and dermatophyte strains, Gonçalves *et al*. (2007) also obtained the highest activity with the sample containing lower amounts of pulegone. The same type of response of EO complex mixtures and individual components was reported in other species, using *M.* x *piperita* EO against *E. coli*, *S. aureus*, and *Candida albicans* (Yadegarinia *et al*., 2006) and with *M. spicata* EO against *L. monocytogenes* (Leonard *et al*., 2010). Given the heterogeneous composition of EOs and the different antimicrobial activities of its components, it seems that different components may have different modes of action and that the activity could be attributed to the presence of minor components or at least to a synergistic effect between components.

Table 2 Minimum inhibitory concentration (MIC, mg/ml) of *M. cervina* essential oils, pure standard compounds, and DMSO, against different bacterial strains

Bacteria strains	MIC (mg/ml)							
	Essential oils			Pure compounds and solvent				Antibiotics[a]
	MC1	MC2	MC3	Pulegone	Isomenthone	Menthone	DMSO	
GRAM−								
Pseudomonas aeruginosa								
ATCC 10554	125	62.5	15.6	125	125	125	>250	<0.25
MR (ID 1833)	125	31.3	31.3	125	125	125	>250	>250
Escherichia coli								
ATCC 11105	2.0	1.0	1.0	3.9	15.6	31.3	125	<0.25
CTX (ID2511)	7.8	1.0	2.0	3.9	62.5	62.5	125	62.5
Acinetobacter baumannii								
ATCC 19606	3.9	2.0	3.9	2.0	31.3	62.5	125	<0.25
MR (ID130)	2.0	1.0	1.0	2.0	15.6	15.6	125	15.6
IMP5 (ID65)	1.0	1.0	2.0	2.0	15.6	15.6	125	<0.25
Klebsiella pneumoniae								
KPC (ID2564)	62.5	3.9	7.8	62.5	62.5	62.5	125	–
CTX-M-15 (ID2510)	31.3	7.8	15.6	62.5	125	125	125	–
TEM-10 (ID683)	62.5	15.6	15.6	31.3	62.5	125	125	–
Salmonella thyphimurium								
CBISA 3969	62.5	31.3	15.6	62.5	7.8	31.3	125	<0.25
Salmonella braenderup								
CBISA 3991	31.3	31.3	7.8	31.3	62.5	62.5	125	<0.25
GRAM+								
Staphylococcus aureus								
ATCC 6533	31.3	15.6	2.0	62.5	15.6	15.6	>250	<0.25
MRSA CIP 106760	62.5	15.6	7.8	62.5	125	125	>250	–
Staphylococcus caprae								
CBISA 3572	62.5	–	7.8	125	125	125	>250	<0.25
Enterococcus faecalis								
CIP 104476	62.5	31.3	15.6	125	125	125	>250	125
Enterococcus faecium								
ID 435628	62.5	62.5	15.6	125	125	125	>250	2.0
Enterococcus hirae								
CIP 5855	62.5	62.5	15.6	125	125	125	>250	2.0
Listeria monocytogenes								
EGDe (CBISA 3992)	62.5	–	7.8	125	125	125	125	<0.25
CECT (CBISA 3004)	62.5	–	7.8	125	125	125	125	<0.25
CBISA 3845	62.5	–	7.8	125	125	125	125	<0.25
CBISA 3077	125.0	–	15.6	125	125	125	125	<0.25

[a] Ampicillin and riphampicin were used as reference compounds for antibacterial activities of Gram-negative and Gram-positive bacteria, respectively

Conclusion

This study demonstrated the potential use of *M. cervina* EOs as well as their components as antibacterial agents, in particular against Gram-negative bacteria, such as *E. coli* and *A. baumannii*, providing an explanation for the reported traditional use of this plant. These results also support the hypothesis that the antibacterial activity of the *M. cervina* EOs is a function of the synergistic effect of their different aromatic monoterpene constituents. The

extraction of active compounds in single or combined forms, from this plant, may lead to their use as food preservatives as well as in pharmaceutical and natural therapies for the treatment of infectious diseases. Nevertheless, further research is required to evaluate the practical value of *M. cervina* EOs applications.

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References

Cos P, Vlietinck AJ, Berghe DV, Maes L (2006) Anti-infective potential of natural products: how to develop a stronger in vitro 'proof-of-concept'. J Ethnopharmacol 106:290–302

Cosentino S, Tuberoso CIG, Pisano B, Satta M, Mascia V, Arzedi E (1999) In vitro *a*ntimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. Lett Appl Microbiol 29: 130–135

Council of Europe (COE) (2007) European Directorate for the Quality of Medicines, 6th edn. European Pharmacopoeia, Strasbourg

Derwich E, Benziane Z, Boukir A (2010) Antibacterial activity and chemical composition of the leaf essential oil of *Mentha rotundifolia* from Marocco. EJEAFChe 9(1):19–28

Flamini G, Cioni PL, Puleio R, Morelli I, Panizzi L (1999) Antimicrobial activity of the essential oil of *Calamintha nepeta* and its constituent pulegone against bacteria and fungi. Phytother Res 13:349–351

Gonçalves M, Vicente A, Cavaleiro C, Salgueiro L (2007) Composition and antifungal activity of the essential oil of *Mentha cervina* from Portugal. Nat Prod Res 21(10):867–871

Gulluce M, Sahin F, Sokmen M, Ozer H, Daferera D, Sokmen A, Polissiou M, Adiguzel A, Ozkan H (2007) Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. Longifolia. Food Chem 103:1449–1456

Hafedh H, Fethi BA, Mejdi S, Emira N, Amina B (2010) Effect of *Mentha longifolia* L. ssp longifolia essential oil on the morphology of four pathogenic bacteria visualized by atomic force microscopy. Afr J Microbiol Res 4(11):1122–1127

Hajlaoui H, Snoussi M, Ben Jannet H, Mighri Z, Bakhrouf A (2008) Comparison of chemical composition and antimicrobial activities of *Mentha longifolia* L. ssp. *longifolia* essential oil from two Tunisian localities (Gabes and Sidi Bouzid). Ann Microbiol 58(3):103–110

Hajlaoui H, Trabelsi N, Noumi E, Snoussi M, Fallah H, Ksouri R, Bakhrouf A (2009) Biological activities of the essential oils and methanol extract of tow cultivated mint species (*Mentha longifolia* and *Mentha pulegium*) used in the Tunisian folkloric medicine. World J Microbiol Biotechnol 25:2227–2238

İşcan G, Krmer N, Kürkcüolu M, Hüsnü K, Başer C, Demrc F (2002) Antimicrobial screening of *Mentha piperita* essential oils. J Agric Food Chem 50(14):3943–3946

Karaman I, Sahin F, Gulluce M, Ogutc H, Sengul M, Adiguzel A (2003) Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. J Ethnopharmacol 85:231–235

Kokkini S (1991) In: Liskens HF, Jackson JF (eds) Essential oils and waxes—chemical races within the genus *Mentha* L. Springer-Verlag, Berlin, pp 63–78

Lawrence BM (2007) The composition from commercially important mints. In: Lawrence BM (ed) Mint. The genus *Mentha*. CRC Press, Boca Raton, pp 217–323

Leonard CM, Virijevic S, Regnier T, Combrinck S (2010) Bioactivity of selected essential oils and some components on *Listeria monocytogenes* biofilms. S Afr J Bot 76(4):676–680

Lis-Balchin M, Deans SG (1997) Bioactivity of selected plant essential oils against *Listeria monocytogenes*. J Appl Bacteriol 82:759–762

Mahboubi A, Haghi G (2008) Antimicrobial activity and chemical composition of *Mentha pulegium* L. essential oil. J Ethnopharmacol 119(2):325–327

McConkey ME, Gershenzon J, Croteau RB (2000) Developmental regulation of monoterpene biosynthesis in the glandular trichomes of peppermint. Plant Physiol 122:215–223

Mimica-Dukic N, Bozin B, Sokovic M, Mihajlovic B, Matavulj M (2003) Antimicrobial and antioxidant activities of three *Mentha* species essential oils. Planta Med 69(5):413–419

Monteiro A, Póvoa O, Marinho S, Rodrigues L, Monteiro P (2007) *Mentha pulegium* e *Mentha cervina*—Os poejos na boa cozinha portuguesa. ISA press, Lisboa

Naigre R, Kalck P, Roques C, Roux I, Michel G (1996) Comparison of antimicrobial properties of monoterpenes and their carbonylated products. Planta Med 62(3):275–277

NCCLS (2006) National committee for clinical laboratory standards: methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 7th edn. Approved Standard M7-A7, Wayne

Ohloff G (1994) *In* Scent and Fragrances: The Fascination of Odors and their Chemical Perspectives. Springer-Verlag, New York

Ormancey X, Sisalli S, Coutiere P (2001) Formulation of essential oils in functional perfumery. Parfum Cosmet Actual 157:30–40

Oumzil H, Ghoulami S, Rhajaoui M, Ilidrissi A, Fkih-Tetouani S, Faid M, Benjouad A (2002) Antibacterial and antifungal activity of essential oils of *Mentha suaveolens*. Phytother Res 16:727–731

Oyedeji O, Afolayan A (2005) Comparative study of the essential oil composition and antimicrobial activity of *Leonotis leonurus* and *L. ocymifolia* in the Eastern Cape. S Afr J Bot 71:114–116

Póvoa O, Farinha N, Marinho S, Nunes P, Godinho D, Mata F, Rodrigues L, Monteiro A (2006) Pennyroyal (M*entha pulegium*) and Hart's Pennyroyal (*Mentha cervina*) biodiversity in Alentejo, Portugal. Acta Hortic 723:91–97

Rodrigues L, Monteiro P, Póvoa O, Teixeira G, Moldão M, Figueiredo AC, Monteiro A (2008) Morphology of secretory structures and essential oil composition in *Mentha cervina* L. from Portugal. Flavour Fragr J 23:340–347

Sahin F, Karaman I, Gulluce M, Ogutcu H, Sengul M, Adiguzel A, Ozturk S, Kotana R (2002) Evaluation of antimicrobial activities *Satureja hortensis* L. J Ethnopharmacol 87:61–65

Silva V, Póvoa O, Espírito-Santo MD, Vasconcelos T, Monteiro A (2009) *Mentha cervina* communities in Portugal. Lazaroa 30: 73–79

Slama T (2008) Gram-negative antibiotic resistance: there is a price to pay. Crit Care 12(Suppl 4):S4

Yadegarinia D, Gachkar L, Rezaei MB, Taghizadeh M, Astaneh SA, Rasooli I (2006) Biochemical activities of Iranian *Mentha piperita* L. and *Myrtus communis* L. essential oils. Phytochemistry 67(12):1249–1255