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Antimicrobial Activity of two *Mentha* Species Essential Oil and its Dependence on Different Origin and Chemical Diversity

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Genus *Mentha* presents group of plants which are the most studied in family *Lamiaceae*. Aboveground parts are used for different purposes in pharmacy, food industry or confectionery. Most important is natural product extracted from leaves - essential oil (EO). The aim of presented experiment was to demonstrate different chemotype and compare antibacterial activity of two *Mentha* species EO. Plant samples were obtained from various environments – from Slovakia and from Italy. Dominant compounds were determined by GC/MS. The results showed high amount of menthol and menthone in tested Slovak peppermint EO. On the other hand, carvone and 1,8-cineole were determinate as dominant compounds in Italian spearmint EO. The antimicrobial activity of the EO was investigated by disc diffusion and broth micro dilution methods. EO was evaluated for their antibacterial activity against 7 microorganisms: *Enterobacter cloacae*, *Salmonella* spp., *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pyogenes*. The determination results of antibacterial activity by agar disk diffusion method ranged from 7 to 14 mm of the growth inhibition zone. MIC of tested mint EO varied from 0.625 to 2.5 µg/mL. In addition, both EO showed relatively the same antibacterial activity against the selected Gram-negative bacteria. However, there is a variation in the antibacterial activity against Gram-positive bacteria.

Keywords: Agar disc diffusion, Bacterial strains, Biodiversity, GC-MS, Natural products.

Mentha spp. is well known genus used by the human throughout history. The major applications include products for oral care, chewing gum, liquors and fragrances. Its potential depends on its multiple properties as carminative, antioxidant, antifungal or antimicrobial [1a]. There was also described as potential nutraceutical and functional food for prevention and treatment diseases [1b, 1c]. The antimicrobial activity of EO is especially focused on the treatment of strains resistant to conventional antibiotics [1d].

Mentha × *piperita* L. (peppermint) is a perennial herb 50-90 cm high, with square-shaped stems and leaves are arranged in opposite pairs [1e]. *Mentha spicata* L. (spearmint) is an herbaceous, rhizomatous, perennial plant growing 30–100 cm tall, with variably hairless to hairy stems and foliage, and a wide-spreading fleshy underground rhizome [1f].

These plants grow in temperate areas of the world, particularly in Europe, North America and North Africa, but nowadays cultivated throughout all the regions in the world. Both species are preferred by customers for their extensive use as culinary herbs, and due to their relatively high resiliency to adverse conditions. The aerial parts are useful to obtain EO. Dominant compounds in peppermint EO are menthol, menthone and 1,8-cineole [1g], while carvone, limonene and menthone were identified as main compounds in spearmint [1h, 1i]. The estimated world consumption of *Mentha* spp. EO is 4 500 tons per year [1j]. International demand of mint EO has been increased in the past few years. The largest producing countries are Bulgaria, Italy, China and USA, which contribute about 90 % of the world total mint oil production [1k].

Menthol is characteristic as dominant compound in peppermint with its well-known penetrating minty odor and cooling taste [2a]. Carvone as the most abundant compound is characteristic in spearmint EO [2b]. Spearmint EO also contains significant amounts of limonene, dihydrocarvone and 1,8-cineol [2c]. Chemotaxonomy is distinguished by the major EO component [2d]. Unlike EO from peppermint, EO of spearmint contains minimal amounts of menthol and menthone. It is also used as a flavoring for toothpaste and confectionery, and is sometimes added to shampoos and soaps. Analysis of the component composition of EO allowed us to distinguish *Mentha* forms belonging to the different chemotype groups. Peppermint chemotypes were specified as menthol, pulegone–menthol and linalool [2e] and four chemotypes of spearmint characterized by the dominant occurrence of linalool, carvone/dihydrocarvone, piperitone oxide/piperitetone oxide and menthone/isomenthone/pulegone [2f]. Five chemotypes groups of minth were generally identified based on dominant compounds as menthol and menthone, piperitenone oxide, linalool, carvone and 3-octanone [2g]. The quantitative composition of EO highly depends on climatic conditions, plant growth stage, collection time, drying conditions, distillation method, etc. [2h]. However, the total character of EO is more influenced by genetic factors confirming that the potential to produce a certain chemical pattern is genetically coded, but the gene expression will be introduced or also repressed by environmental factors [2i, 2j].

Studies have reported many factors that influence development of bacterial resistance against treatment with conventional antibiotics [3a]. Factors associated with it are well documented and known, among which are most important antibiotic overuse or use improper prescribed antibiotics combined with inadequate clinical diagnosis [3b]. Therefore, there is increased demand for the development of alternative strategies to conventional antibiotic therapy [3c]. *Mentha* spp. EO was found to inhibit growth of several tested micro-organisms [3d]. Antibacterial activity of EO is closely related to the chemical variability of EO [3e]. Many studies have been carried out for screening antibacterial activity of *Mentha* spp. EO, but no one was related to the chemical diversity of EO. The presented study demonstrated a variation in the chemical composition and the antibacterial activity between two *Mentha* spp. EO.

The results of the chemical analysis of *Mentha* spp. EO is shown in Table 1. The total content of identified components represented 98.8% in Slovak sample (EO^{Sk}) and 91.1% in Italian sample (EO^{It}). 22 compounds were identified in Slovak sample (EO^{Sk}), from which three were in content less than 0.1 % and four compounds below the limit of detection comparing to Italian sample (EO^{It}). The major constituents in EO^{Sk} were menthol (49.3%), menthone (22.4%), 1,8-cineol (9.4%), menthyl acetate (6.4%), isomenthone (5.7%) and β-caryophyllene (1.4%). However, 13 compounds were determined in Italian sample (EO^{It}), from which as dominant were characterized carvone (62.4%), 1,8-cineole (6.2%), menthone (5.8%), β-caryophyllene (5.3%), methyl acetate (4.5%) and limonene (4.2%). Thirteen components were below the limit of detection.

Menthol was the major component of the studied EO^{Sk}. Main components and their general limits for peppermint EO are in accordance with the published ranges [2k]. Spearmint EO^{It} composition was compared with other analyses [2l], where dominated carvone (40 - 75 %), limonene (1.5 - 20 %) and 1,8-cineole (2.5 – 5 %). The variation in quantitative and qualitative composition could be due to different geographical sources, different climate conditions and genotype of tested *Mentha* spp. EO [2m]. The existence of different chemotypes, based on qualitative differences within taxon is a common feature in most *Mentha* species and hybrids [2n].

The most commonly used methods for study antibacterial activity of *Mentha spp.* EO are well diffusion method [3f] and agar disk diffusion method [1a, 3g]. The agar disk diffusion method was used for screening antibacterial activity of *Mentha* EO against 7 bacterial strains, five Gram-negative and two Gram-positive (Table 2). In this study EO^{SK} and EO^{It} exhibited an antibacterial activity against all the selected bacterial strains. Both samples showed a 7-10 mm/10μL inhibition zone against the tested strains. The most sensitive were *E. coli* and *E. coli* (ATCC 25922) strains (9-10 mm). The less sensitive was *K. pneumoniae* for EO^{Sk} (7.0±0.6 mm) and *S. pyogenes* for EO^{It} (7.0±0.5 mm). The highest inhibition zone (10 mm) were observed against two bacterial strains (*E. coli* and *S. pyogenes*) for EO^{Sk} and against three bacterial strains (*K. pneumoniae*, *E. coli* ATCC 25922 and *S. aureus*) for EO^{It}.

The present study showed relatively the same antibacterial activity against all tested Gram-negative bacterial strains with inhibition zones diameters ranged from 7.0 ± 0.6 to 10.0 ± 0.2 mm and from 9.0 ± 0.2 to 10.0 ± 0.4 mm, respectively, except for *E. coli* which is more sensitive to EO^{Sk} than EO^{It}. However, there is a variation in the antibacterial activity against Gram-positive bacteria. *Staphylococcus aureus* is more sensitive to EO^{It} than EO^{Sk}. Furthermore, *Streptococcus pyogenes* is more sensitive to EO^{Sk} than EO^{It}.

Table 1: Composition of *Mentha* EO in Slovak (EO^{Sk}) and Italian (EO^{It}) samples.

N.	Ri^{exp}	Ri^{lit}	Compound names	EO^{Sk}	EO^{It}	Identif.
1	934	936	α-Pinene	0.4	0.5	1,2,3
2	979	978	β-Pinene	0.9	0.7	1,2,3
3	1020	1024	1,8-Cineole	9.4	6.2	1,2,3
4	1025	1025	Limonene	-	4.2	1,2,3
5	1026	1029	β-Ocimene	t	-	1,2
6	1048	1051	γ-Terpinene	0.2	-	1,2,3
7	1078	1082	Terpinolene	0.2	-	1,2
8	1130	1132	Isopulegol	0.1	-	1,2
9	1134	1136	Menthone	22.4	5.8	1,2,3
10	1142	1146	Isomenthone	5.7	0.2	1,2
11	1170	1172	Menthol	49.3	0.2	1,2,3
12	1178	1176	α-Terpineol	0.5	0.7	1,2,3
13	1212	1214	Carvone	-	62.4	1,2,3
14	1216	1215	Pulegone	0.7	0.2	1,2,3
15	1125	1226	Piperitone	0.3	-	1,2
16	1278	1280	Menthyl acetate	6.4	4.5	1,2
17	1284	1386	β-Bourbonene	0.1	0.2	1,2
18	1390	1389	β-Elemene	t	-	1,2
19	1419	1420	β-Farnesene	0.2	-	1,2
20	1422	1421	β-Caryophyllene	1.4	5.3	1,2
21	1470	1472	γ-Gurjunene	-	-	1,2
22	1474	1474	γ-Muurolene	t	-	1,2
23	1476	1479	Germacrene D	0.3	-	1,2
24	1492	1494	Valencene	0.1	-	1,2
25	1503	1503	α-Chamigrene	-	-	1,2
26	1521	1520	δ-Cadinene	0.2	-	1,2
			Total identified	**98.8**	**91.1**	

- = below the limit of detection; t = traces (less than 0.1%); *Ri^{exp} and Ri^{lit} are the retention indices determined relative to a series of n-alkanes (C10–C35) on BR-5 in our experiment and compared with literature on DB-5 capillary columns; Identification: 1, comparison of retention indices with published data; 2, comparison of mass spectra with those listed in the NIST 02 library and with published data; 3, co-injection with authentic compound.

The minimum inhibitory concentration (MIC) is cited by most researchers as a measure of the antibacterial performance of EOs [2f]. The MIC values of *Mentha* spp. EO are shown in Table 2. The MIC of EO were determinate against Gram-negative (*Enterobacter cloacae*, *Salmonella* spp., *Klebsiella pneumoniae*, *Escherichia coli*) and Gram-positive (*Staphylococcus aureus*, *Streptococcus pyogenes*) bacterial strains. The MIC of both EO for the tested bacteria ranged from 0.625-2.5 μg/mL. Our results showed MIC values comparable to reported results [1a]. In their study is presented MIC of *Enterobacter cloacae* (1.0 μg/mL), *Staphylococcus aureus* (0.75 μg/mL), *Escherichia coli* (ATCC25922) (0.20 μg/mL) and *Klebsiella pneumoniae* (0.50 μg/mL).

Table 2: Diameters of inhibition zones (mm) and MIC values of *Mentha* EO.

Bacterial groups	Bacterial strains	Inhibition zone (mm) ± SD (n=3)		MIC (μg/ml) of EO	
		EO^{Sk}	EO^{It}	EO^{Sk}	EO^{It}
Gram-negative	*Klebsiella pneumoniae*	7.0 ± 0.6	10.0 ± 0.4	2.50	1.25
	E. coli	10.0 ± 0.2	9.0 ± 0.2	1.25	2.50
	Enterobacter cloacae	9.0 ± 0.4	9.0 ± 0.6	1.25	1.25
	Salmonella spp.	8.0 ± 0.3	9.0 ± 0.3	1.25	1.25
	E. coli ATCC 25922	9.0 ± 0.5	10.0 ± 0.4	1.25	1.25
Gram-positive	*Staphylococcus aureus*	8.0 ± 0.2	10.0 ± 0.3	1.25	0.65
	Streptococcus pyogenes	10.0 ± 0.2	7.0 ± 0.5	0.62	2.50

*EO^{Sk}- Essential oils from Slovak peppermint, EO^{It} – essential oil from Italian spearmint.

There were reported differences of susceptibility of variety bacterial strains. Gram-negative organisms are slightly less susceptible than gram-positive bacteria [3h]. Results obtained in study [3i] showed not selective antibacterial activity of spearmint EO on the basis of the cell-wall differences of bacterial microorganisms (Gram-positive or Gram-negative bacteria).

Many publications presented strong antibacterial activity of *Mentha* EO. Their activities are assessed by the presence of dominant

compounds as menthol, menthone, carvone etc. [2c, 3j]. Testing individual components exhibited a variable degree of antimicrobial activity against different bacterial strains [3k**]**. There is evident that some bacterial strains are more sensitive to EO with different dominant compounds.

The antibacterial activity of EO^{Sk} in the disc diffusion could be due to the presence of high menthol content [3l] which also support the study, where menthol was dominant compound in *M. arvensis* and presented higher antimicrobial activity comparing to *M. piperita*, in which menthone was dominant compound. Isomenthone accompanied both dominant compounds in mentioned EO [2c]. The antibacterial activity of EO^{It} in the disc diffusion against tested bacteria could be explained by high content of carvone. Carvone, as tested individual component, showed antimicrobial activity against *S. aureus,* while menthol presented no inhibition [3m]. This is in accordance with our findings.

In any cases, also the synergistic effect of the components, found in small concentration, cannot be ruled out for activity of EO [3l]. A previous study confirmed that minor components play a role in antibacterial activity, possibly by producing synergistic effects with other components [3h, 4a**]**. Considering the large number of different groups of chemical compounds present in EO, the activity of terpenes would be expected to relate to the structural configuration and to their functional groups [3m] and it is most likely that their antibacterial activity is not attributable to one specific mechanism but that there are several targets in the cell [4b, 4c]. The comparison of the chemical composition and the antibacterial activity between two *Mentha* spp. EO was investigated in this study. The present study showed a variation in the chemical composition between EO^{Sk} and EO^{It}. In addition, both EO exhibited an antibacterial activity against the selected bacterial strains. Relatively the same antibacterial activities of both EO against Gram-negative bacteria were demonstrated. However, there is a variation in the antibacterial activity against Gram-positive bacteria. More specific studies are required to investigate the active components present in EO of different origin and their mechanism of action to justify their further used as antibacterial agents.

Experimental

Plant material and Essential oils (EO): Two *Mentha* plant samples originated from Slovakia and Italy were used in this study. Sample from Slovakia, as a plant source was used peppermint cultivar named *Perpeta* which was grown by private company Agrokarpaty, Plavnica (Slovakia). Italian spearmint was collected in Peloritani Mountains, Sicily (Italy) and was identified at University of Messina. The EO from both samples were extracted by hydro distillation in a laboratory at University of Prešov according the procedures previously described [5a].

GC/MS analysis: Both samples of EO were analyzed by a gas chromatography/mass spectrometry (GC/MS) for quantitative and qualitative properties in laboratory at University of Prešov. GC/MS analyses were carried out on devices Varian 450-GC and 220-MS. Separation was provided on a capillary column BR 5ms (30 m× 0.25mm ID, 0.25 μm film thickness). Injector type 1177 was heated to a temperature of 220°C. Injection mode was split less (1 μL of a 1:1000 n-hexane solution). Helium was used as a carrier gas at a constant column flow rate of 1.2 mL min−1. Column temperature was programmed in four steps: (1) 50°C for 10 min, (2) 100°C at 3°C min−1, (3) isothermal for 5 min and (4) 150°C at 10°C min−1. The total time for analysis was 87.67 min. The MS trap was heated to 200°C, manifold 50°C and transfer line 270°C. Mass spectra were scanned every 1 s in the range 40–650 m/z. The retention indices were determined in relation to the Rt values of an homologous series of n-alkanes (C10–C35) under the same operation conditions. Constituents were identified by comparison of their retention indices (RI) with published data in different literature. Further identification was made by comparison of the mass spectra with either those stored in NIST 02 library or with those from the literature [5b]. Components relative concentrations were obtained by percentage of peak area normalization.

Bacterial strains: The bacterial strains *Enterobacter cloacae* (S54b/16), *Salmonella* sp. (S13b/16), *Klebsiella pneumoniae* (S12b/16), *Escherichia coli* (S34/16) were isolated from poultry in the Regional Veterinary Laboratory of Mostaganem, Algeria. *Staphylococcus aureus* and *Streptococcus pyogenes* were from the Department of Biomedical and Dental Sciences and Morphofunctional Imaging, University of Messina, Italy. The strains were identified using Api20E systems (bioMerieux, France). The reference strain *Escherichia coli* ATCC 25922 (American Type Culture Collection, Rockville, MD, USA) was also used in this study.

Disk diffusion method: The antibacterial activity of *Mentha* spp. EO was determined by agar disc diffusion assay according to the method previously described [5c]. The agar plates had been inoculated with selected bacterial strains. Well isolated, single bacterial colonies growth overnight was transferred into physiological water. The turbidity of the bacterial suspensions was adjusted to 0.5 McFarland standards. The bacterial suspensions were swabbed on the surface of Mueller Hinton agar with sterile cotton swabs. The sterile paper discs (6 mm in diameter, Thermo Fisher, Italy) were separately impregnated with 10 μl of EO. Samples were prepared in triplicates. After 24 h of incubation at 37°C, the diameters of the growth inhibition zone were measured.

Minimal inhibitory concentration (MIC): The determination of the minimal inhibitory concentration (MIC) was performed with a broth micro dilution assay, using a serial microplate dilution method [4d] with slight modifications. MIC was determined using 96-well microplates, serial twofold dilutions of peppermint EOs in Mueller-Hinton broth (MHB) were prepared over the range of 5 to 0.0097 μg/mL. Compounds to be investigated were dissolved in MHB. Subsequently, 100 μL of an actively growing culture of the tested strain were added to each dilution. The microplates were sealed and incubated 24 h at 37°C. The MIC was defined as the lowest concentrations without visible growth, at the end of incubation. MHB and bacterial culture were used as a positive control and the medium without bacteria as a negative control. After incubation the optical density was measured at a wavelength of 630 nm by microplate's reader (Fluostar Omega, BMG Labtech, Italy). Three replicates were done for each EO. Escherichia coli strain ATCC 25922 was used as quality control in MIC determination.

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References

[1] (a) Desam NR, Al-Rajab AJ, Sharma M, Moses MM, Reddy GR, Albratty M. (**2017**) Chemical constituents, in vitro antibacterial and antifungal activity of *Mentha×piperita* L. (peppermint) essential oils. *Journal of King Saud University*, doi: http://dx.doi.org/10.1016/j.jksus.2017.07.013; (b)

Santini A, Cammarata SM, Capone G, Ianaro A, Tenore GC, Pani L, Novellino E. (**2018**) Review Nutraceuticals: opening the debate for a regulatory framework. *British Journal of Clinical Pharmacology*, ***84***, 659–672; (c) Sevindik M. (**2018**) Pharmacological Properties of *Mentha* Species. *Journal of Traditional Medicine & Clinical Naturopathy*, ***7***: 259. doi: 10.4172/2573-4555.1000259; (d) Severino P, Acioli FRD, Cardoso Cordeiro J, Teixeira MdC, Santini A, Kovačević AB, Souto EB. (**2017**) Essential oils with antimicrobial properties formulated in lipid carriers – Review of the state of the art. In: "*Essential oils and nanotechnology for the cure of microbial diseases*", Rai M, Derita M, Zacchino S (Editors) CRC Press, Taylor and Francis Group, Boca Raton, FL, USA. ISBN 978-1-1386-3072-7, Chapter 1:1-13; (e) Briggs C. (**1993**) Peppermint: medicinal herb and flavoring agent. *Canadian Pharmaceutical Journal*, ***126***, 89-92; (f) Chauhan RS, Kaul MK, Shahi AK, Kumar A, Ram G, Tawa A. (**2009**) Chemical composition of essential oils in *Mentha spicata* L. accession [IIIM(J)26] from North-West Himalayan region, India. *Industrial Crops and Products, **29***, 654–656; (g) Schmidt E, Bail S, Buchbauer G, Stoilova I, Atanasova T, Stoyanova A, Krastanov A, Jirovetz L. (**2009**) Chemical composition, olfactory evaluation and antioxidant effects of essential oil from *Mentha x piperita*. *Natural Product Communications*, ***4***, 1107-1112; (h) Díaz-Maroto MC, Pérez-Coello MS, González Viñaz MA, Cabezudo MD. (**2003**) Influence of drying on the flavor quality of spearmint (*Mentha spicata* L.). *Journal of Agricultural and Food Chemistry*, ***51***, 1265-1269; (i) Buleandra M, Oprea E, Popa DE, David IG, Moldovan Z, Mihai I, Badea IA. (**2016**) Comparative chemical analysis of *Mentha piperita* and *M. spicata* and a fast assessment of commercial peppermint teas. *Natural Product Communications*, ***11***, 551-555; (j) Baser KH and Buchbauer G. (**2010**) *Handbook of Essential oils*. CRC Press, New York; (k) Dwivedi S, Khan M, Srivastava SK, Syamasunnder KV, Srivastava A. (**2004**) Essential oil composition of different accessions of *Mentha* × *piperita* L. grown on the northern plains of India. *Flavour and Fragrance Journal*, ***19***, 437-440.

[2] (a) Pushpangadan P, Tewari SK**.** (**2006**) Peppermint. In *Handbook of Herbs and Spices*. Volume 3. Woodhead publishing in food science, technology and nutrition. CRC Press: Boca Raton FL (USA). 460– 481; (b) Bakkali F, Averbeck S, Averbeck D, Idaomar M. (**2008**) Biological effects of essential oils – A review. *Food and Chemical Toxicology*, ***46***, 446–475; (c) Hussain AI, Anwar F, Nigam PS, Ashraf M, Gilani A. (**2010**) Seasonal variation in content, chemical composition and antimicrobial and cytotoxic activities of essential oils from four *Mentha* species. *Journal of the Science of Food and Agriculture*, ***90***, 1827-1836; (d) Kurilov DV, Kirichenko EB, Bidyukova GF, Olekhnovich LS, Ku LD. (**2009**) Composition of the essential oil of introduced Mint forms of *Mentha piperita* and *Mentha arvensis* Species. *Doklady Biological Sciences*, ***429***, 538-540; (e) Talbaoui AT, Jamaly N, Aneb M, Il Idrissi A, Bouksaim M, Gmouh S, Amzazi S, El Moussaouiti M, Benjouad A, Bakri Y. (**2012**) Chemical composition and antibacterial activity of essential oils from six Moroccan plants. *Journal of Medicinal Plants Research*, ***6***, 4593-4600; (f) Kofidis G, Bosabalidis A and Kokkini S. (**2004**) Seasonal variation of essential oils in a linalool-rich chemotype of *Mentha spicata* grown wild in Greece. *Journal of Essent Oil Research, **16***, 469–472; (g) Ludwiczuk A, Kiełtyka-Dadasiewicz A, Sawicki R, Golus J, Ginalska G. (**2016**) Essential oils of some *Mentha* species and cultivars, their chemistry and bacteriostatic activity. *Natural Product Communications*, ***11***, 1015-1018; (h) Tuttolomondo T, Dugo G, Ruberto G, Leto C, Napoli EM, Cicero N, Gervasi T, Virga G, Leone R, Licata M, La Bella S. (**2015**) Study of quantitative and qualitative variations in essential oils of Sicilian *Rosmarinus officinalis* L. *Natural Products Research*, ***29***, 1928-1934; (i) Franz Ch. (**2010**) Genetic versus climatic factors influencing essential oil formation, In: *Handbook of Essential oils*. CRC Press, New York. 89; (j) Gruľová D, De Martino L, Mancini E, Tkáčiková Ľ, Šalamon I, Fejer J, De Feo V. (**2016**) Phytotoxic and antibacterial activity of essential oil of new peppermint cultivar. *Natural Product Communications*, ***11***, 1721-1724; (k) European Pharmacopoeia. (**2004**) 5th edition, Council of Europe, Strasbourg, France, 1, 217-218; (l) Chauhan SS, Prakash O, Padalia RC, Vivekanand, Pant AK, Mathela CS. (**2011**) Chemical diversity in *Mentha spicata*: Antioxidant and potato sprout inhibition activity of its essential oils. *Natural Product Communications*, ***6***, 1373-1378; (m) Massotti V, Juteau F, Bessiere JM, Viano J. (**2003**) Seasonal and phenological variations of the essential oil from the narrow endemic species *Artemisia molinieri* and its biological activities. *Journal of Agriculture and Food Chemistry*, ***51***, 7115-7121; (n) Kokkini S. (**1991**) Chemical races within the genus *Mentha* L. In Modern Methods of Plant Analysis, New Series 12; Linskens HF, Jackson JF, Eds.; Springer: Berlin; pp 61-75.

[3] (a) Xavier KB, Bassler BL. (**2003**) Lux Squorumsensing: more than just a numbers game. *Current Opinion in Microbiology*, ***6***, 191–197; (b) Shalayel MHF, Asaad AM, Qureshi MA, Elhussein AB. (**2017**) Anti-bacterial activity of peppermint (*Mentha piperita*) extracts against some emerging multi-drug resistant human bacterial pathogens. *Journal of Herbal Medicine*, ***7***, 27–30; (c) Zeng Z, Qian L, Cao L, Tan H, Huang Y, Xue X. (**2008**) Virtual screening for novel quorum sensing inhibitors to eradicate biofilm formation of *Pseudomonas aeruginosa*. *Applied Microbiology and Biotechnology*, ***79***, 119–126; (d) Jirovetz L, Buchbauer G, Bail S, Denkova Z, Slavchev A, Stoyanova A, Schmidt E and Geissler M. (**2009**) Antimicrobial activities of essential oils of mint and peppermint as well as some of their main compounds. *Journal of Essential Oil Research*, ***21***, 363–366; (e) Almeida Barbosa LC, Filomeno CA and Teixeira RR. (**2016**) Chemical variability and biological activities of *Eucalyptus* spp. essential oils. *Molecules*, ***21***, 1671; doi:10.3390/molecules21121671; (f) Afridi MS, Ali J, Abbas S, Rehman SU, Khan FA, Khan MA, Shahid M. (**2016**) Essential oil composition of *Mentha piperita* L. and its antimicrobial effects against common human pathogenic bacterial and fungal strains. *Pharmacology Online*, ***91***, 90-97; (g) Toroglu S. (**2011**) *In-vitro* antimicrobial activity and synergistic/antagonistic effect of interactions between antibiotics and some spice essential oils. *Journal of Environmental Biology, **32***, 23-29; (h) Burt S. (**2004**) Essential oils: their antibacterial properties and potential applications in foods – a review. *International Journal of Food Microbiology, **94***, 223–253; (i) Brahmi F, Adjaoud A, Marongiu B, Falconieri D, Yalaoui-Guellal D, Madani K, Chibane M. (**2016**) Chemical and biological profiles of essential oils from *Mentha spicata* L. leaf from in Bejaia Algeria. *Journal of Essential Oil Research*, DOI: 10.1080/10412905.2015.1118411; (j) Mimica-Dukic N, Bozin B, Sokovic M, Mihajlovic B and Matavulj M. (**2003**) Antimicrobial and antioxidant activities of three *Mentha* species essential oils. *Planta Medica, **69***, 413–419; (k) Sivropoulou A, Kokkini S, Lanaras T and Arsenakis M. (**1995**) Antimicrobial activity of mint essential oils. *Journal of Agriculture and Food Chemistry, **43***, 2384–2388; (l) Iscan G, Kirimer N, Kurkcuoglu M, Baser HC, Demirci F. (**2002**) Antimicrobial screening of *Mentha piperita* essential oils. *Journal of Agriculture and Food Chemistry*, ***50***, 3943-1946; (m) Gallucci MN, Oliva M, Casero C, Dambolena J, Luna A, Zygadlo J and Demo M. (**2009**) Antimicrobial combined action of terpenes against the food-borne microorganisms *Escherichia coli*, *Staphylococcus aureus* and *Bacillus cereus*. *Flavour and Fragrance Journal*, ***24***, 348–354;

[4] (a) Mulyaningsih S, Sporer F, Zimmermann S, Reichling J, Wink M. (**2010**) Synergistic properties of the terpenoids aromadendrene and 1,8-cineole from the essential oil of *Eucalyptus globulus* against antibiotic-susceptible and antibiotic-resistant pathogens. *Phytomedicine*, ***17***, 1061–1066; (b) Skandamis P, Koutsoumanis K, Fasseas K, Nychas G-JE. (**2001**) Inhibition of oregano essential oil and EDTA on *Escherichia coli* O157:H7. *Italian Journal of Food Science*, ***13***, 65–75; (c) Carson CF, Mee BJ, Riley TV. (**2002**) Mechanism of action of *Melaleuca alternifolia* (tea tree) oil on *Staphylococcus aureus* determined by time-kill, lysis, leakage and salt tolerance assays and electron microscopy. *Antimicrobial Agents and Chemotherapy*, ***46***, 1914–1920;

[5] (a) Gruľová D, De Martino L, Mancini E, Salamon I, De Feo V. (**2015**) Seasonal variability of the main components in essential oil of *Mentha* × *piperita* L. *Journal of the Science of Food and Agriculture*, ***95***, 621-627; (b) Adams RP. (**2007**) *Identification of Essential Oil Components by Gas Chromatography/Mass Spectroscopy*, 4th ed.; Allured Publishing Corporation: Carol Stream, IL, USA.; (c) Bauer AW, Kirby WM, Sherris JC, Turck M. (**1966**) Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, ***45***, 493-496; (d) Eloff JN. (**1998**) A sensitive and quick method to determine the minimum inhibitory concentration of plant extracts for bacteria. *Planta Medica*, ***60***, 1-8.