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Antimicrobial and antioxidant properties of the essential oils and methanol extract from *Mentha longifolia* L. ssp. *longifolia*

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Abstract

This study was designed to evaluate antimicrobial and antioxidant activities of the essential oil and methanol extract from *Mentha longifolia* ssp. *longifolia*. The essential oil showed strong antimicrobial activity against all 30 microorganisms tested whereas the methanol extract almost remained inactive. In contrast, the extract showed much better activity than the essential oil in antioxidant activity assays employed, e.g. in the inhibition of free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) and β-carotene/linoleic acid systems. In the former, the extract was able to reduce the stable free radical DPPH with an IC_{50} of 57.4 μg/ml while that of the oils was 10700 μg/ml. When compared to BHT, a synthetic antioxidant, both showed weaker antioxidative potential. Similarly, in β-carotene/linoleic acid assay, these samples were not effectively able to inhibit the linoleic acid oxidation; exhibiting only 24% and 36% inhibitions at 2 mg/ml, respectively; both were far below than that of BHT. Total phenolic constituent of the extract was 4.5 g/100 g as gallic acid equivalent. GC–MS analysis of the oil resulted in the identification of 45 constituents, *cis*-piperitone epoxide, pulegone and piperitenone oxide being the main components.

Keywords: Antimicrobial activity; Antioxidant activity; *Mentha longifolia* ssp. *longifolia*; GC–MS-analysis

1. Introduction

The genus *Mentha* L. (Lamiaceae) comprises more than 25 species, mainly perennial herbs growing wildly in damp or wet places throughout temperate regions of Eurasia, Australia and South Africa (Lange & Croteau, 1999). Except hybrids, six *Mentha* species including *M. pulegium*, *M. arevensis*, *M. aquatica*, *M. longifolia*, *M. piperita* and *M. suaveolens* are recorded from the Turkish flora (Baser, Ozek, Kurkcuoglu, & Tumen, 1992; Rasooli & Rezaei, 2002).

Three *Mentha* species, *M. x piperita* L. (peppermint), *M. arvensis* L. (cornmint) and *M. spicata* L. (spearmint), are commonly cultivated in the world for essential oil production that is used extensively in the liquor and confectionary industries, flavoring, perfume production and medicinal purposes (Iscan, Kirimer, Kurkcuoglu, Baser, & Demirci, 2002; Lin et al., 1999; Moreno, Bello, Primo-Yufera, & Esplugues, 2002).

Leaves, flowers and the stem of *Mentha* spp. are frequently used in herbal teas or as additives in commercial

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spice mixtures for many foods to offer aroma and flavour (Kothari & Singh, 1995; Moreno et al., 2002). In addition, *Mentha* spp. has been used as a folk remedy for treatment of nausea, bronchitis, flatulence, anorexia, ulcerative colitis, and liver complaints due to its antinflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue, and anticatharrhal activities (Cowan, 1999; Iscan et al., 2002; Moreno et al., 2002). Furthermore, it is well-documented that the essential oils or/and extracts from some *Mentha* species including *M. spicata*, *M. x piperita*, *M. arvensis*, *M. rotundifolia*, *M. suaveolens* and *M. pulegium* possess antimicrobial and antioxidant properties (Daferera, Ziogas, & Polissiou, 2003; Economou, Oreopolou, & Thomopoulos, 1991; Kaur & Kapoor, 2002). However, there has been no attempt to study the biological activities of the essential oils and extracts from *Mentha longifolia* ssp. *longifolia* growing wildly in the north-eastern part of Anatolia.

In recent years, multiple drug/chemical resistance in both human and plant pathogenic microorganisms have been developed due to indiscriminate use of commercial antimicrobial drugs/chemicals commonly used in the treatment of infectious diseases (Davis, 1994; Service, 1995). On the other hand, foodborne diseases are still a major problem in the World, even in well-developed countries, like USA (Mead et al., 1999). Food spoilage caused by a variety of microorganisims has often been recognised as inconvenient and one of the most important concerns for the food industry. The contamination of raw and/or processed foods with microflora can take place at various stages from production to sale and distribution (Deak et al., 1996). Thus, the food industry at present uses chemical preservatives to prevent the growth of food spoiling microbes (Alzoreky & Nakahara, 2003).

Therefore, the objectives of this study were: (1) to analyze the chemical composition of a hydrodistilled essential oil and total phenolic constituent in methanol extract of *M. longifolia* ssp. *longifolia* plants collected from the north eastern Anatolia region of Turkey by a GC/MS system in order to determine the essential oil chemotype; (2) to investigate the antimicrobial and antioxidant activities of essential oil and methanol extracts of *M. longifolia* ssp. *longifolia*.

2. Materials and methods

2.1. Plant material

M. longifolia L. Hudson subsp. *longifolia* plants at flowering stage were collected from Köse, Gümüşhane, Turkey. The taxonomic identification of plant materials was confirmed by a senior plant taxonomist, Meryem Şengül, of the Department of Biology, Atatürk University, Erzurum, Turkey. The voucher specimen has been deposited at the Herbarium of the Department of Biology, Atatürk University, Erzurum-Turkey (ATA HERB 9732).

The leaves of plant material were separated from the stem, dried in the shade, and ground in a grinder with a 2 mm diameter mesh.

2.2. Preparation of the extracts

2.2.1. Isolation of the essential oil

The air-dried and finely ground material was submitted for 3 h to water-distillation using a British type Clevenger apparatus (ILDAM Ltd., Ankara, Turkey) (yield: 2.31 ml/100 g). The obtained essential oil (EO) was dried over anhydrous sodium sulphate and, after filtration, stored at +4 °C until tested and analysed.

2.2.2. Preparations of the methanol extract (MeOH)

A portion of plant material (500 g) were successively extracted with 1 l of methanol (Merck, Darmstadt, Germany) by using a Soxhlet extractor (Isolab, Wetheim, Germany) for 72 h at a temperature not exceeding the boiling point of the solvent (Lin et al., 1999). The methanol extracts were filtered using Whatman filter paper (No: 1) and then concentrated in vacuo at 40 °C using a Rotary Evaporator (Buchi, Flawil, Switzerland). The residue obtained was lyophilized in a Modulyo freeze-dryer (Edwards, Crawkey, Sussex, UK) and the resulting powdered material was stored at −80 °C until tested.

2.3. GC–MS analysis conditions

The analysis of the essential oil was performed using a Hewlett Packard 5890 II GC (Hellamco SA, Athens, Greece), equipped with a HP-5 MS capillary column (30 m × 0.25 mm i.d., 0.25 μm) and a HP 5972 mass selective detector. For GC–MS detection an electron ionization system with ionization energy of 70 eV was used. Helium was the carrier gas, at a flow rate of 1 ml/min. Injector and MS transfer line temperatures were set at 220 and 290 °C, respectively. Column temperature was initially kept at 50 °C for 3 min, then gradually increased to 150 °C at a 3 °C/min rate, held for 10 min and finally raised to 250 at 10 °C/min. Diluted samples (1/100 in acetone, v/v) of 1.0 μl were injected manually and in the splitless mode. The components were identified based on the comparison of their relative retention time and mass spectra with those of standards, NBS75K library data of the GC–MS system and literature data (Adams, 2001). The results were also confirmed by the comparison of the compounds elution order with their relative retention indices on non-polar phases reported in the literature (Adams, 2001). Pure α-pinene, β-pinene, β-myrcene, α-terpinene, γ-terpinene, *p*-cymene, limonene, α-terpineol, linalool, *d*-camphor, menthone, pulegone, thymol, carvacrol, and β-caryophyllene, were purchased from the Sigma–Aldrich Co (St. Louis, Missouri).

2.4. Antioxidant activity

2.4.1. DPPH assay

The hydrogen atom or electron donation ability of the corresponding extracts and some pure compounds was measured from the bleaching of purple colored methanol solution of DPPH. This spectrophotometer assay (Pharmacia, Uppsala, Sweden) uses stable radical diphenylpicrylhydrazyl (DPPH) (Sigma, Aldrich) as a reagent (Burits & Bucar, 2000; Cuendet, Hostettmann, & Potterat, 1997). Aliquots (50 μl) of various concentrations of the extracts in methanol was added to 5 ml of a 0.004% methanol solution of DPPH. After a 30 min incubation period at room temperature the absorbance was read against a blank at 517 nm. Inhibition free radical DPPH in percent (I %) was calculated in following way:

$$I\ \% = (A_{\text{blank}} - A_{\text{sample}}/A_{\text{blank}}) \times 100$$

where A_{blank} is the absorbance of the control reaction (containing all reagents except the test compound), and A_{sample} is the absorbance of the test compound. Extract concentration providing 50% inhibition (IC_{50}) was calculated from the graph plotted inhibition percentage against extract concentration. Synthetic antioxidant reagent butylated hyroxytoluene (BHT) was used as the positive control and all tests were carried out in triplicate.

2.4.2. β-Carotene–linoleic acid assay

In this assay antioxidant capacity is determined by measuring the inhibition of the volatile organic compounds and the conjugated diene hydroperoxides arising from linoleic acid oxidation (Dapkevicius, Venskutonis, Van Beek, & Linssen, 1998).

A stock solution of β-carotene/linoleic acid (Sigma–Aldrich) was prepared as follows. First, 0.5 mg of β-carotene was dissolved in 1 ml of chloroform (HPLC grade), then 25 μl of linoleic acid and 200 mg of Tween 40 (Merck) were added. The chloroform was subsequently evaporated using a vacuum evaporator (Büchi, Flawil, Switzerland). Then 100 ml of distilled water saturated with oxygen (30 min at 100 ml min^{-1}) was added with vigorous shaking. Aliquots (2.5 ml) of this reaction mixture were transferred to test tubes, and a 350 μl portion of the extracts (2 g l^{-1} in ethanol) was added before incubating for 48 h at room temperature. The same procedure was repeated with BHT at the same concentration and a blank containing only 350 μl of ethanol. After the incubation period, the absorbencies of the mixtures were measured at 490 nm. Antioxidant capacities of the samples were compared with those of BHT and the blank.

2.4.3. Assay for total phenolics

Total phenolic constituent in methanol extract of *M. longifolia* ssp. *longifolia* was performed employing the literature methods (Chandler & Dodds, 1983; Slinkard & Singleton, 1977) involving Folin-Ciocalteu reagent and gallic acid (both Sigma–Aldrich) as standard. Briefly, an aliquot (0.1) ml of extract solution containing 1 mg of extract was transferred to a volumetric flask, 46 ml distilled water and 1 ml Folin-Ciocalteu reagent was added and the flask was shaken thoroughly. After 3 min, 3 ml of solution 2% Na_2CO_3 was added and the mixture was allowed to stand for 2 h with intermittent shaking. Absorbance was measured at 760 nm. The same procedure was repeated for all standard gallic acid solutions (0–1000 μg in 0.1 ml) and a standard curve was obtained according to the equation

$$\text{Absorbance} = 0.0012 \times \text{Gallic acid } (\mu g) + 0.0033.$$

2.5. Antimicrobial activity

2.5.1. Microbial strains

The essential oil and the extract were individually tested against a panel of microorganisms including a total of 38 microbial cultures belonging to 23 bacteria and 15 fungi and yeast species. The list of microorganisms used is given in Tables 3 and 4. Microorganisms were provided by the Department of Clinical Microbiology, Faculty of Medicine; and Plant Diagnostic Laboratory, Faculty of Agriculture at Atatürk University, Erzurum, Turkey. Identity of the microorganisms used in this study was confirmed by Microbial Identification System in Biotechnology Application and Research Center at Atatürk University.

2.5.2. Disc-diffusion assay

The dried plant extracts were dissolved in the same solvent (methanol) to a final concentration of 30 mg/ml and sterilized by filtration by 0.45 μm Millipore filters. Antimicrobial tests were then carried out by the disc diffusion method (Murray, Baron, Pfaller, Tenover, & Yolke, 1995) using 100 μl of suspension containing 10^8 CFU/ml of bacteria, 10^6 CFU/ml of yeast and 10^4 spore/ml of fungi spread on nutrient agar (NA), sabouraud dextrose agar (SDA), and potato dextrose agar (PDA) medium, respectively. The discs (6 mm in diameter) were impregnated with 10 μl of essential oil or the 30 mg/ml extracts (300 μg/disc) placed on the inoculated agar. Negative controls were prepared using the same solvents employed to dissolve the plant extracts. Ofloxacin (10 μg/disc), sulbactam (30 μg) + cefperazone (75 μg) (105 μg/disc) and/or netilmicin (30 μg/disc) 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 37 °C for 24 h for clinical bacterial strains, 48 h for yeast and 72 h for fungi isolates. Plant associated microorganisms were incubated at 27 °C. Antimicrobial activity was evaluated by measuring the zone of inhibition against the test organisms. Each assay in this experiment was repeated twice.

2.5.3. Micro-well dilution assay

The minimal inhibition concentration (MIC) values were studied for the bacterial strains which were sensitive

to the essential oil and/or extracts in disc diffusion assay. The inocula of the bacterial strains were prepared from 12 h broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. The essential oils of *M. longifolia* ssp. *longifolia* dissolved in 10% dimethylsulfoxide (DMSO) were first diluted to the highest concentration (500 μg/ml) to be tested, and then serial two-fold dilutions were made in a concentration range from 7.8 to 500 μg/ml in 10 ml sterile test tubes containing nutrient broth. MIC values of *M. longifolia* ssp. *longifolia* essential oil against bacterial strains were determined based on a micro-well dilution method (Gulluce, Adiguzel, Ogutcu, Sengul, & Sahin, 2004a; Gulluce et al., 2004b).

The 96-well plates were prepared by dispensing into each well 95 μl of nutrient broth and 5 μl of the inoculum. A 100 μl aliquot from the stock solutions of *M. longifolia* essential oil initially prepared at the concentration of 500 μg/ml was added into the first wells. Then, 100 μl from their serial dilutions was transferred into six consecutive wells. The last well containing 195 μl of nutrient broth without compound and 5 μl of the inoculum on each strip was used as the negative control. The final volume in each well was 200 μl. Maxipime (Sefalosporin, Bristol-Myers Squibb, Istanbul, Turkey) at the concentration range of 500–7.8 μg/ml was prepared in nutrient broth and used as standard drug for positive control. The plate was covered with a sterile plate sealer. Contents of each well were mixed on plate shaker at 300 rpm for 20 s and then incubated at appropriate temperatures for 24 h. Microbial growth was determined by absorbance at 600 nm using the EL ×800 universal microplate reader (Biotek Instrument inc, Highland Park, Vermont, USA) and confirmed by plating 5 μl samples from clear wells on nutrient agar medium. The essential oil tested in this study was screened two times against each organism. The MIC was defined as the lowest concentration of the compounds to inhibit the growth of microorganisms.

2.5.4. MIC agar dilution assay

MIC values of the fungi isolates were studied based on the agar dilution method as described previously (Gul, Ojanen, & Hanninen, 2002). The essential oils of *M. longifolia* ssp. *longifolia* were added aseptically to sterile molted PDA medium containing Tween 20 (Sigma) (0.5%,v/v) at the appropriate volume to produce the concentration range of 7.8–500 μg/ml. The resulting PDA agar solutions were immediately poured into petri plates after vortexing. The plates were spot inoculated with 5 μl (10^4 spore/ml) of each fungi isolate. Amphotericin B (Sigma A 4888) was used as a reference antifungal drug. The inoculated plates were incubated at 27 °C and 37 °C for 72 h for plant and clinical fungi isolates, respectively. At the end of incubation period, the plates were evaluated for the presence or absence of growth. MIC values were determined as the lowest concentration of the essential oil where absence of growth recorded. Each test was repeated at least twice.

3. Results and discussion

The chemical composition of *M. longifolia* ssp. *longifolia* essential oil was analysed by employing GC–MS, leading to comparison of the relative retention times and the mass spectra of oil components with those of authentic samples and mass spectra from data library. As shown in Table 1.

Table 1
Chemical composition for *Mentha longifolia* ssp. *longifolia* essential oil

	R_t (min)[a]	K.I.[b]	Component[c]	% Composition
1	9.766	939	α-Pinene[e]	0.1
2	11.699	979	β-Pinene[e]	0.2
3	12.462	991	β-Myrcene[e]	0.1
4	12.819	991	3-Octanol[f]	0.1
5	13.631	1017	α-Terpinene[e]	tr[d]
6	14.068	1025	*p*-Cymene[e]	0.4
7	14.226	1029	Limonene[e]	0.4
8	14.761	1037	(*Z*)-β-Ocimene[f]	0.2
9	15.267	1050	(*E*)-β-Ocimene[f]	tr
10	15.762	1060	γ-Terpinene[e]	0.7
11	16.258	1070	*cis*-Sabinene hydrate[f]	0.1
12	17.953	1097	Linalool[e]	0.2
13	20.074	1146	Camphor[e]	1.6
14	20.748	1153	Menthone[e]	7.9
15	21.234	1163	Isomenthone[f]	6.6
16	21.600	1175	Pinocamphone[f]	0.2
17	21.710	1177	Terpinen-4-ol[f]	0.6
18	22.611	1189	α-Terpineol[e]	0.4
19	22.730	1193	Dihydro carvone[f]	0.4
20	25.099	1237	Pulegone[e]	15.5
21	25.268	1243	Carvone[f]	4.9
22	25.962	1254	*cis*-Piperitone epoxide[f]	18.4
23	26.051	1256	*trans*-Piperitone epoxide[f]	4.1
24	27.062	1289	Bornyl acetate[f]	0.5
25	27.359	–	Sabinyl acetate[*f]	0.2
26	27.766	1290	Thymol[e]	6.6
27	28.152	1299	Carvacrol[e]	0.4
28	29.738	1343	Piperitenone[f]	1.0
29	30.293	1352	Thymol acetate[f]	0.3
30	31.245	1369	Piperitenone oxide[f]	14.7
31	31.621	1388	β-Bourbonene[f]	0.2
32	31.998	1392	Nepetalactone[f]	0.8
33	33.217	1419	β-Caryophyllene[e]	2.6
34	33.554	1432	β-Copaene[f]	tr
35	34.644	1455	α-Caryophyllene[f]	0.5
36	35.595	1480	γ-Muurolene[f]	1.1
37	36.478	1500	Bicyclogermacrene[f]	0.4
38	36.924	1506	β-Bisabolene[f]	0.2
39	37.202	1514	γ-Cadinene[f]	tr
40	37.568	1523	*d*-Cadinene[f]	0.2
41	39.937	1578	Spathulenol[f]	0.6
42	40.125	1583	Caryophyllene oxide[f]	0.6
43	40.512	1595	Salvial-4(14)-en-1-one[f]	tr
44	40.908	1608	β-Atlantol[f]	tr
45	41.166	1608	Humulene epoxide II[f]	0.1
			Total	93.8

[a] Retention time (as minutes).
[b] K.I. Kovats Index on DB-5 column in reference to *n*-alkanes (37).
[c] Compounds listed in order of elution from a HP-5 MS column.
[d] Trace ⩽ 0.06%.
[e] Identified by reference compound.
[f] Tentatively identified.
[*] Correct isomer not identified.

GC/MS analysis resulted in the identification of forty five compounds representing 93.8% of the oil. The main components were oxygenated monoterpenes, all *p*-menthane derivatives. Moreover, the essential oil composition of *M. longifolia* ssp. *longifolia* is characterized mainly by the presence of C-3 substituted compounds, including *cis*-piperitone epoxide (18.4%), pulegone (15.5%), piperitenone oxide (14.7%), menthone (7.9%), isomenthone (6.6%), *trans*-piperitone epoxide (4.1%) and secondly by the C-2 substituted compounds, including carvone (4.9%). *M. longifolia* ecotypes are reported to contain either high amounts of carvone and dihydro carvone (Kokkini, Karousou, & Lanaras, 1995; Monfared, Nabid, & Rustaiyan, 2002) or piperitenone oxide and piperitone oxide to the composition of their essential oil. From the view of distribution and essential oil variation of *M. longifolia* plants, the investigated ecotype is familiar to those observed from Jordan, Morocco, and Iran (Ghoulami, Il Idrissi, & Fkih-Tetouani, 2001; Jaimand & Rezaee, 2002).

In our study, we have investigated the free radical scavenging activity and lipid oxidation inhibition of *M. longifolia* extracts *in vitro*. Free radical scavenging activities of the extracts were measured in DPPH assay and the reaction followed a concentration dependent pattern. Free radical scavenging increases with increasing extract concentration and extract concentrations providing 50% inhibition (IC_{50}) are given in Table 2. The free radical scavenging activity of methanol extract was superior to oil ($IC_{50} = 57.4$ μg/ml). Activity might be related to its phenolic content like phenolic acids e.g. rosmarinic acid and polyphenols as reported in a previous study (Mimica-Dukic, Popovic, Jakovljevic, Szabo, & Gasic, 1999). Therefore, total phenolic constituent of methanol extract was determined as gallic acid equivalent. Forty-five micrograms gallic acid equivalent total phenolic in 1 mg extract (4.5%) was determined that might be responsible for the radical scavenging activity of methanol extracts. In the case of inhibition of linoleic acid assay, both methanol extract and essential oil were not able to effectively inhibit the linoleic acid oxidation, and only 24% and 36% inhibitions were achieved at 2 mg/ml concentrations respectively, which were far below the positive control BHT at the same concentration (Fig. 1). Activity might be improved at higher concentrations but this was not considered here. Although methanol extract and the essential oil possessed weaker inhibition effect on linoleic acid oxidation the methanol extract exhibited particularly strong free radical scavenging activity related to its total phenolic constituent.

Table 2
Effects of *Mentha longifolia* ssp. *longifolia* methanol extract, essential oil and positive control BHT on the *in vitro* free radical (DPPH) scavenging

Sample	DPPH, IC_{50} (μg/ml)
Methanol extract	57.4 ± 0.5
Essential oil	10700 ± 5.0
BHT	19.8 ± 0.5

The antimicrobial activities of *M. longifolia* ssp. *longifolia* essential oil and extracts against microorganisms examined in the present study and their potency were qualitatively and quantitatively assessed by the presence or absence of inhibition zones and zone diameter, and MIC values. The results are given in Tables 3 and 4. The results showed that the essential oil of *M. longifolia* ssp. *longifolia* had great potential for antimicrobial activities against all 15 bacteria, and 14 fungi and a yeast species tested. On the other hand, the methanol extract from aerial parts of *M. longifolia* ssp. *longifolia* plants showed no antimicrobial activities (Tables 3 and 4). The maximal inhibition zones and MIC values for bacterial strains, which were sensitive to the essential oil of *M. longifolia* ssp. *longifolia*, were in the range of 8–22 mm, and 15.62–125 μl/ml, respectively (Table 3). The maximal inhibition zones and MIC values of the yeast and fungi species sensitive to the essential oil of *M. longifolia* ssp. *longifolia*, were 12–35 mm and 31.25–125 μl/ml, respectively (Table 4).

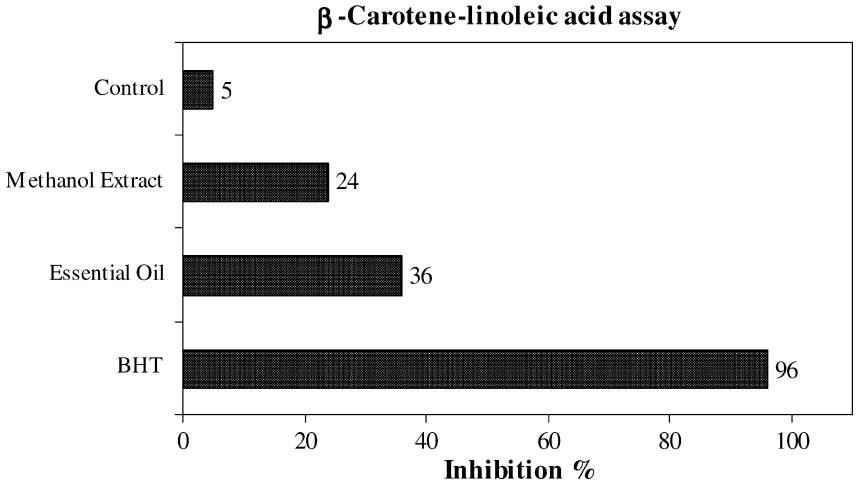

Fig. 1. Antioxidant activity of *M.* longifolia ssp. *longifolia* extract and essential oil defined as inhibition percentage in β-carotene–linoleic acid assay.

Table 3
Antimicrobial activities of *Mentha longifolia* ssp. *longifolia* essential oil against the bacterial strains tested

Test microorganisms	Plant extract (MeOH)		Essential oil		Antibiotics	
	DD[b]	MIC[d]	DD[c] (mm)	MIC[d]	DD[a] (mm)	MIC[e] (max)
Acinetobacter baumanii-A8	–	–	14	31.25	18 (OFX)	31.25
Bacillus macerans-M58	–	–	22	15.62	19 (OFX)	15.62
Bacillus megaterium-M3	–	–	9	–	9 (SCF)	15.62
Bacillus subtilis-ATCC-6633	–	–	8	62.50	28 (OFX)	62.50
Bacillus subtilis-A57	–	–	–		28 (OFX)	125
Brucella abortus-A77	–	–	–	–	12 (SCF)	62.50
Burkholdria cepacia-A225	–	–	8	62.50	22 (SCF)	125
Clavibacter michiganense-A227	–	–	–	–	25 (SCF)	16.62
Enterobacter cloacae-A135	–	–	11	62.50	20 (NET)	31.25
Enterococcus faecalis-ATCC-29122	–	–	12	62.50	18 (SCF)	31.25
Escherichia coli-A1	–	–	18	31.25	20 (OFX)	62.50
Klebsiella pneumoniae-A137	–	–	14	31.25	12 (OFX)	125
Proteus vulgaris-A161	–	–	9	125	12 (OFX)	125
Proteus vulgaris-KUKEM1329	–	–	12	62.50	13 (OFX)	125
Pseudomonas aeruginosa-ATCC9027	–	–	–	–	22 (NET)	31.25
Pseudomonas aeruginosa-ATCC27859	–	–	–	–	22 (NET)	15.62
Pseudomonas syringae pv.*tomato* A35	–	–	–	–	24 (OFX)	125
Salmonella enteritidis-IK27	–	–	8	62.50	27 (SCF)	62.50
Staphylococcus aureus-A215	–	–	21	15.62	22 (SCF)	31.25
Staphylococcus aureus-ATCC-29213	–	–	14	31.25	22 (SCF)	62.50
Staphylococcus epidermis-A233	–	–	13	62.50	12 (SCF)	15.62
Streptococcus pyogenes-ATCC-176	–	–	–	–	10 (OFX)	62.50
Streptococcus pyogenes-KUKEM-676	–	–	–	–	13 (OFX)	31.25
Xanthomonas campestris-A235	–	–	–	–	20 (SCF)	31.25

–: No inhibition zone and/or MIC value measured.
[a] DD = Diameter of disc diffusion (mm); OFX = Ofloxacin (10 μg/disc); SCF = sulbactam (30 μg)+cefoperazona (75 μg) (105 μg/disc) and NET = Netilmicin, (30 μg/disc) were used as positive reference standards antibiotic discs (Oxoid).
[b] DD = Inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 300 μg/disc of methanol extract.
[c] DD = Inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 10 μl of essential oil.
[d] Minimal inhibitory concentrations as (μg/ml).
[e] MIC = Maxipine (μg/ml) was used as reference antibiotic in micro well dilution assay (Sigma).

Table 4
Anticandidal and antifungal activities of *Mentha longifolia* ssp. *longifolia* essential oil against the yeast and fungi isolates tested

Test yeast and fungi	Plant extract (MeOH)		Essential oil		Antibiotics	
	DD[b]	MIC[d]	DD[c] (mm)	MIC[d]	DD[a]	MIC[e] (Amp B)
Yeast *Candida albicans*-A117	–	–	28	125	– (NET)	31.25
Fungi						
Alternaria alternata	–	–	32	125	– (NET)	15.62
Aspergillus flavus	–	–	35	31.25	– (NET)	15.62
Aspergillus variecolor	–	–	23	31.25	– (NET)	62.50
Fusarium acuminatum	–	–	35	62.50	– (NET)	62.50
Fusarium oxysporum	–	–	35	62.50	– (NET)	125
Fusarium solani	–	–	–	–	– (NET)	125
Fusarium tabacinum	–	–	20	31.25	– (NET)	62.50
Moliniana fructicola	–	–	23	62.50	– (NET)	62.50
Penicillum spp.	–	–	32	31.25	– (NET)	31.25
Rhizopus spp.	–	–	15	125	– (NET)	125
Rhizoctonia solani	–	–	35	62.50	– (NET)	31.25
Sclorotinia minor	–	–	20	31.25	– (NET)	125
Sclorotinia sclerotiorum	–	–	23	31.25	– (NET)	62.50
Trichophyton mentagrophytes	–	–	35	31.25	– (NET)	15.62
Trichophyton rubrum	–	–	12	31.25	– (NET)	31.25

[a] DD = Diameter of disc diffusion (mm); NET = Netilmicin, (30 μg/disc) were used as positive reference standards antibiotic discs (Oxoid).
[b] DD = Inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 300 μg/disc of methanol extract.
[c] DD = Inhibition zone in diameter (mm) around the discs (6 mm) impregnated with 10 μl of essential oil.
[d] Minimal inhibitory concentrations as (μg/ml).
[e] MIC = Amphotericin B (μg/ml) was used as reference antibiotic in MIC agar dilution (Sigma).

Based on these results, it is possible to conclude that the essential oil has stronger and broader spectrum of antimicrobial activity as compared to the methanol extract. There is evidence in the literature that Gram-positive bacteria are more sensitive to plant oil and extracts than Gram-negative bacteria (Cosentino et al., 1999; Karaman et al., 2003; Sahin et al., 2002). However, the results in this study showed that essential oil of *M. longifolia* ssp. *longifolia* did not have selective antimicrobial activities on the basis of the cell well differences of bacterial microorganisms. This result may be explained by the high content of *cis*-piperitone epoxide (18.4%), pulegone (15.5%) and piperitenone oxide (14.7%) in the essential oil of *M. longifolia* ssp. *longifolia* analysed in the present study. Antibacterial and antifungal activities of these substances have been reported in the previous studies (Karaman et al., 2003; Kitic, Jovanovic, Ristic, Palic, & Stojanovic, 2002; Sahin et al., 2002). This is the first study to provide data that essential oil of *M. longifolia* ssp. *longifolia* plants evaluated against a wide range of microorganisms possess potential antibacterial, antifungal and anticandidal activities that are comparable to standard drugs. This result may indicate that essential oil of *M. longifolia* ssp. *longifolia* may be used as natural preservatives in food against the well known causal agents of foodborne diseases and food spoilage such as *Escherichia coli*, *Enterobacter* spp., *Bacillus* spp., *Salmonella* spp., *Staphylococcus aureus*, *Candida* spp., *Fusarium* spp., *Aspergillus* spp., *Rhizopus* spp., and *Penicillium* spp. isolates.

4. Conclusion

The results of this work have shown that *M. longifolia* ssp. *longifolia* possess compounds with antimicrobial and antioxidant properties. A further study *in vivo* conditions is also necessary to confirm antimicrobial and antioxidant activities of *M. longifolia* ssp. *longifolia*, which may be used for preservation and/or extension the self-life of raw and processed foods as well as pharmaceuticals and natural therapies of infectious diseases in human, and management of plant diseases. In addition, the present study is provided an additional data for supporting the use of *M. longifolia* ssp. *longifolia* plants as tea or additive in foods, and traditional remedies for the treatment of infectious diseases.

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References

Adams, R. P. (2001). *Identification of essential oils components by gas chromatography/quadrupole mass spectroscopy*. Allured Publishing Corporation, Illinois, USA, pp. 456.

Alzoreky, N. S., & Nakahara, K. (2003). Antimicrobial activity of extracts from some edible plants commonly consumed in Asia. *International Journal of Food Microbiology, 80*, 223–230.

Baser, K. H. C., Ozek, T., Kurkcuoglu, M., & Tumen, G. (1992). Composition of the essential oil of Origanum sipyleum of Turkish origin. *Journal of Essential Oil Research, 4*(2), 139–142.

Burits, M., & Bucar, F. (2000). Antioxidant activity of *Nigella sativa* essential oil. *Phytotheraphy Research, 14*, 323–328.

Chandler, S. F., & Dodds, J. H. (1983). The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum lacinitum*. *Plant Cell Reports, 2*, 105.

Cosentino, S., Tuberoso, C. I. G., Pisano, B., Satta, M., Mascia, V., Arzedi, E., et al. (1999). In vitro antimicrobial activity and chemical composition of Sardinian *Thymus* essential oils. *Letters in Applied Microbiology, 29*, 130–135.

Cowan, M. M. (1999). Plant products as antimicrobial agents. *Clinical Microbiology Reviews, 12*, 564–582.

Cuendet, M., Hostettmann, K., & Potterat, O. (1997). Iridoid glucosides with free radical scavenging properties from *Fagraea blumei*. *Helvetica Chimica Acta, 80*, 1144–1152.

Daferera, D. J., Ziogas, B. N., & Polissiou, M. G. (2003). The effectiveness of plant essential oils on the growth of *Botrytis cinerea*, *Fusarium* sp. and *Clavibacter michiganensis* subsp. Michiganensis. *Crop Protection, 22*, 39–44.

Dapkevicius, A., Venskutonis, R., Van Beek, T. A., & Linssen, P. H. (1998). Antioxidan activity of extracts obtained by different isolation procedures from some aromatic herbs grown in Lithuania. *Jounal of the Science of Food and Agriculture, 77*, 140–146.

Davis, J. (1994). Inactivation of antibiotics and the dissemination of resistance genes. *Science, 264*, 375–382.

Deak, T., & Beuchat, L. R. (1996). *Handbook of food spoilage (pp. 224)*. New York, USA: CRC Press (pp. 224).

Economou, K. D., Oreopolou, O., & Thomopoulos, C. D. (1991). Antioxidant activity of some plant extracts of the family *Labiatae*. *Journal of the American Oil Chemists' Society, 68*, 109–113.

Ghoulami, S., Il Idrissi, A., & Fkih-Tetouani, S. (2001). Phytochemical study of *Mentha longifolia* of Morocco. *Fitoterapia, 72*(5), 596–598.

Gulluce, M., Adiguzel, A., Ogutcu, H., Sengul, M., & Sahin, F. (2004a). Antimicrobial effects of *Quercus ilex* L. extract. *Phytotherapy Research, 18*, 208–211.

Gulluce, M., Sokmen, M., Sahin, F., Sokmen, A., Adiguzel, A., & Ozer, H. (2004b). Biological activities of the essential oil and methanolic extract of *Micromeria fruticosa* (L) Druce ssp. *serpyllifolia*(Bieb) PH Davis plants from the eastern Anatolia region of Turkey. *Journal of The Science of Food and Agriculture, 84*, 735–741.

Gul, H. I., Ojanen, T., & Hanninen, O. (2002). Antifungal evaluation of bis Mannich bases derived from acetophenones and their corresponding piperidinols and stability studies. *Biological and Pharmaceutical Bulletin, 25*, 1307–1310.

Iscan, G., Kirimer, N., Kurkcuoglu, M., Baser, K. H. C., & Demirci, F. (2002). Antimicrobial screening of *Mentha piperita* essential oils. *Journal of Agricultural and Food Chemistry, 50*(14), 3943–3946.

Jaimand, K., & Rezaee, M. B. (2002). Chemical constituents of essential oils from *Mentha longifolia* (L.) Hudson var. *asiatica* (Boriss.) Rech. f. from Iran. *Journal of Essential Oil Research, 14*(2), 107–108.

Karaman, I., Sahin, F., Gulluce, M., Ogutçu, H., Sengul, M., & Adiguzel, A. (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedrus* L. *Journal of Ethnopharmacology, 85*, 231–235.

Kaur, C., & Kapoor, H. C. (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology, 37*(2), 153–161.

Kitic, D., Jovanovic, T., Ristic, M., Palic, R., & Stojanovic, G. (2002). Chemical composition and antimicrobial activity of the essential oil of *Calamintha nepeta* (L.) Savi ssp. *glandulosa* (Req.) P.W. Ball from Montenegro. *Journal of Essential Oil Research, 14*(2), 150–152.

Kokkini, S., Karousou, R., & Lanaras, T. (1995). Essential oils of spearmint (Carvone-rich) plants from the island of Crete (Greece). *Biochemical Systematics and Ecology, 23*(4), 425–430.

Kothari, S. K., & Singh, U. B. (1995). The effect of row spacing and nitrogen fertilization on scotch spearmint (*Mentha gracilis* Sole). *Journal of Essential Oil Research, 7*, 287–297.

Lange, B. M., & Croteau, R. (1999). Genetic engineering of essential oil production in mint. *Current Opinion in Plant Biotechnology, 2*, 139–144.

Lin, J., Opoku, A. R., Geheeb-Keller, M., Hutchings, A. D., Terblanche, S. E., Jager, A. K., et al. (1999). Preliminary screening of some traditional Zulu medicinal plants for anti-inflammatory and anti-microbial activities. *Journal of Ethnopharmacology, 68*, 267–274.

Mead, P. S., Slutsker, L., Dietz, V., McCaig, L. F., Breese, J. S., Shapiro, C., et al. (1999). Food related illness and dead in the United States. *Emerging Infectious Diseases, 5*, 607–625.

Mimica-Dukic, N., Popovic, M., Jakovljevic, V., Szabo, A., & Gasic, O. (1999). Pharmacological studies of *Mentha longifolia* phenolic extracts II. Hepatoprotective activity. *Pharmaceutical Biology, 37*(3), 221–224.

Monfared, A., Nabid, M. R., & Rustaiyan, A. (2002). Composition of a carvone chemotype of *Mentha longifolia* (L.) Huds. from Iran. *Journal of Essential Oil Research, 14*(1), 51–52.

Moreno, L., Bello, R., Primo-Yufera, E., & Esplugues, J. (2002). Pharmacological properties of the methanol extract from *Mentha suaveolens* Ehrh. *Phytotherapy Research, 16*, 10–13.

Murray, P. R., Baron, E. J., Pfaller, M. A., Tenover, F. C., & Yolke, R. H. (1995). *Manual of clinical microbiology* (7th ed.). Washington, DC: ASM (pp. 1773).

Rasooli, I., & Rezaei, M. B. (2002). Bioactivity and chemical properties of essential oils from *Zataria multiflora* Boiss and *Mentha longifolia* (L.) Huds. *Journal of Essential Oil Research, 14*(2), 141–146.

Sahin, F., Karaman, I., Gulluce, M., Ogutcu, H., Sengul, M., Adiguzel, A., et al. (2002). Evaluation of antimicrobial activities *Satureja hortensis* L. *Journal of Ethnopharmacology, 87*, 61–65.

Service, R. F. (1995). Antibiotics that resist resistance. *Science, 270*, 724–727.

Slinkard, K., & Singleton, V. L. (1977). Total phenol analyses: automation and comparison with manual methods. *American Journal of Enology and Viticulture, 28*, 49–55.