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Original Article

Chemical composition and antimicrobial activity of peppermint (*Mentha piperita* L.) Essential oil

Mohaddese Mahboubi* and Nastaran Kazempour

Department of Microbiology, Medicinal Plants Research Center of Barij, Kashan, 1187-87135 Iran.

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Abstract

Peppermint with antiseptic and known healing properties is a plant from the *Labiatae* family. In this study, we analyzed the chemical composition of essential oil from the flowering aerial part of peppermint by GC and GC/MS. Its antimicrobial activity was evaluated against bacteria, fungi and yeast by micro broth dilution assay. The fractional inhibitory concentration (FIC) and FIC Index (FICI) and related isobologram curve were determined by check board micro titer assay. The results exhibited that the MIC, MLC value of peppermint oil against different kinds of microorganisms were in the range of 0.125-2 and 0.125- >64 µl/ml, respectively. *Candida albicans* was the most sensitive microorganism and *Pseudomonas aeruginosa* was the less sensitive ones. The oil showed synergistic activities with vancomycin, gentamycin, and amphotericin B with the FICI less of 0.5. This oil could be used as natural antibiotics and may decrease the effective dose of antibiotics.

Keywords: menthol, peppermint, synergism, antimicrobial activity

1. Introduction

Mentha piperita L. or peppermint with vernacular name of "nana felfeli", a plant from the Labiatae family, is traditionally used as an antiseptic, stimulant, carminative agent or it is further used as a flavoring agent in cosmetic and pharmaceutical industries throughout the world.

According to its antiseptic activities, there are some investigations on its antimicrobial activities. The peppermint essential oil and its ethanol extract exhibited antifungal activity against *Candida albicans*, *C. tropicalis*, *C. glabrata* and *C. parasilosis*, but its infusion did not have any anti-fungal activity (Carretto *et al.,* 2010). Peppermint oil showed good antimicrobial activity against *Aspergilus niger*, *Rhizopus solani* and *Alternaria alternate* (Hussain *et al*., 2010), *Pseudomonas syringe*, *Xanthamonas campestris*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium* (Iscan *et al*., 2002). The antibacterial activity of peppermint leave's juice against Gram negative bacilli was higher than that of its stem juice (Saeed and Tariq, 2005). The antimicrobial activity of peppermint oil against *C. albicans* and *E. coli* was higher than that of *S. aureus*. This oil has good antioxidant activity in two systems of DPPH free radical scavenging and β- carotene/linoleic acid systems (Yadegarinia *et al.,* 2006). Other pharmacological activities, such as antiviral activity against *Influenza*, *Herpes* and other viruses (Kerman and Kucera, 1967) and its antihelmentic effect is confirmed, The antihelmintic effect of peppermint methanol extract is comparable with Albendazole with mechanism of paralysis and death of worms (Girme *et al.*, 2006).

Because of differences in the chemical composition of peppermint essential oil from different parts of world we analyzed the chemical composition of peppermint oil and its antimicrobial effect against a large number of microorganisms and its synergistic effects with vancomycin, gentamycin and amphotericin B against *Staphylococcus aureus*, *E. coli*, *Candida albicans,* and *Aspergilus niger*, respectively.

* Corresponding author.
Email address: mahboubi1357@yahoo.com

2. Materials and Methods

2.1 Plant materials and extraction of peppermint oil

Mentha piperita aerial parts at the beginning of the flowering stage were collected from a research farm of the Medicinal Plant Research Center of Barij in June 2011 and authenticated by H. Hosseini; the herbarium sample is kept under the number 174-1. The aerial samples were grinded and subjected to hydrodistillation by Clevenger type apparatus for 3 hrs. The yellow essential oil was separated and dried by sodium disulfate. The essential oil was kept in a dark vial at a cold place until analysis.

2.2 Essential oil and GC/GC-MS analysis

Essential oil from *Mentha piperita* were analyzed using GC-FID and GC/MS. The GC apparatus was Agilent technology (HP) 6890 system, capillary column of HP-5MS (60 m × 0.25 mm, film thickness 0.25 µm). The oven temperature program was initiated at 40°C, held for 1 min then raised up to 230°C at a rate of 3°C/min, and maintained for 10 min. Helium was used as the carrier gas at a flow rate 1.0 ml/min. The detector and injector temperatures were 250 and 230°C, respectively. GC/MS analysis was conducted on a HP 6890 GC system coupled with a 5973 network mass selective detector with a capillary column the same as above, carrier gas helium with flow rate 1 ml/min with a split ratio equal to 1/50, injector and oven temperature programmed was identical to GC. The compounds of the oil were identified by comparison of their retention indices (RI), mass spectra fragmentation with those stored in the Wiley 7n.1 mass computer library, NIST (National Institute of Standards and Technology) and with data published in the literature (Adams, 2001).

2.3 Microbial strains

This research included following strains: *Staphylococcus aureus* ATCC 25923, *Staphylococcus epidermidis* ATCC 14990, *Staphylococcus saprophyticus* ATCC 15305, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* ATCC 25788, *Streptococcus salivarius* ATCC 9222, *Streptococcus sanguis* ATCC 10566, *Streptocoocus pyogenes* PTCC 1447, *Streptococcus pneumoniae* ATCC 33400, clinical isolate of *Streptococcus agalactiae, Streptococcus sobrinus* ATCC 27607, *Streptococcus mutans* ATCC 35668, *Bacillus cereus* PTCC 1247, *Bacillus subtilis* ATCC 6051, *Escherichia coli* ATCC 8739, *Enterobacter aerogenes* ATCC 13048, *Proteus vulgaris* PTCC 1079, *Salmonella typhimurium* ATCC 14028, *Pseudomonas aeruginosa* ATCC 9027, *Shigella flexeneri* NCTC 8516, *Shigella dysenteriae* PTCC 1188, *Klebsiella pneumoniae* ATCC 10031, *Candida albicans* ATCC 10231, *Candida glabrata* ATCC 90030, a farm isolate of *Aspergillus flavus*, *Aspergillus niger* ATCC 16404, *Aspergillus parasiticus* ATCC 15517. The bacteria and fungi were cultured on Brain Heart infusion Agar and Sabouraud dextrose Agar, respectively. The microbial suspensions were prepared in normal saline and the turbidity of microbial suspensions was adjusted to 0.5 McFarland by spectrophotometrical method (wave length 600 and 520 nm for bacteria and fungi, respectively).

2.4 Antimicrobial evaluation by micro broth dilution assay

Micro broth dilution assay were done as recommended instruction of NCCLS (NCCLS 2009). We used the standard antibiotics such as vancomycin, gentamycin, and amphotericin B as positive controls for Gram positive, Gram negative bacteria and fungi, respectively. The peppermint oil was dissolved in dimethyl sulfoxide as solvent; then the oil was diluted in distilled water in two serial dilutions. The oil concentrations were in the ranges of 16- 0.25 µl/ml. 100 µl of each dilution from the oil were poured in each well, then the 100 µl of above microbial suspension was added to 20 ml Muller Hinton broth (for non-fastidious bacteria), RPMI 1640 (for fungi) or Todd Hewitt broth (fastidious bacteria) as broth media. Then 100 µl of diluted suspension were added to each serial dilution of oil. The plates were incubated at unsuitable conditions. After that, the first dilution of oil that did not show any turbidity was used as minimal inhibitory concentration (MIC), and the first well that did not show any growth on solid media was used as minimal lethal concentration (MLC).

2.5 Check board titer test for evaluation of synergistic effect

Eight serial, two fold dilutions of peppermint oil and each antibiotics (vancomycin, gentamycin and amphotericin B) were prepared as used in the MIC tests. 50 µl of each dilution of oil was added to the wells of 96-well plates in vertical orientation and 10 µl of antibiotic dilutions was added in horizontal orientation. 100 µl of microbial suspension (10^4, 10^6 CFU/ml for fungi and bacteria, respectively) was added to each well and incubated at 35°C for 24 hrs. Fractional inhibitory concentrations (FICs) were calculated as the MIC of the combination of oil and antibiotic divided by the MIC of oil or antibiotic alone. The FIC index was the sum of FIC from oil and antibiotic and interpreted as showing synergistic effect when it was $\leq$0.5, as indifferent when it was >0.5-2 and as antagonistic when it was >2.0. The synergic effect is shown graphically by applying published Isobole methods (Wagner and Ulrich-Merzenich, 2009).

3. Results and Discussion

3.1 Chemical composition of peppermint oil

Forty three components were identified in peppermint oil accounting for 99.8% of total oil. Menthol (36.9%), menthone (28.8%) and methyl acetate (4.5%) were the main components of peppermint oil followed by carveone (3.8%), neomenthol (3.8%), 1,8-cineole (3.8%) and limonene (3.29%)

(see Table 1). Many articles reported menthol as the main component of peppermint oil (Iscan *et al.,* 2002; Behnam *et al.,* 2006; Sokovic *et al.,* 2009; Hussain *et al.,* 2010) and menthone and limonene were the second component of peppermint oil from the Iscan *et al.* (2002) and Hussain *et al.* (2010) studies, respectively, but one report showed β-terpinene, piperitone oxide as the main components of peppermint oil (Yadegarinia *et al.,* 2006). Therefore menthol is not always the primary component of peppermint oil. The chemical composition of peppermint oil from this study is comparable to Iscan *et al.* (2002). Menthol and menthone are the main components of the oil.

3.2 Antimicrobial activity of peppermint oil

The results for MIC and MLC (µl/ml) assay are shown in Table 2. The MIC and MLC values of peppermint oil were in the ranges of 0.125-1, 0.125- > 64 µl/ml, respectively. The MIC and MLC values for Gram positive bacteria were 0.25-1 and 0.5- >64 µl/ml. The peppermint oil exhibited inhibitory effect against *B. cereus* (MIC, MLC = 0.25, > 64 µl/ml). The MLC values of peppermint oil against *E. faecalis* and *E. faecium* were higher than those of the other Gram positive bacteria. The oil had bactericidal effect against *S. mutans*, *S. typhimurium*, *P. aeruginosa*, *Sh. flexeneri*, *Sh. dysenteriae, C. albicans* and *A. parasiticus*. Among the tested micro-organisms, the MIC and MLC values of *P. aeruginosa* were higher than those of others (MIC and MLC = 16 µl/ml). *C. albicans* showed more sensitivity to peppermint oil and the oil had bactericidal effect on it (MIC = MLC = 0.125 µl/ml). Among the fungi, *A. niger* was more sensitive than other fungi, such as *A. flavus* and *A. parasiticus* (0.5 versus 1 µl/ml). *K. pneumoniae* and *E. aerogenes* with MIC and MLC values 0.25 and 0.5 µl/ml were more sensitive than those of other Gram negative bacteria. The MIC value of *B. cereus* was smaller than that of other microorganisms, but its MLC value was higher than that of the resistant bacteria such as *P. aeruginosa* (> 64 µl/ml versus 16 µl/ml).

The chemical composition of essential oils may have an effect on their antimicrobial activities; therefore, a comparison between the results of our study with other investigations is not always possible. The chemical composition analysis of peppermint from this study is similar to Iscan *et al.* (2002) that showed the presence of menthol and menthone as the main components of peppermint oil (Iscan *et al.,* 2002). Iscan *et al.* (2002) showed *C. albicans* as the most sensitive microorganism (MIC = 0.312 mg/ml) to peppermint oil followed by *S. epidermidis* (MIC =0.625 mg/ml), *B. cereus*, *S. thyphimurium*, *E. aerugenes*, *S. aureus,* and *E. coli* (MIC = 1.25 mg/ml). *P. aeruginosa* with MIC value of 5 mg/ml was more resistant that others (Iscan *et al.,* 2002). Iscan and his colleagues has not been reported the MLC values of essential oils for the tested microorganisms, but the MIC values of peppermint oil from their study are comparable to our study.

3.3 Evaluation of synergistic activity of peppermint oil and antibiotics

For the evaluation of the synergistic effect of peppermint oil with vancomycin, amphotericin B and gentamycin,

Table 1. Chemical composition of peppermint essential oil

(%)	RI*	Compound
0.05	765	3-hexen-1-ol
0.04	843	3-methylcyclohexanone
0.05	849	α-phellandrene
0.55	855	α-pinene
0.38	892	Sabinene
0.80	895	β-pinene
0.32	912	β- myrcene
0.29	913	3-octanol
0.06	921	Phellandrene
0.19	934	α- terpinene
0.25	937	o-Cymene
3.75	945	1,8-cineole
3.29	948	Limonene
0.20	955	β- ocimene Y
0.32	973	α- terpinene
0.51	976	Trans sabinene hydrate
0.11	1001	α- terpinolene
0.39	1013	Linalool L
0.08	1016	Amyl isovalerate
28.8	1057	L -menthone
1.90	1065	Menthofuran
3.8	1069	Neomenthol
36.9	1090	Menthole
0.63	1094	β- fenchyl alcohol
0.17	1097	Dihydro-carveol
3.82	1127	carveone
0.57	1133	Cis-3-hexenyl isovalerate
0.76	1135	Piperitone
0.06	1139	Hexyl n-valerate
1.26	1165	Trans-anethole
4.54	1183	Methyl acetate
0.25	1192	p-menth-3-ene
0.14	1211	Dihydrocarveol acetate
0.33	1294	β- bourbonene
0.11	1312	n-decyl acetate
1.61	1320	Trans caryophyllene
0.24	1347	Trans beta farnesene
1.30	1359	β- cubebene
0.27	1369	bicyclogermacrene
0.06	1379	γ- cadinene
0.12	1386	σ- cadinene
0.12	1429	Caryophyllene oxide
0.39	1444	Veridiflorol

* Retention Index

Table 2. Antimicrobial activity of peppermint oil against microbial species by microbroth dilution assay.

	Peppermint oil (µl/ml)		Vancomycin (µg/ml)		Gentamycin (µg/ml)		Amphorericin B (µg/ml)	
	MIC	MLC	MIC	MLC	MIC	MLC	MIC	MLC
S. aureus	1	2	0.25	0.25	-	-	-	-
S. epidermidis	1	2	0.25	0.5	-	-	-	-
S. saprophyticus	1	2	0.5	1	-	-	-	-
E. faecalis	2	4	1	2	-	-	-	-
E. faecium	2	4	1	2	-	-	-	-
S. pyogenes	1	2	0.125	0.25	-	-	-	-
S. pneumoniae	1	2	0.25	0.5	-	-	-	-
S. agalactiae	1	2	0.5	1	-	-	-	-
S. sobrinus	0.25	0.5	0.25	0.5	-	-	-	-
S. mutans	0.25	0.25	0.25	0.25	-	-	-	-
B. cereus	0.25	>64	0.25	8	-	-	-	-
B. subtilis	0.5	1	0.125	0.25	-	-	-	-
S. salivarius	1	2	0.5	1	-	-	-	-
S. sanguis	0.25	0.5	2	4	-	-	-	-
E. coli	1	1	-	-	0.25	0.25	-	-
E. aerugenes	0.25	0.5	-	-	0.25	0.5	-	-
S. typhimurium	2	2	-	-	1	2	-	-
P. aeruginosa	16	16	-	-	0.25	0.5	-	-
Sh. flexeneri	0.5	0.5	-	-	0.25	0.5	-	-
Sh. dysentariae	0.5	0.5	-	-	0.25	0.5	-	-
K. pneumoniae	0.25	0.5	-	-	0.25	0.25	-	-
C. albicans	0.125	0.125	-	-	-	-	0. 25	0.5
C. glabrata	0.25	0.5	-	-	-	-	1	1
A. flavus	1	2	-	-	-	-	0.5	1
A. niger	0.5	1	-	-	-	-	0.5	0.5
A. parasiticus	1	1	-	-	-	-	0.5	1

MIC= Minimal Inhibitory Concentration, MLC= Minimal Lethal Concentration

Table 3. Fractional Inhibitory Concentration (FIC) and FIC Indices.

	FIC peppermint oil	FIC Antibiotic	FICI
S. aureus	0.125	0.0625	0.1875
E. coli	0.0035	0.05	0.0535
C. albicans	0.028	0.1	0.128
A. niger	0.014	0.025	0.039

we measured the FIC and FICI of peppermint oil and each antibiotic against *S. aureus*, fungi (*C. albicans*, *A. niger*) and *E. coli*, respectively. The results were shown in Table 3. The FIC values for peppermint oil against *S. aureus*, *E. coli*, *C. albicans* and *A. niger* were 0.125, 0.0035, 0.028, and 0.014, respectively. The FIC of vancomycin, gentamaycin, amphotericin B were 0.0625 (*S. aureus*), 0.05 (*E. coli*), 0.1 (*C. albicans*), and 0.025 (*A. niger*). The FICI of vancomycin, gentamaycin, amphotericin B with peppermint oil were 0.1875, 0.0535, 0.128, and 0.039 against *S. aureus*, *E. coli*, *C. albicans* and *A. niger*, respectively. Therefore, peppermint oil has synergistic effects with vancomycin, gentamaycin, and amphotericin B.

There is one report that evaluates the synergistic activity of peppermint with 13 antibiotics against *S. aureus* using the Kirby & Bauer method. Tetracyclin, chloramphenicol, netilmicin, erythromycin, gentamycin and oxacillin exhibited synergistic effect while vancomycin, penicillin, cefoxitin, cotrimoxazol and ofloxacin have no synergism (Betoni *et al*., 2006). In our study, we evaluated the synergistic effect of peppermint oil and some antibiotics by check board microtitre assay. Although the methods and plant

materials were different, our essential oil showed synergistic effect with vancomycin and gentamycin, while with the disc diffusion method of Betoni *et al.* (2006) the extract has synergistic effects with gentamycin and it has no effects on vancomycin.

The synergic effect of peppermint oil and antibiotics are shown graphically by the isobologram diagram as shown in Figure 1. The peppermint oil and three antibiotics are shown by a convex isobole. The FICI of peppermint oil and chemical antibiotics such as vancomycin, gentamycin and amphotericin B was lower than 0.5 and showed convex isobole. Therefore, peppermint may be used as a synergistic agent for lowering the dose of antibiotics.

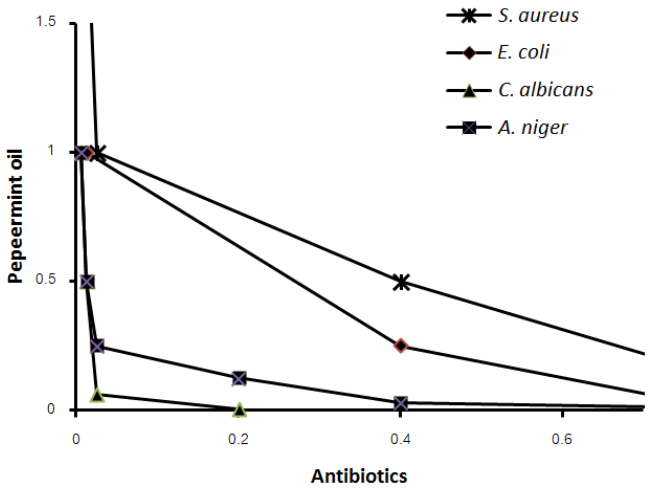

Figure 1. Isobole curve revealing the synergistic effect of peppermint oil and antibiotics.

4. Conclusion

In conclusion, peppermint oil with menthol and menthone as the main components exhibits high antimicrobial activities against Gram positive, Gram negative, yeast and fungi especially against *C. albicans*. The combination of peppermint oil with antibiotics could be used to reduce the effective dose of antibiotics and its related side effects. Further research is needed for demonstration its efficiency against clinical resistant isolate of microorganisms or evaluating the efficacy in clinical practice on infectious patients.

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