ORIGINAL ARTICLE

African peppermint (*Mentha piperita*) from Morocco: Chemical composition and antimicrobial properties of essential oil

Chraibi Marwa, Kawtar Fikri-Benbrahim, Douae Ou-Yahia, Abdellah Farah[1]

Department of Biology, Laboratory of Microbial Biotechnology, Faculty of Science and Technology Saïss, Sidi Mohamed Ben Abdellah University, [1]Department of Chemistry, Laboratory of Organic Chemistry, Faculty of Science and Technology Saïss, Sidi Mohamed Ben Abdellah University, Fez, Morocco

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ABSTRACT

To replace and avoid synthetic chemicals toxicity, there is a growing interest in the investigation of natural products from plant origin for the discovery of active compounds with antimicrobial properties. This work was devoted to determine chemical composition and antimicrobial properties of the EO of *M. piperita* harvested in the garden of the National Institute of Medicinal and Aromatic Plants of Morocco. Experiments have been conducted at the Microbial Biotechnology Laboratory at the Sciences and Technology Faculty, Sidi Mohamed Ben Abdellah University, Fez, Morocco. *M. piperita* oil was screened for its antimicrobial activity against seven bacteria and two fungi using broth microdilution method. Chemical EO analysis was performed using CPG/MS. The EO revealed menthol (46.32%), menthofuran (13.18%), menthyl acetate (12.10%), menthone (7.42%), and 1,8-cineole (6.06%) as the main constituents. The tested EO exhibited strong inhibitory effect against all tested microorganisms with minimum inhibitory concentrations ranging from 0.062% to 0.5% (v/v), except for *Pseudomonas aeruginosa* that was the least sensitive and was only inhibited by concentrations as high as 0.5% (v/v). The studied EO showed an antimicrobial potential. This reinforces its use as an alternative to chemical additives that can be applied to the food and drug industry.

Key words: Antimicrobial activity, chemical composition, essential oil, *Mentha piperita*, Morocco

INTRODUCTION

The extensive use of chemical antimicrobial and antiseptic agents in human mediation as well as in animal breeding led to the selection of resistant strains permitting the development of resistance, which is a biological phenomenon very hard to remove. There are a few decades; several diseases appeared under control using antibiotics. Scientific and technological progress believed in a possible eradication of many diseases;[1] however, the resistance developed increasingly by microorganisms and the regular emergence of new infectious agents have denied this optimistic prognosis.

Faced with this phenomenon, the discovery of new antibacterial molecules that could provide an alternative to

Address for correspondence:
Prof. Kawtar Fikri-Benbrahim,
Laboratory of Microbial Biotechnology, Faculty of Science and Technology Saïss, Sidi Mohamed Ben Abdellah University,
P.O. Box 2202, Fez, Morocco.
E-mail: kawtar.fikribenbrahim@usmba.ac.ma

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the use of conventional antibiotics which became ineffective seems an absolute necessity. The research tracks are numerous, but the exploration of natural resources appears to be more promising because they constitute, by their biodiversity, the largest reserve of active substances and especially the medicinal and aromatic plants, which are the source of high-value products, such as essential oils (EOs).

The genus *Mentha*, known as peppermint, is a cultivated natural hybrid of *Mentha aquatic L.* (water mint) and *Mentha spicata L.*(spearmint). Although being a native genus of the Mediterranean region, it is cultivated all over the world and the EO of this plant has been reported by other works for its insecticidal,[2] antimicrobial,[3] antioxidant effects.[4]

The present study aims to investigate the chemical composition of Moroccan *M. piperita* EO, to assess its antimicrobial activity against seven bacteria and two fungi causing spoiling and pathogenicity, in an attempt to contribute to the use of these as alternative products for microbial control and food preservation.

MATERIALS AND METHODS

Plant material

Fresh aerial part of *Mentha piperita* was harvested from the National Institute of the Medicinal and Aromatic Plants garden in Taounate city (34°32′11″ N, 4°38′24″W, altitude: 600 m) (Morocco).

Essential oils extraction

The fresh aerial parts of *M. piperita* (leaves and stems) were subjected to hydrodistillation for 3 h using a Clevenger-type apparatus. The recovered EO was kept in the dark at 4°C until further use.

Chemical analysis of essential oil

The EO was analyzed using gas chromatography (GC) coupled to mass spectrometry (MS) (GC/MS) (PolarisQ ion trap MS). Hence, analyses were performed on a Hewlett-Packard (HP 6890) gas chromatograph (flame ionization detector [FID]). The temperature was programmed from 50°C after 5 min initial hold to 200°C at 4°C/min. Chromatography carrier gas was N_2 (1.8 ml/min), split mode was used (flow: 72.1 ml/min. ratio: 1/50), temperature of injector and detector was 250°C, and final hold time was 48 min. The machine was led by a computer system type "HP ChemStation," managing its functioning and allowing to follow the evolution of chromatographic analyses. Diluted samples (1/20 in methanol) of 1 µl were injected manually.[3]

Bacterial strain

Tested bacteria include seven isolates of *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Micrococcus luteus* ATCC 14452, *Staphylococcus aureus* ATCC 29213, *Bacillus subtilis* ATCC 6633, *Salmonella typhimurium*, and *Bacillus cereus*. Before use, strains were revivified by subcultures in Luria-Bertani (LB) plates at 37°C for 24 h.

The tested yeast includes *Candida albicans* and *Candida tropicalis*. Their revivification was made by subculturing each strain in yeast extract-peptone-glucose (YPG) agar plates at 30°C for 48 h.

These strains belong to the microbial biotechnology laboratory.

Determination of minimum inhibitory concentration

The minimum inhibitory concentrations (MICs) were determined in 96-well microplate using the microdilution assay according to Balouiri *et al*.[5] with slight modifications. Bacteriological agar at 0.15% (w/v) was used as an emulsifier of the EO in the culture medium. For bacteria, the EO was serially diluted in Muller-Hinton broth supplemented with agar to obtain final concentrations ranging between 8% and 0.007% (v/v). The 12th well was considered as growth control (free-EO control). Then, 50 µl of bacterial inoculum, previously prepared and adjusted to 0.5 McFarland, were added to each well to reach the final concentration of 10^6 CFU/ml. After incubation at 37°C for 24 h, 10 µl of resazurin was added to each well as bacterial growth indicator. After a further incubation at 37°C for 2 h, the bacterial growth was revealed by coloration change from purple to pink.

For yeasts, the microdilution assay was conducted according to the protocol previously described by Clinical and Laboratory Standards Institute[6] with slight modifications. First, the EO was serially diluted in YPG broth, supplemented with agar at 0.15% (w/v), to achieve final concentrations ranging between 4 and 0.003% (v/v). The 12th well was also considered as growth control. Then, 50 µl of inoculum was added to each well at final concentration of 10^3 CFU/ml. Finally, the microplate was incubated at 30°C for 48 h.

Determination of minimal (bactericidal/fungicidal) concentration

The minimal bactericidal/fungicidal concentration (MBC/MFC) was determined by spotting 3 µL from each negative well on LB agar plates for bacteria and YPG for yeasts and incubating at 37°C for 24 h and 30°C for 48 h, respectively.[7]

RESULTS

Chemical composition

The studied EO has been previously subjected to the GC-MS analysis. Twenty-seven constituents, representing 99.51% of the total EO of *M. piperita* L., were identified. Its major constituents were menthol (46.32%), menthofuran (13.18%),

menthyl acetate (12.10%), menthone (7.42%), and 1.8-cineole (6.06%) [Table 1].

Antimicrobial effect of essential oil from *Mentha piperita*

As shown in Table 2, the EO of *M. piperita* exercised an important inhibitory activity against all studied bacteria, especially *M. luteus* and *B. subtilis* which showed a high sensitivity to this oil and were inhibited from very low concentrations of 0.062% (v/v) and 0.125% (v/v), respectively. Furthermore, the concentration of 0.25% (v/v) was sufficient to stop the growth of *S. aureus* and *B. cereus*.

For Gram-negative bacteria, *M. piperita* EO was more effective against EO and *S. typhimurium* with a MIC of 0.5% (v/v). In contrast, *P. aeruginosa* was the least sensitive and was only inhibited by concentrations as high as 0.5% (v/v).

With regard to fungal strains, *M. piperita* EO exhibited a remarkable antifungal effect against both yeast strains tested with an MIC value of 0.062% (v/v) [Table 3].

Regarding the MBC values of *M. piperita* EO tested [Table 4], we found that MBC values could well be similar to their MIC values against *S. aureus*, *B. cereus*, and *M. luteus* and 2-fold higher toward *B. subtilis*, *S. typhi*, and EO and *C. albicans*. In addition, the MFC of *C. tropicalis* was 4-fold higher than its MIC value [Table 5].

Table 1: Chemical composition of *Mentha piperita* essential oil

RI	Constituents	Picsaera (%)
931	α-thujene	0.31
939	α-pinene	0.32
967	Verbenene	0.02
975	Sabinene	1.38
979	β-pinene	0.53
1005	α-phellandrene	0.01
1026	P-cymene	0.03
1031	Limonene	3.01
1033	1,8-cineole	6.06
1070	Cis-sabinene hydrate	0.24
1098	Linalool	0.05
1123	Chrysanthenone	0.42
1152	Menthone	7.42
1164	Menthofuran	13.18
1165	Neomenthol	4.79
1171	Menthol	46.32
1177	terpinen-4-ol	0.04
1189	α-terpineol	0.03
1243	Carvone	1.02
1273	Neomenthylacetate	0.43
1295	Methyl acetate	12.10
1305	Isomenthylacetate	0.82
1352	α-terpinyl acetate	0.03
1388	β-bourbonene	0.37
1409	α-gurjunene	0.03
1418	β-caryophyllene	0.55
1460	α-humulene	0.01
	Total	99.51

RI: Retention index

DISCUSSION

The present study has demonstrated the potential of *M. piperita* EO as an antimicrobial agent in the liquid phase.

The analysis results of *M. piperita* EO showed that its chemical composition is broadly similar to EO of *M. piperita* from Turkey regarding menthol, menthone, menthyl acetate, and menthofuron as main compounds.[8]

Hence, it is clear that menthol is the principal major component of the studied EO, which is in agreement with several other works.[9,10] Regarding other components, our results diverge from those published by other authors; in fact, the team research of Laghouiter *et al*.[11] has found that *M. piperita* from South Algeria is composed by trans-carveol (58.98%), D-limonene (19.94%), carvone (2.07%), and 4-terpineol (3.01%); hence, Yadegarinia *et al*.[12] showed that EO of *M. piperita* from Iran presented a significantly different chemical composition, consisting of α-terpinene (19.7%), isomenthone (10.3%),

Table 2: The minimum inhibitory concentrations of *Mentha piperita* essential oil against bacterial strains tested

Strains	Concentrations (%v/v)										
	8	4	2	1	0.5	0.25	0.125	0.062	0.031	0.015	0.007
Staphylococcus aureus	-	-	-	-	-	-	+	+	+	+	+
Bacillus subtilis	-	-	-	-	-	-	-	+	+	+	+
Bacillus cereus	-	-	-	-	-	-	+	+	+	+	+
Micrococcus luteus	-	-	-	-	-	-	-	-	+	+	+
Escherichia coli	-	-	-	-	-	+	+	+	+	+	+
Salmonella typhi	-	-	-	-	-	+	+	+	+	+	+
Pseudomonas aeruginosa	-	+	+	+	+	+	+	+	+	+	+

+: Microbial growth, -: No growth

Table 3: The minimum inhibitory concentrations of *Mentha piperita* essential oil against fungal strains tested

Strains	Concentrations (%v/v)										
	4	2	1	0.5	0.25	0.125	0.062	0.031	0.015	0.007	0.003
Candida albicans	-	-	-	-	-	-	-	+	+	+	+
Candida tropicalis	-	-	-	-	-	-	-	+	+	+	+

+: Microbial growth, -: No growth

Table 4: The minimum bactericidal concentrations of *Mentha piperita* essential oil against bacterial strains tested

Strains	Concentrations (%v/v)										
	8	4	2	1	0.5	0.25	0.125	0.062	0.031	0.015	0.007
Staphylococcus aureus	-	-	-	-	-	-	+	+	+	+	+
Bacillus subtilis	-	-	-	-	-	-	+	+	+	+	+
Bacillus cereus	-	-	-	-	-	-	+	+	+	+	+
Micrococcus luteus	-	-	-	-	-	-	-	-	+	+	+
Escherichia coli	-	-	-	-	+	+	+	+	+	+	+
Salmonella typhi	-	-	-	-	+	+	+	+	+	+	+
Pseudomonas aeruginosa	+	+	+	+	+	+	+	+	+	+	+

+: Microbial growth, -: No growth

Table 5: The minimum fungicidal concentrations of *Mentha piperita* essential oil against fungal strains tested

Strains	Concentrations (%v/v)										
	4	2	1	0.5	0.25	0.125	0.062	0.031	0.015	0.007	0.003
Candida albicans	-	-	-	-	-	-	+	+	+	+	+
Candida tropicalis	-	-	-	-	-	+	+	+	+	+	+

+: Microbial growth, -: No growth

trans-carveol (14.5%), piperitenone oxide (19.3%) and β-caryophyllene (7.6%).

In fact, the compositions of EO vary significantly because of different species and chemotypes, geographical origin, plant's age and maturity season, and extraction procedure.[13-15]

M. piperita EO is known for its antimicrobial properties which have been reported in several studies.[16,4,8] These antibacterial activities could be mainly attributed to its chemical composition, which is very rich with oxygenated monoterpenes (92.95%) known for their higher efficiency and broader spectrum of antimicrobial activity.

In addition, Bakkali *et al*.[17] stipulate that the major compounds determine the biological EO activity. However, the antimicrobial power might also be attributed to the synergy between the various components of this oil.[18] Furthermore, the influence of minor compounds on antimicrobial activity should be also taken into consideration.

Moreover, among the identified compounds, some were previously reported to have antimicrobial activity including menthol and menthone that have been proven effective against human pathogenic[16,19] and also 1,8-cineole[20] and menthofuran.[21]

In addition, the antimicrobial effects of an EO can be attributed to its action mechanisms at cytoplasmic membrane level. In fact, EOs are selectively absorbed and interfered with the functions of biological cell membrane which involve lysis and loss of membrane integrity, causing damage to essential processes for cell survival.[22]

Hence, the antibacterial activity of EO tested in this study was more marked against Gram-positive than Gram-negative bacteria, which can be explained by the structure of the cell envelope. In fact, Gram-negative bacteria possess an additional membrane which gives them a double protection against toxic agents and hydrophobic compounds. However, the absence of this structure in Gram-positive bacteria makes them sensitive to attacks from external agents.[23]

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Conflicts of interest
There are no conflicts of interest.

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