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ORIGINAL ARTICLE

CHEMICAL COMPOSITION, ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *MENTHA GATTEFOSSEI* MAIRE ESSENTIAL OIL

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Abstract

The aim of this study was to investigate the chemical composition, the *in vitro* antioxidant and antimicrobial activities of the essential oil obtained from dried aerial parts of *Mentha gattefossei* Maire (*Lamiaceae* Family) cultivated in the Republic of Moldova. *Mentha gattefossei* essential oil (MgEO) was isolated by hydrodistillation and its chemical profile was evaluated by GC-MS and GC-FID analysis. The main compounds of MgEO were pulegone (57.36%), neomenthone (28.74%) and D-limonene (1.20%). MgEO exhibited good free radical scavenging activities (EC_{50} = 3.64 ± 0.07 μg/mL in DPPH assay and EC_{50} = 254.12 ± 8.40 μg/mL in $ABTS^{\bullet+}$ assay) and it showed a weak reducing capacity. The essential oil possessed strong antimicrobial activity against *Streptococcus pneumoniae* (ATCC 49619) and it was moderate active against *Staphylococcus aureus* (ATCC 25923). This is the first report on the chemical composition and bioactivity of the essential oil from *Mentha gattefossei* cultivated in the Republic of Moldova.

Rezumat

Acest studiu a avut drept obiectiv investigarea compoziţiei chimice a activităţii antioxidante *in vitro*, precum şi a acţiunii antimicrobiene, a uleiului volatil obţinut din părţile aeriene uscate de *Mentha gattefossei* Maire (familia *Lamiaceae*) (MgEO) cultivată în Republica Moldova. Compoziţia chimică a uleiului volatil obţinut prin hidrodistilare a fost analizată prin GC-MS şi GC-FID. Componenţii principali identificaţi ai MgEO au fost: pulegona (57,36%), neomentona (28,74%) şi D-limonen (1,20%). MgEO a prezentat o bună activitate de chelare a radicalilor liberi (CE_{50} = 3,64 ± 0,07 μg/mL în testul DPPH şi CE_{50} = 254,12 ± 8,40 μg/mL în testul $ABTS^{\bullet+}$) şi o slabă capacitate reducătoare. Uleiul volatil manifestă o activitate antimicrobiană puternică asupra *Streptococcus pneumoniae* (ATCC 49619) şi este moderat activ asupra *Staphylococcus aureus* (ATCC 25923). Acesta este primul studiu referitor la compoziţia chimică şi bioactivitatea uleiului volatil obţinut din *Mentha gattefossei* cultivată în Republica Moldova.

Keywords: *Mentha gattefossei*, essential oil, chemical composition, bioactivities

Introduction

The genus *Mentha* (*Lamiaceae* Family) includes 18 species and 11 naturally occurring hybrids distributed all over the world, mainly in the temperate regions of Europe and Asia [8]. Mint species are one of the most useful aromatic and medicinal plants with a significant economic value for food, pharmaceutical, flavour industries, cosmetics, perfumery, confectionery and alcoholic beverages. In therapeutics, mint plants are used mainly for their spasmolytic, carminative, cholagogue, expectorant, antiemetic and antidiarrheal properties [15]. Many of these effects as well as mint specific sensory qualities are chiefly bounded to the composition of essential oil. *Mentha gattefossei* Maire (Menthe de Perse) is endemic to Morocco and it is used for medicinal purposes and as a food source and also for the extraction of essential oil [5]. This species is listed among the rarest plants in the world and it is mentioned in the IUCN (World Conservation Union) Red List of Threatened Plants as Near Threatened being a plant of global conservation interest [2, 3]. This paper reports for the first time the chemical composition and the antioxidant and antimicrobial activities of the essential oil isolated from *Mentha gattefossei* species cultivated in Republic of Moldova within *ex situ* conservation programmes at international level.

Materials and Methods

Chemicals

All chemicals were of analytical grade and were purchased from Sigma-Aldrich GmbH (Steinheim, Germany) and Merck (Darmstadt, Germany).

Plant material

The aerial parts of *Mentha gattefossei* were harvested during flowering stage in July 2017 from the experimental field of Botanical Garden, Academy of Sciences of Moldova, Chişinău, Republic of Moldova. *Mentha gattefossei* plants were obtained from seeds received by the international exchange with the Botanical Garden from Coimbra, Portugal. A voucher specimen was deposited at the Department of Pharmacognosy, "Grigore T. Popa" University of Medicine and Pharmacy, Iaşi, Romania (MG1/Pharmacog/2017).

Essential oil isolation

100 g of powdered air-dried aerial parts of plants were hydro-distilled for 3 h using a modified Clevenger-type apparatus. The obtained essential oil was subsequently dried over anhydrous sodium sulphate and stored in a sealed dark glass at -4°C until analysis. The extraction yield of MgEO calculated on a dry weight basis was 2.5 % (v/w).

GC and GC-MS analysis

GC analysis was accomplished using an Agilent 6890N gas chromatograph equipped with a flame ionization detector (FID) and a HP-5MS capillary column (30 m × 0.25 mm internal diameter, 0.25 μm film thickness). The injection volume was 0.2 μL MgEO and the split ratio was 50:1. Helium was used as carrier gas at a flow rate of 1.8 mL/min. The following temperature programme was performed: 4°C/min from 60°C to 220°C, 20°C/min from 220°C to 320°C. The GC-MS analysis was carried out on the same instrument coupled with an Agilent 5975C mass spectrometer selective detector with electron impact ionization. The column and GC-MS conditions were the same as the ones described above except that helium flow rate was 0.5 mL/min and the split ratio was 20:1. Mass spectra were acquired in the scan mode (mass range 15 - 450 *m*/*z*) [1, 16].

Identification of components

The components of MgEO were identified by comparison of their Kovats Index (KI) relative to standard solution of C8 - C23 n-alkanes under the same chromatographic conditions, and by matching their mass spectral data with those National Institute of Standards and Technology (NIST) library [12]. Concentration of individual components was calculated based on GC-FID peak areas without correction factors.

In vitro antioxidant assays

DPPH (2,2-diphenyl-1-picryl-hydrazyl radical) radical scavenging assay. The DPPH radical scavenging capability was estimated according to the modified method of Mighri *et al.* [11, 16]. The methanolic dilutions of MgEO with concentrations ranging from 5 μg/mL to 80 μg/mL were used. Butylhydroxianisole (BHA) was used as positive control in all anti-oxidant assays.

ABTS [*2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic) acid*] *radical cation scavenging assay*. The assay was carried out as described by Re *et al.* with minor changes [16], using methanolic dilutions of MgEO with concentrations ranging from 5 to 80 mg/mL.

Reducing Power Assay. The method described by Oyaizu was used to determine the reducing power [16]. The methanolic dilutions of MgEO with concentrations ranging between 0.6336 μg/mL and 10.1384 μg/mL were used.

Antimicrobial activity

Bacterial species. The antimicrobial activity of MgEO was investigated against the following standard bacterial strains: *Pseudomonas aeruginosa* (ATCC 27853) (Gram-negative), *Escherichia coli* (ATCC 25922) (Gram-negative), *Staphylococcus aureus* ATCC 25923 (Gram-positive) and *Streptococcus pneumoniae* (ATCC 49619) (Gram-positive).

Disc diffusion method. *In vitro* antimicrobial activity of MgEO was determined by using the agar-diffusion assay [4]. The test was performed on sterile Petri plates (90 mm diameter) using Mueller Hinton agar inoculated with microbial suspension at a density adjusted to a 0.5 McFarland standard (10^6 CFU/mL). The inoculum was spread on the plates using sterile swabs. The wells with 50 mm diameter have been made in agar and every well was completely filled with 50 μL MgEO. Then, the plates were aerobically incubated for 18 - 24 h, at 35°C. As positive controls, there were used antibiotic discs of amoxicillin (25 μg/disc) and ciprofloxacin (5 μg/disc) placed on the medium surface. After incubation, the growth inhibition zones were measured and recorded in mm.

Statistical analysis

All tests were performed in triplicate, and the results are expressed as mean ± SD. EC_{50} (μg/mL), which is the concentration of MgEO/positive control that reduces 50% of the free-radical concentration, was calculated through linear interpolation between values above and below 50% activity. In reducing power assay, EC_{50} value represents the concentration of MgEO/positive control that leads to an absorbance of 0.5.

Results and Discussion

Chemical composition of essential oil

The hydrodistillation of dried aerial parts of *Mentha gattefossei* gave a green oil with strong minty and herbaceous flavour. Twenty-three compounds were identified representing about 90.87% of total oil composition (Table I). The major components were pulegone (57.36%), neomenthone (28.74%) and D-limonene (1.20%). Oxygenated monoterpenes constitute the dominant fraction of MgEO (87.03%) followed by monoterpene hydrocarbons (2.73%). The monoterpene-ketones prevail in the composition of oxygenated monoterpenes (86.53% from 87.03%). To the best of our knowledge, only one study reported data about the chemical composition of *Mentha gattefossei* essential

oil identifying pulegone (56.9%), menthone (30%) and piperitenone (3.4%) as the main compounds [6]. Our results are in agreement with those previously reported. Also, pulegone was detected as major component in the essential oils of *Mentha pulegium* (76% - 78%), *M. canadensis* (1.5% - 81.5%), *M. cervina* (31.7% - 60.8%), or *M. arvensis* (54.6%) while menthone is one of the main components of *M. piperita* essential oil (8.1% - 31.6%) [9, 10]. Among *Mentha* species, *Mentha gattefossei* is closely related to *M. cervina* as suggests the evidence of chloroplast DNA sequences for assessing the phylogenetic relationships in *Mentha* genus [3].

Table I

Chemical composition of *Mentha gattefossei* essential oil

Peak	Compound	RI[a]	%
1	α-Pinene	939	0.52
2	Camphene	952	0.03
3	β-Pinene	980	0.85
4	β-Myrcene	990	0.08
5	Octanal	1002	0.05
6	Pseudolimonene	1018	0.01
7	*p*-Cymene	1024	0.08
8	**D-Limonene**	1027	**1.20**
9	β-Phellandrene	1030	0.03
10	Eucalyptol	1033	0.33
11	γ-Terpinene	1062	0.01
12	α-Campholenal	1127	0.02
13	Hexenyl isobutanoate	1142	0.41
14	Pinocarveol	1146	0.11
15	**Neomenthone**	1158	**28.74**
16	Myrtenol	1184	0.04
17	**Pulegone**	1236	**57.36**
18	Piperitone	1251	0.43
19	*o*-Cymen-5-ol	1334	0.03
20	Phenylbutyrate	1245	0.05
21	Mint furanone	1291	0.01
22	Hydroxyacetophenone	1447	0.06
23	Caryophyllene oxide	1578	0.42
Group of constituents (%)			
Monoterpene hydrocarbons		2.73	
Oxygenated monoterpenes		87.03	
Sesquiterpenes		0.42	
Aromatic compounds		0.22	
Non-terpenoid aliphates		0.47	
Total identified (%)		**90.87**	

[a]Retention indices relative to C8 - C20 n-alkanes calculated on HP-5MS capillary column

In vitro antioxidant activity

MgEO was screened for its antioxidant activities *in vitro* using free radicals (DPPH and ABTS•$^+$) scavenging assays and also the reducing power assay. DPPH scavenging activity of MgEO increased dose-dependently from 36.88 ± 0.81% at 2.5 μg/mL to 81.51 ± 0.63% at 40 μg/mL (Figure 1A). Also, ABTS•$^+$ scavenging activity of MgEO ranged from 13.40 ± 0.33% at 50 μg/mL to 84.56 ± 0.11% at 800 μg/mL after 6 min reaction time (Figure 1B). The positive control, BHA almost completely scavenged the DPPH radical (94.96 ± 0.35%) at 10 μg/mL and it exhibited 100% ABTS•$^+$ scavenging effects at 200 μg/mL (Figure 1). MgEO was more effective as DPPH free radical scavenger than as ABTS•$^+$ inhibitor (Table II). Also, MgEO was similarly effective as BHA in terms of DPPH scavenging activity (EC_{50} = 3.64 ± 0.07 *vs.* EC_{50} = 3.30 ± 0.15) (Table II). Regarding the reducing power assay, MgEO showed a weak reducing capacity. Thus, EC_{50} in this assay could not be calculated because MgEO showed an absorbance value of 0.2 at a concentration of 0.4848 μg/mL, and the increase of the tested concentration was not possible due to low solubility of the oil in the reaction medium. The antioxidant activity of mint essential oils was largely investigated but this is the first study on the antioxidant properties of *Mentha gattefossei* essential oil. Although a direct comparison cannot be achieved, we must say, however, that the MgEO showed more efficient free radical scavenging properties than other *Mentha* essential oils with high concentrations of monoterpene ketones such as pulegone and menthone. Thus, Kamkar *et al.* (2010) reported for the essential oil from Iranian *Mentha pulegium* (with 40.5% pulegone and 35.4% menthone) an EC_{50} value of 14736 μg/mL in DPPH assay. In our study, EC_{50} of MgEO in DPPH assay was 3.64 μg/mL (Table II) [7].

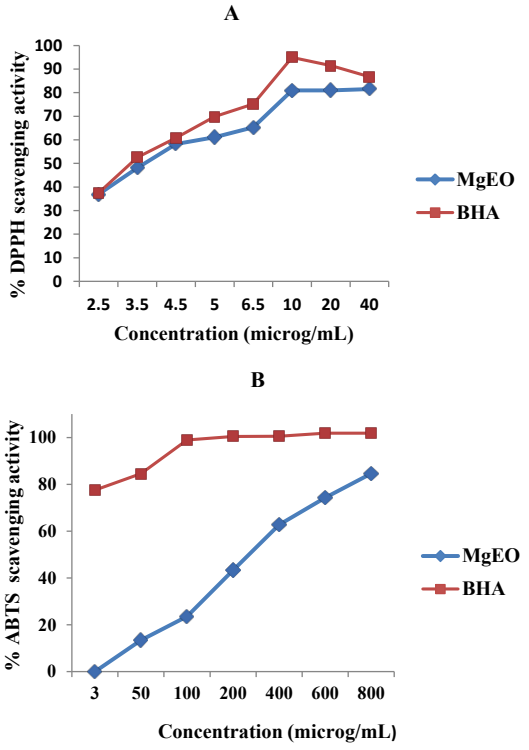

Figure 1.

Free radial scavenging activity of *Mentha gattefossei* essential oil (MgEO): A) DPPH radical scavenging activity; B) ABTS radical cation scavenging activity; BHA, butylhydroxyanisole as positive control

Table II

Antioxidant activity of *Mentha gattefossei* essential oil

Essential oil/positive control	EC_{50} (μg/mL)		
	DPPH assay	$ABTS^{\bullet+}$ assay	Reducing power assay
MgEO	3.64 ± 0.07	254.12 ± 8.40	-
BHA	3.30 ± 0.15	1.9 ± 0.10	4.26 ± 0.10

In vitro antimicrobial activity

The agar-diffusion assay showed a strong antibacterial activity (inhibition zone ≥ 20 mm) of MgEO against *Streptococcus pneumoniae* (ATCC 49619) bacteria but lower than antibiotics used as positive controls (Table III). Also, MgEO showed moderate activity on *Staphylococcus aureus* (ATCC 25923). The standard strains Gram-negative bacteria of *Pseudomonas aeruginosa* and *Escherichia coli* were non-sensitive to MgEO (Table III). Although pulegone, the major component of MgEO, has a potent biocide activity on both Gram-positive and Gram-negative bacteria, however the essential oil behaves differently. The antimicrobial efficacy of the MgEO itself is determined by its entire chemical composition. In this respect, other important components of MgEO such as neomenthone and limonene have proven to be less effective against the tested microorganisms [14]. The higher antimicrobial effects of MgEO against Gram-positive than Gram-negative bacteria, could be explained by the structure of cell envelope. Thus, the structure and the composition of cell wall and outer membrane of Gram-negative bacteria are more complex and contribute significantly to the occurrence of certain resistance to the passage of antimicrobial agents such as essential oils [13].

Table III

The antibacterial activity of *M. gattefossei* essential oil (MgEO) and conventional antibiotics against the tested bacteria

Bacterial strains		Diameter of inhibition zone (mm)		
		MgEO	Standard drugs	
			Amoxicillin	Ciprofloxacin
G-	*Pseudomonas aeruginosa* (ATCC 27853)	0.00 ± 0.00	0.00 ± 0.00	26.33 ± 0.57
G-	*Escherichia coli* (ATCC 25922)	0.00 ± 0.00	25.00 ± 0.57	40.00 ± 0.57
G+	*Staphylococcus aureus* (ATCC 25923)	10.00 ± 0.00	25.00 ± 0.00	30.00 ± 0.57
G+	*Streptococcus pneumoniae* (ATCC 49619)	20.66 ± 1.15	40.00 ± 0.00	30.00 ± 0.00

Conclusions

The results from the present study indicated that the essential oil of *Mentha gattefossei* plants cultivated in the Republic of Moldova is a rich source of monoterpene-ketones as pulegone and neomenthone. Further, it showed good free radical scavenging properties and promising antimicrobial activity on *Streptococcus pneumoniae*.

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