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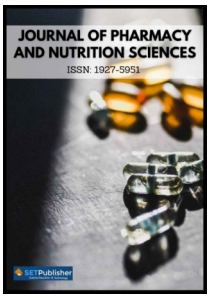

Effects of Controlled Culture Conditions on Chemical Composition and Antimicrobial Activities of *Mentha rotundifolia* Essential Oils

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Abstract:

The study objectives were the determination of *Mentha rotundifolia* essential oils composition using GC/MS analysis and the evaluation of their antimicrobial activities. In addition, the determination of the relationships between plants acclimatization conditions, essential oils composition and antimicrobial activities.

Essential oils extracted via hydrodistillation method from wild plants and acclimatized plants in different culture conditions of *Mentha rotundifolia*. Five selected pathogenic microbial strains were used to evaluate EOs *in vitro* antimicrobial activities.

Essential oils GC/MS analysis revealed the dominance of the oxygenated monoterpene (piperitenone oxide). A significant effect of plant culture conditions in acclimatization room on essential oils composition compared to the control was observed. We also noted that antimicrobial activities of extracted essential oils from acclimatized plants were higher than those from field-grown *M. rotundifolia* plants.

Content of Piperitenone Oxide in acclimatized plantlets is 93.07% for plantlets cultured at 16°C and a photoperiod of 16 hours light / 8 hours dark, statistically higher than wild plants where it is around 78%. We also noted the presence of Limonene (5.7%) in plantlets grown at 35°C which is significantly different than that of the wild plants (1.55 %). Essential oils antimicrobial activities showed that the plantlets grown at 16 °C or in total darkness were more active towards the tested strains. While, the EO of plantlets cultured at 35 °C were the least active.

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1. INTRODUCTION

Mint preserves an infinite variety of uses and occupies a large place in phytotherapeutics. In terms of chemical principles, the smell and activity of most species of mint is due to their essential oils (EOs) or mint essences [1]. EOs are very complex mixtures of organic molecules belonging to the most diverse classes. These molecules are generally hydrocarbons (such as monoterpenes and sesquiterpenes) and oxygen-containing compounds [2].

The agri-food sector uses large quantities of additives. The excessive use of chemical preservatives, such as antimicrobial substances, which are suspect because of their carcinogenic potential, teratogenic actions or residual toxicity, has resulted in increasing pressure on agri-food industry stakeholders to use chemical food additives [3]. Some studies have demonstrated the value of the use of alternative additives. In particular as a preservative of biological origin, due to their antimicrobial potential, for foodstuffs. Many natural compounds, such as phenols and organic acids, considered in this context. Several spices, herbs and extracts possess antimicrobial activity [3] and antioxidant properties [4].

The cultivation of aromatic and medicinal plants, for bioactive constituents extracting, can cope with certain constraints linked to climates, seasons, water availability, diseases and to the scarcity of plants with spontaneous growth. Such limits have led to the use of *in vitro* plant culture techniques for the production of its bioactive compounds [5, 6]. Above ground, technologies significantly reduce the overexploitation of endangered wildlife.

The composition of an essential oil is very fluctuating and depends on many parameters, whether natural, intrinsic, extrinsic or technological [7]. Knowing that, the antimicrobial activity of an EO linked to its composition, the aim of our study is to play on the acclimatization culture conditions in order to influence the chromatographic profile of the EOs of the selected *vitro plants*.

Plant cell cultures are an attractive alternative source for the plant for the production of high value secondary metabolites. *In vitro* plant cultures constitute a promising platform for the production of numerous valuable secondary compounds [5]. The ex-situ biotechnology process could enable to implement a standardized process for the production of ex-situ EO, staggered throughout the year. This would allow the exploitation and preservation of the biological resources in a sustainable way [6].

2. MATERIAL AND METHODS

2.1. Plant Material

The botanical identification of *Mentha rotundifolia* (L.) Huds wild plants was carried out by Pr. Laouar H., Department of Biology, University Ferhat Abbas, Sétif, Algeria. Spontaneous populations of mint were harvested in their natural environment and at an early flowering development stage at Beni Hamidene (long. 6.55, lat. 36.5167, elevation 300 m), 16 km north-west of Constantine, Algeria. Aerial parts of plant samples were transported to laboratory the same day of harvesting in kraft paper bags. The plant material intended for EOs extraction was dried at room temperature.

In vitro plants of *M. rotundifolia* were obtained according to the protocol recommended by Benahmed *et al*., [8, 9] from Wild plants. *Vitro-plants* cultured in an acclimatization room set at 70 % humidity under different acclimatization conditions (Table **1**).

Table 1: Culture Conditions in Acclimatization Room

Plant material	Acclimatization conditions (temperature, light/dark)
Wild plant	/
Culture A	(23°C, 16h/8h)
Culture B	(16°C, 16h/8h)
Culture C	(35°C, 16h/8h)
Culture D	(23°C, 8h/16h)
Culture E	(23°C, total darkness)

2.2. Extraction of Essential Oils

The EO from wild and acclimatized plants was obtained by hydrodistillation of dry plant material (100 g of leaf and stem) using a Clevenger type apparatus. Anhydrous sodium sulfate (Na_2SO_4) used to remove water micro-droplets after extraction. The extracted essential oils were stored in brown hermetic tubes at 4°C.

2.3. GC-MS Analysis

For GC/MS analysis, a Hewlett Packard Agilent 6890 plus equipped with a mass-selective detector Hewlett

Packard Agilent 5973 with quadruples mass analyzer and an HP-5MS-fused silica column (30 m × 0.25 mm × 0.25 µm film thickness) employed. The column temperature programmed as follows: 60°C holds 8 min to 250°C at 2°C/min and then holds 10 min. Helium (N6) carrier gas used at a flow rate of 0.5 ml/min. The detector maintained at 250°C. Sample injection volume was 0.2 µl with a split ratio of 10:1. Mass spectra recorded in the electron-impact (EI) mode at 70 eV by 1.8 scans/s; the mass range used was m/z 30-550; ion source and connecting parts temperatures were 230°C and 270°C respectively [9].

Compounds identification was based on comparison of their mass spectra with those of reference standards in mass spectra libraries (NIST02.L, NBS/Wiley 7N.1), and those published in the literature [10], as well as on comparison of their retention indices (RIs) relative to C8-C28 (n-alkanes).

Quantitative analysis made by the normalization method from the electronic integration of the TIC peak areas without the use of correction factors.

2.4. Antimicrobial Assay

Essential oils tested against five selected pathogenic microbial strains obtained from the American Type Culture Collection (ATCC). These include two gram-positives strains bacteria {*Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923)}, one gram-negative strain bacterium {*Salmonella enteritidis* (ATCC 13076)}, one fungus {*Aspergillus brasiliensis* (ATCC 6404)}, and one yeast {*Candida albicans* (ATCC 10231)}. Those strains supplied by the Pasteur Institute, Algiers (Algeria). The bacterial strains maintained on agar slant at 4°C until EOs antimicrobial activities evaluation.

Microbial cultures growths made in their appropriate medium, Muller-Hinton agar (MHA) for bacteria and Sabouraud dextrose agar (SDA) for fungi as previously reported [11]. Three to five well-isolated and perfectly identical colonies from young bacterial and fungal (18 and 48 h, respectively) cultures were removed by loops and placed into tubes containing 5 ml sterile physiological water at 0.9%. After agitation, the measured transmittance values of bacterial and fungal suspensions cultures adjusted between 22-32% and 2-3%, respectively. These values correspond to a concentration of (10^7 to 10^8 CFU/ml). Petri dishes of 9 cm diameter first filled with 15 ml of the appropriate agar culture medium and left to rest until agar solidification. Then 200 µl of each of the microbial suspensions added aseptically to 50 ml of each of the appropriate liquid culture medium. After agitation, 4 ml of the inoculated agar medium poured as a second layer on the Petri dishes. After agar solidification, blank sterile disks S70150A (diam. 6 mm) impregnated with 10 µL of the EO was placed on the surface of MHA and SDA inoculated culture media. Petri dishes left on the bench for 30 minutes to allow essential oils diffusion. Incubation carried out at 37°C (24 hours) and 25°C (48 hours) for bacteria and fungi cultures respectively. The diameters of growth inhibition zone (mm) carefully measured with a digital caliper. Each treatment repeated three times.

2.5. Data Analysis

All the measurements were replicated three times for each assay and the results were presented as mean ± SD. Data was analysed (ANOVA test and Tukey test) using windows SPSS version 20.0. For compared treatments, P-values ($P<0.05$) were regarded as statistically significant.

3. RESULTS AND DISCUSSION

3.1. Essential Oils Composition

Results of GC-MS analysis of *M. rotundifolia* EOs from natural grown and acclimatized plants grown under different culture conditions (temperature and light) summarized in Table **2**.

According to the results of the qualitative and quantitative analysis, it appears that *M. rotundifolia* EO characterized by the dominance of the oxygenated monoterpene (piperitenone oxide) with a content of 78.70% followed by two monoterpene hydrocarbons: Limonene, β-Pinene with contents of 1.55 and 1.06 % respectively and sesquiterpene (trans-Caryophyllene) 1.36%. These results are close to those reported by Lorenzo *et al*. [12], who's content of the majority constituent of *M. rotundifolia* from Uruguay Piperitenone oxide is 80.8%. Similarly, Riahi *et al*., [4] showed that the leaves' oil of *Mentha rotundifolia* collected from the Bizerte region in Tunisia during the vegetative stage showed: Pulegone (32.09, 2.36 %), Piperitenone oxide (17.28, 3.41 %) and Caryophyllene (3.21, 26.67%) as the main constituents. Another study reported that the main compounds of the oils obtained from *M. rotundifolia* aerial parts at flowering stage and sampled from the National Park of El-Kala (North-East Algeria) was characterized by Rotundifolone 65.99 %

Table 2: Essential Oils Chemical Composition of *Mentha rotundifolia* Wild and Acclimatized Plants

Compounds	RI	Percentage					
		Wild plants	Acclimated plants				
			Culture A	Culture B	Culture C	Culture D	Culture E
Ethyl 2-methylbutanoate	843	0,05	0,31	0,16	0,12	0,07	0,19
α-Pinene	932	0,99	0,83	0,37	0,62	0,64	0,87
Camphene	946	0,18	-	-	-	-	-
Sabinene	971	0,39	0,36	0,14	0,13	0,30	-
l-β-Pinene	974	1,06	0,91	0,45	0,68	0,73	0,90
1-Octen-3-ol	979	0,32	0,57	-	-	0,17	0,21
β-Myrcene	990	0,54	0,43	0,09		0,32	
Limonene	1027	1,55	0,95	0,54	5,78	0,46	0,68
Eucalyptol (1,8 cineole)	1030	0,14	0,26	0,11	-	0,15	0,24
Cis-Ocimene	1037	0,15	0,12	-	-	0,09	-
γ-Terpinene	1057	0,05	-	-	-	-	-
Trans-Sabinene hydrate	1266	0,11	-	-	-	-	-
1-Nonen-3-ol	1081	0,06	-	-	-	-	-
Cis-Sabinene hydrate	1097	0,08	-	-	-	-	-
Octen-1-ol, acetate	1110	0,60	-	0,45	0,48	0,73	1,84
1 Octen 3 yl acetate	1114	-	1,57	-	-	-	-
3-Octyl acetate	1126	0,04	0,31	0,10	0,11	-	0,40
Cyclohexanone, 3-vinyl-3-methyl-	1132	0,33	-	-	-	0,17	-
2,4,5-Trimethylfluorobenzene	1134	-	0,25	-	-	-	0,32
Borneol	1163	0,93	-	--	-	-	-
L-Menthone	1163	-	-	0,13	0,32	-	-
4-Terpineol	1175	0,34	0,14	-	0,22	-	-
p-Cymen-8-ol	1185	0,17	0,36	0,13	0,21	0,51	0,88
α-Terpineol	1189	0,43	0,08	-	0,19	0,17	0,16
α-Methyl cinnamic aldehyde	1210	0,13	0,06	-	-	-	-
(E)-2,3'-Dimethyl-5-(1',3'-butadien-1'-yl) furan	1216	0,39	-	-	-	-	-
Trans-(+)-Carveol	1219	-	-	-	0,24	-	-
Cis-3-Hexenyl isovalerate	1237	0,21	0,09	-	-	-	-
Pulegone	1238	-	-	-	0,18	-	-
l-Carvone	1243	0,07	-	-	0,21	-	-
1,1'-Bicyclopentyl	1263	0,53	-	-	-	0,55	0,16
3-Dodecyne	1263	-	0,66	-	-	-	-
3-methylcyclohex-3-en-1-one	1263	-	-	-	0,62	-	-
3-methyl- 2-Cyclopenten-1-one,	1265	0,28	-	0,19	-	0,23	-
2,4-Dimethylfuran	1266	-	0,27	-	0,36	-	0,31
Isophorone	1271	0,35	0,16	-	0,38	-	-
(1'-butenyl) Thiophene	1287	0,23	0,22	-	-	-	-
2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-(2.alpha.,4a.alpha.,8a.alpha.)	1291	0,29	0,11	0,14	0,12	-	-

(Table 2). Continued.

Compounds	RI	Percentage					
		Wild plants	Acclimated plants				
			Culture A	Culture B	Culture C	Culture D	Culture E
Car-3-en-2-one	1340	-	0,33	-	-	-	-
Piperitenone	1341	0,22	-	-	-	0,32	0,31
Piperitenone oxide	1384	78,70	86,88	93,07	84,24	91,87	87,22
3-Cyclohexen-1-carboxaldehyde, 3,4-dimethyl-	1396	-	-	0,17	-	-	-
Cis-Jasmone	1401	-	-	0,49	0,52	-	0.31
2-hydroxy-7-methoxy-4-methylcyclohepta-2,4,6-trien-1-one	1404	-	1,06	-	-	0,69	--
4-hydroxy-3-methoxybenzaldehyde oxime	1415	-	0,12	0,47	-	0,16	0,70
Trans-Caryophyllene	1420	1,36	-	-	-	-	-
Trans-β-Farnesene	1457	0,45	-	-	-	-	-
(+)-Epi-bicyclosesquiphellandrene	1462	0,29	0,10	-	-	-	-
β-Cubebene	1480	0,67	-	-	-	-	-
1s,cis-Calamenene	1520	0,42	0,22	0,24	0,10	0,15	0,16
α-Cadinene	1536	0,10	0,07	-	-	-	-
Trans-3,4-Dimethyl-1-cyclohexenecarbaldehyde	1555	-	0,18	-	-	-	0,21
3-Chloro-4-t-butyl-6-methylpyridazine	1572	0,41	0,25	-	0,18	0,23	-
Cyclohexanecarboxylic acid, 1-methyl-2-oxo-, ethyl ester	1572	-	-	0,26	-	-	0,38
Caryophyllene oxide	1581	0,85	-	0,11	0,13	-	0,19
Humulene oxide	1607	0,12	-	-	-	-	-
2H-1,4-Benzothiazin-3(4H)-one	1616	-	-	-	0,89	0,62	0,72
α-Cadinol	1653	0,21	0,14	0,15	0,09	-	-
14-Norcadin-5-en-4-one isomer B	1685	0,22	0,09	0,13	0,09	-	-
Monoterpenes		5,97	3,60	1,59	7,21	3,09	2,61
Oxygenated Monoterpenes		81,28	89,99	94,43	85,92	93,02	89,59
Sesquiterpenes		4,47	0.53	0,50	0,32	0,15	0,35
Others		3,74	4,34	2,20	3,76	2,90	4,81

Notes: RI: retention index (normalized peak area abundances without correction factors); Culture A: 23 ± 2°C/16hours light: 8hours dark; Culture B: 16 ± 2°C /16hours light: 8hours dark; Culture C: 35± 2°C/16hours light: 8hours dark; Culture D: 23± 2°C/8 hours light: 16hours dark; Culture E: 23 ± 2°C/0hours light: 24hours dark.

[13]. In addition, Bouhabila *et al*. [14] showed that there were some important differences between the three oils depending on the organ of the plants, particularly in the amounts of sesquiterpenes.

The chemical composition of the EOs of *M. rotundifolia* growing in various parts of the world has been the subject of numerous studies and different chemotypes defined [15, 16]. One of them is particularly rich in Piperitenone oxide. This oxygenated monoterpene has very interesting antimicrobial (antibacterial and antifungal) properties [17], also, acts as an agent delaying the reproduction of the malaria vector Anopheles stephensis [18]. Piperitenone oxide is also of interest for the synthesis of allylic heterocycles, pyrazoles, pyrazolines and alcohols [19].

The oxygenated monoterpene Piperitenone oxide characterizes several chemotypes of certain *Mentha sp*. such as *M. spicata* [20], *M. longifolia* [21] and *M. rotundifolia* [22].

Our study shows a similarity between *M. rotundifolia* different analyzed EOs regarding to their constituents.

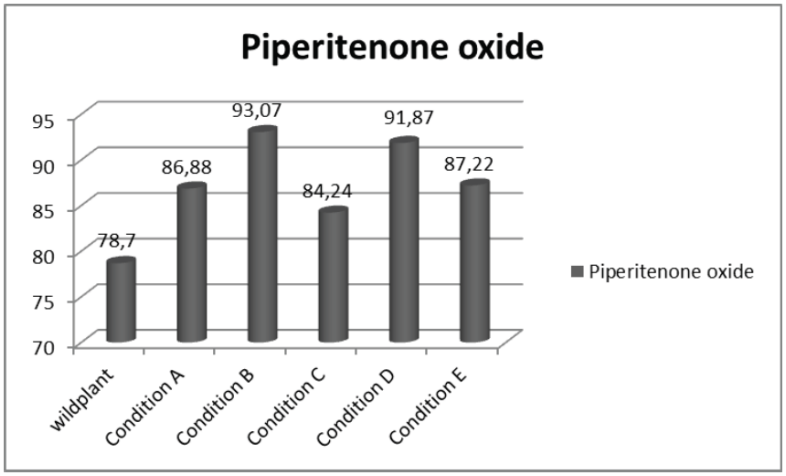

Figure 1: Piperitenone oxide content of essential oils under different acclimatization conditions.

Table 3: Diameter of Inhibition Zones (mm)

EO	Strains	Diameter of inhibition (mm)*
Wildplant	*Staphylococcus aureus*	17,33 ± 1,15d
	Bacillus subtilis	30,00 ± 2,00bc
	Salmonella enteritidis	24,33 ± 2,08cd
	Aspergillus brasiliensis	70,00 ± 0,00a
	Candida albicans	34,00 ± 5,29b
Culture A	*Staphylococcus aureus*	21,67 ± 0,58c
	Bacillus subtilis	29,33 ± 3,21bc
	Salmonella enteritidis	23,33 ± 3,06c
	Aspergillus brasiliensis	53,33 ± 5,77a
	Candida albicans	35,33 ± 6,43b
Culture B	*Staphylococcus aureus*	19,67 ± 0,58d
	Bacillus subtilis	39,00 ± 1,73b
	Salmonella enteritidis	26,00 ± 2,65cd
	Aspergillus brasiliensis	70,00 ± 0,00a
	Candida albicans	32,67 ± 5,03bc
Culture C	*Staphylococcus aureus*	22,67 ± 0,58c
	Bacillus subtilis	19,00 ± 1,73c
	Salmonella enteritidis	17,33 ± 1,15c
	Aspergillus brasiliensis	55,33 ± 5,03a
	Candida albicans	32,00 ± 5,29b
Culture D	*Staphylococcus aureus*	16,33 ± 0,58c
	Bacillus subtilis	33,33 ± 2,89b
	Salmonella enteritidis	23,33 ± 3,51c
	Aspergillus brasiliensis	46,00 ± 3,46a
	Candida albicans	31,33 ± 1,15b
Culture E	*Staphylococcus aureus*	20,00 ± 3,61c
	Bacillus subtilis	36,33 ± 4,04b
	Salmonella enteritidis	24,67 ± 0,58c
	Aspergillus brasiliensis	70,00 ± 0,00a
	Candida albicans	34,00 ± 2,00b

Notes: Values are expressed as mean ± standard deviation; 70: total inhibition; Culture A: 23°C / 16h light: 8h dark; Culture B: 16°C / 16h light: 8h dark; Culture C: 35°C / 16h light: 8h dark; Culture D: 23°C / 8h light: 16h dark; Culture E: 23°C / 0h light: 24h dark.
a, b, c, and d: Statistically groups classification of strains. Groupe “a” is the most sensitive.

However, the percentage of these constituents differs between the acclimatized plants and the wild plants. The percentage of Piperitenone Oxide in acclimatized plantlets is statistically significant difference (Figure **1**), with a content of 93.07% for plantlets cultured at 16°C and a photoperiod cycle of 16 hours light / 8 hours dark (culture B) than that of wild plants with 78.70%. Note also the presence of Limonene with a percentage of 5.7% in plantlets grown at 35°C (culture C) which is statistically higher than that of the wild plant (1.55 %). Limonene serves as a common olefinic precursor of EO terpenes in mint [23].

Similarly, a reduction in the light exposure time (culture D) or a total darkness (culture E), while maintaining a temperature of 23°C, leads to significant increases in the levels of piperitenone. However, during an increase of the culture temperature to 35°C (culture C) or its lowering to 16°C (culture B) while maintaining a photoperiod cycle of 16 hours light / 8 hours dark, as well that in the control, piperitenone is not detected. The absence of piperitenone in the cultures mentioned above indicates that it is completely converted to piperitenone oxide. Piperitenone considered as an essential intermediate in the biosynthesis of C3-oxygenated p-menthane monoterpenes in *Mentha* species [23].

3.2. Antimicrobial Activity

EOs of *Mentha rotundifolia* antibacterial activity against five reference microbial strains {*Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 25923), *Salmonella enteritidis* (ATCC 13076), *Aspergillus brasiliensis* (ATCC 6404), *Candida albicans* (ATCC 10231)} estimated by the method of disk diffusion were listed in Table **3** and pictures of some results were shown in Figure **2**. Among the microorganisms tested *A. brasiliensis* was the most sensitive to all EOs of *M. rotundifoila* with mean diameters of inhibition of

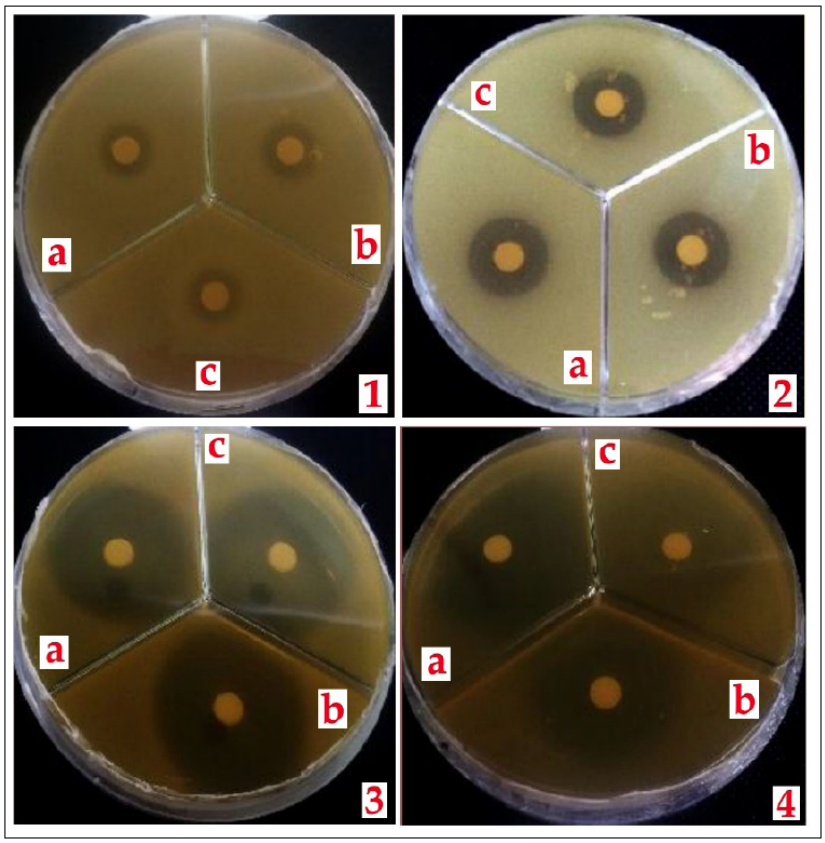

Figure 2: Antimicrobial activities of mint essential oil against some pathogens

a, b and c: repetitions;

1: EO of *M. rotundifolia* acclimatized at 23°C and 16h obscurity against *B. subtilis*;

2: EO of *M. rotundifolia* acclimatized at 16°C and 16h light against *S. aureus*;

3: EO of *M. rotundifolia* acclimatized at 23°C and 16h light against *C. albicans*;

4: EO of *M. rotundifolia* acclimatized at 35°C and 16h light against *S. enteritidis*.

Table 4: Statistical Classification of Homogeneous Groups

Essential oils	Strains	Classification into homogeneous groups of EO activity	Classification into homogeneous groups of susceptibility of strains
Wild plant	*Staphylococcus aureus*	35.13±19.9 AB	
	Bacillus subtilis		
	Salmonella entertidis		
	Aspergillus brasiliensis		
	Candida albicans		
Culture A	*Staphylococcus aureus*	32,60 ± 12,39BC	19.61 ± 2,66d
	Bacillus subtilis		
	Salmonella entertidis		
	Aspergillus brasiliensis		
	Candida albicans		
Culture B	*Staphylococcus aureus*	37,47 ± 18,26A	31,17 ± 6.97b
	Bacillus subtilis		
	Salmonella entertidis		
	Aspergillus brasiliensis		
	Candida albicans		
Culture C	*Staphylococcus aureus*	29,27 ± 14,76C	23.17 ± 3.49c
	Bacillus subtilis		
	Salmonella entertidis		
	Aspergillus brasiliensis		
	Candida albicans		
Culture D	*Staphylococcus aureus*	30,07 ± 10,59C	60.78 ±10,34a
	Bacillus subtilis		
	Salmonella entertidis		
	Aspergillus brasiliensis		
	Candida albicans		
Culture E	*Staphylococcus aureus*	37,00 ± 18,30A	33,22 ± 4,12b
	Bacillus subtilis		
	Salmonella entertidis		
	Aspergillus brasiliensis		
	Candida albicans		

Notes: Culture A: 23°C / 16h light: 8h dark; Culture B: 16°C / 16h light: 8h dark; Culture C: 35°C / 16h light: 8h dark; Culture D: 23°C / 8h light: 16h dark; Culture E: 23°C / 0h light: 24h dark.
A, B and C: Statistically groups classification of mint EO considering culture conditions. Group “A” is the most interesting.
a, b, c, and d: Statistically groups classification of strains. Groupe “a” is the most sensitive.

60.78±10.34 mm. All the tested bacteria have a moderate sensitivity to the *M. rotundifoila* EOs.

The study of acclimatization conditions (temperature and photoperiod) effects on *M. rotundifoila* EOs antimicrobial activities showed that the plantlets grown at 16 °C (culture B), plantlets grown in total darkness (culture E) and that of control (culture A) are more active towards the tested strains. While, the EO of plantlets cultured at 35 °C (culture C) are the least active (Table **4**).

The great antifungal power of *M. rotundifoila* essential oils is directly related to the content of the major constituents, Piperitenone oxide, which it has moderate antifungal activity [24]. In general, oxygenated

monoterpenes are significantly more reactive than hydrocarbon monoterpenes [25]. This may explain the important antimicrobial activities of the studied EOs.

The antimicrobial activity of EOs depends on their chemical composition [26]. Their biological activities often attributed to their major constituents. El Arch *et al*. [16] reported that *M. rotundifolia* essential oil inhibits all bacteria (*S. aureus*, *B. subtilis* and *E. coli*) and all tested fungi (*A. niger*, *P. parasiticus* and *Trametespini*), which confirms our results. In addition, the essential oil of *M. rotundifolia* has been found to have significant antibacterial activity and therefore can used as a natural antimicrobial agent [27].

4. CONCLUSION

Micropropagation techniques of medicinal plants in controlled conditions (temperature and photoperiod) can be an interesting alternative for producing important plant material characterized by the same aromatic flavor (characteristic) as plants grown in nature.

Mint EOs can be produced using *in vitro* plant culture systems and successful experiments to improve antimicrobial properties or manipulate their chemical profiles suggest that future commercial exploitation may be possible.

Our results showed that the production of mint essential oils under controlled conditions, at a temperature of 16°C and a photoperiod of 16 hours of light and 8 hours of darkness, allows obtaining a reproducible chromatographic profile (chemical composition). Also, a better antimicrobial activity than essential oil extracted from wild plants.

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CONFLICT OF INTEREST

The authors do not declare any conflict of interest.

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