Chemical Composition and Antimicrobial and Antioxidant Activities of *Mentha* (*longifolia* L. and *viridis*) Essential Oils

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ABSTRACT: The study was aimed to investigate essential oil chemical composition (gas chromatography/flame ionization detection [GC-FID] and gas chromatography/mass spectrometry [GC-MS]) and antioxidant (1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) and 2,2'-azinobis-3-ethylbenzothiazoline-6-sulphonate [ABTS] assays) and antimicrobial (Gram-positive and Gram-negative bacteria, fungi, and yeast) activities of essential oils extracted from leaves of *Mentha longifolia* L. and *Mentha viridis*. GC-MS analysis revealed that *M. longifolia* was constituted by pulegone (54.41%) as a major component followed by isomenthone (12.02%), 1,8-cineole (7.41%), borneol (6.85%), and piperitenone oxide (3.19%). *M. viridis* was rich in carvone (50.47%), 1,8-cineole (9.14%), and limonene (4.87%). The antioxidant activity by ABTS assay showed IC_{50} values of 476.3 ± 11.7 and 195.1 ± 4.2 mg/L for *M. longifolia* and *M. viridis*, respectively, the DPPH assays have resulted in a moderate IC_{50} (>8000 mg/L and 3476.3 ± 133 mg/L for *M. longifolia* and *M. viridis*, respectively). Antimicrobial activity showed that *Listeria monocytogenes* and *Klebsiella pneumoniae* bacteria were more inhibited by the 2 essential oils tested. *Escherichia coli* was least susceptible. A strong activity was also observed on fungi and yeasts. Carvone, thymol, and piperitone oxide have not been detected in Tunisian *M. longifolia*. Camphor is reported for the 1st time for *M. viridis*. Antioxidant and antibacterial activities were correlated to chemical composition.

Keywords: antioxidant activity, antimicrobial activity, essential oil, *Mentha longifolia* L., *Mentha viridis* L.

Introduction

The genus *Mentha* L. (Lamiaceae), is widely distributed in all continents (except in South America and Antarctica) (Chambers 1992). The centers of variety of this genus are Europe, Australia, Central Asia, and North Africa (Harley and Brighton 1977; Chambers 1992). It groups spontaneous and cultivated forms. It is represented by 18 species and about 100 varieties and cultivars divided into 5 sections: Audibertia, Eriodontes, Mentha, Preslia, and Pulegium. The systematic of the genus is not very elucidated because of the strong morphologic variations, levels of ploïdie ($2n = 2x = 24$ to $2n = 6x = 96$), and hybridizations intra- and interspecific (or between spontaneous and cultivated forms).

Several studies have reported the research of phylogenic relations between different species on the basis of morphologic markers (Malinvaud 1880; Hüsnü 2002), chemical (Lawrence 1978; Chalchat and others 1997; Raeis Vasco and others 1999), caryologic (Harley and Brighton 1977; Singh and Sharma 1986), or molecular. Others researchers have aimed also the identification of specific composites of essential oils for its economic value and its use in pharmaceutical, cosmetic, food, confectionary, and beverage industries (Hendriks 1998; Kanatt and others 2007).

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In Tunisia, the genus *Mentha* L. is represented by the species *M. rotundifolia* L., *M. longifolia* L. Huds., *M. spicata* (*M. viridis*) L., *M. aquatica* L., and *M. pulegium* L. (Maire 1938; Bonnier and De-Layens 1953; Pottier-Alapetite 1981). Many naturalized species (*M. piperita*, *M. spicata*, *M. longifolia*, and so on) are cultivated for family usages or for small commerce.

M. longifolia or wild mint is a fast-growing, perennial herb, growing wildly in the northeastern part of Tunisia and cultivated in all regions. The species possesses antimicrobial and antioxidant properties (Economou and others 1991; Kaur and Kapoor 2002; Daferera and others 2003; Gulluce and others 2007). However, there has been no attempt to study antimicrobial and antioxidant activities of essential oils and extracts of *M. longifolia* from Tunisia.

M. viridis (green mint) is cultivated everywhere in Tunisia (humid to arid and semi-arid climate). It is considered as a simple variety obtained by culture of the wild mint. Green mint essential oil contains monoterpenoids like carvone, limonene, menthone, menthol, pulegone, and dihydrocarveol. Some of them were found to possess high antioxidant activity (Elmasta and others 2006). This composition can vary considerably according to the origin of the plant.

The green mint acquires a very powerful action on the nervous system (Bruneton 1993; Adsersen and others 2006). The boiled leaves extract has an anti-infectious antiflatulence effect and anti-inflammatory action (notably of the digestive system), it was counseled in the viral hepatitis and colitis, gastric acidities, aerophagia, to stimulate the digestion; furthermore, it presents some invigorating and stimulating qualities (Saleem and others 2000; Kouhila and others 2001; Kumar and Chattopadhyay 2007; Arumugam and others 2008).

Apart from being a stimulant and carminative, the mint plant is also known for its insecticidal (Papachristos and Stamopoulos 2002), antimicrobial, antispasmodic, and antiplatelet properties (Samarth and Kumar 2003; Ozgen and others 2006; Tognolini and others 2006).

Actually, essential oils and their components are gaining increasing interest because of their relatively safe status, their wide acceptance by consumers, and their exploitation for potential multi-purpose functional use (Ormancey and others 2001; Yadegarinia and others 2006). Many researchers have reported the antimicrobial, antifungal, and antioxidant properties of essential oils (Baratta and others 1998; Vardar-Unlü and others 2003; Mimica-Dukic and others 2004; Singh and others 2005; Sacchetti and others 2005). In fact, essential oils from mint species have strong antioxidant activity and good antimicrobial activity against *Staphylococcus aureus, Escherichia coli, Candida albicans,* and *Salmonella enteritidis* (Yadegarinia and others 2006; Gulluce and others 2007; Mahboubi and Haghi 2008). However, activity against fungi *Aspergillus ochraceus* and yeast species as *Saccharomyces cerevisiae* has not been reported previously only for *Menta arvensis* (Basilico and Basilico 1999). The *Mucor ramamnianus* has not been tested until now with essential oils of *M. longifolia* L. and *M. viridis* L. The search for antioxidant and antimicrobial activities of Tunisian *Mentha* species essential oils is also not explored.

Therefore, the objectives of this study were to analyze chemical composition of hydrodistilled essential oils of *M. longifolia* and *M. viridis* by a GC-MS system to determine the essential oils chemotypes and investigate their antimicrobial and antioxidant activities.

Materials and Methods

Chemicals used

All chemicals used were of analytical reagent grade. All reagents were purchased from Sigma-Aldrich and Fluka (Saint-Quentin, France).

Essential oil isolation

Fresh leaves of *M. longifolia* L. and *M. viridis* L. cultivated species were collected (May 2007) from the region of Gabes Tunisia.

About 100 g of fresh leaves from each sample of *Mentha* were placed in 400 mL distilled water and submitted to hydrodistillation for 3 h using a Clevenger-type 5 apparatus (British Pharmacopoeia 1980). The condensation of each essential oil gives 2 phases: an organic phase was formed practically by essential oils (Hpo) to which we added anhydrous sodium sulfate to eliminate water traces and an aqueous phase that contains a negligible quantity of essential oil. Essential oils were stored at 4 °C until tested and analyzed.

Gas chromatography and gas chromatography-mass spectrometry

Quantitative and qualitative analysis of essential oils was carried out by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). Gas chromatography analyses were carried out on a Varian Star 3400 (Les Ulis, France) Cx chromatograph fitted with a fused silica capillary DB-5MS (5% phenylmethylpolysyloxane, 30 m × 0.25 mm, film thickness 0.25 μm). Chromatographic conditions were a 60 to 260 °C temperature rise with a gradient of 5 °C/min and 15 min isotherm at 260 °C. A 2nd gradient was applied to 340 °C at 40 °C/min. Total analysis time was 57 min. For analysis, essential oils were dissolved in petroleum ether. One microliter of sample was injected in the split mode ratio of 1 : 10. Helium (purity 99.999%) was used as carrier gas at 1 mL/min. The injector was operated at 200 °C. The mass spectrometer (Varian Saturn GC-MS/MS 4D) was adjusted for an emission current of 10 μA and electron multiplier voltage between 1400 and 1500 V. Trap temperature was 150 °C and that of the transfer line was 170 °C. Mass scanning was from 40 to 650 amu.

Compounds were identified by comparison of their KI (Kovats indices) relative to C5-C24 *n*-alkanes obtained on a nonpolar DB-5MS column, with those provided in the literature, by comparison of their mass spectra with those recorded in NIST 08 (Natl. Inst. of Standards and Technology) and reported in published articles and by co-injection of available reference compounds. The samples were analyzed in duplicate. The percentage composition of essential oils was computed by the normalization method from the GC peak areas, assuming identical mass response factor for all compounds. Results were calculated as mean values of 2 injections from essential oil, without using correction factors.

Free radical scavenging activity: DPPH test

Antioxidant scavenging activity was studied using 1,1-diphenyl-2-picrylhydrazyl free radical (DPPH) as described by Blois (1958) with some modifications. Briefly, 1.5 mL of various dilutions of the test materials (2 mg/mL vitamin C were diluted 1/200, 1/400, 1/800, 1/1000, 1/1200; 16 mg/mL of essential oil *M. longifolia* were diluted 1/2, 1/3, 1/4, 1/5, 1/10; 16 mg/mL of essential oil *M. viridis* were diluted 1/2, 1/3, 1/4, 1/5, 1/10) were mixed with 1.5 mL of 0.2 mM methanolic DPPH solution. After an incubation period of 30 min at 25 °C, the absorbance at 520 nm was measured. The wavelength of maximum absorbance of DPPH was recorded as $A_{(\text{sample})}$, using a spectrophotometer (Helios, Unicam, Cambridge, U.K.). A blank experiment was also carried out applying the same procedure to a solution without the test material and the absorbance was recorded as $A_{(\text{blank})}$. The free radical scavenging activity of each solution was then calculated as percent inhibition according to the following equation:

$$\%\,\text{inhibition} = ([A_{(\text{blank})} - A_{(\text{sample})}]/A_{(\text{blank})}) \times 100$$

Antioxidant activity of standard or essential oils was expressed as IC_{50}, defined as the concentration of the test material required to cause a 50% decrease in initial DPPH concentration. Ascorbic acid was used as a standard. All measurements were performed in triplicate.

ABTS radical scavenging assay

The radical scavenging capacity of the 2,2-AzinoBis-3-ethylbenzoThiazoline-6-Sulphonate radical cation (ABTS) was determined as described by Re and others (1999). ABTS was generated by mixing a 7 mM of ABTS at pH 7.4 (5 mM NaH_2PO_4, 5 mM Na_2HPO_4, and 154 mM NaCl) with 2.5 mM potassium persulfate (final concentration) followed by storage in the dark at room temperature for 16 h before use. The mixture was diluted with ethanol to give an absorbance of 0.70 ± 0.02 units at 734 nm using a spectrophotometer. For each sample, diluted methanol solution (2 mg/mL of vitamin C were diluted 1/200, 1/400, 1/800, 1/1000, 1/1200; 6 mg/mL of essential oil *M. longifolia* were diluted 1/4, 1/5, 1/10, 1/20, 1/50; 2 mg/mL of essential oil *M. viridis* were diluted 1/4, 1/5, 1/10, 1/20, 1/50) of the essential oil (100 μL) was allowed to react with fresh ABTS solution (900 μL), and then the absorbance was measured 6 min after initial mixing. Ascorbic acid was used as a standard. The capacity of free radical scavenging was expressed by IC_{50} (mg/L) values, which represents the

concentration required to scavenge 50% of ABTS radicals. The capacity of free radical scavenging IC_{50} was determined using the same equation previously used for the DPPH method. All measurements were performed in triplicate.

Microbial strains

All strains were obtained from the Laboratory of Chemical Engineering, Bioprocess Systems Microbiens Dept. of High Natl. School of Agronomy of Toulouse. The different essential oils were

Table 1 — Chemical composition (percent) of essential oils extracted from *Mentha longifolia* and *Mentha viridis* leaves using hydrodistillation methods.

Nr	KI[a]	Compounds	*M. longifolia* (%)	*M. viridis* (%)
1	936	α-pinene	0.52	0.50
2	976	sabinene	0.70	0.20
3	985	β-myrcene	0.04	0.19
4	990	3-octanol	—	0.54
5	1011	δ-3-carene	0.18	0.34
6	1025	p-cymene	0.04	0.33
7	1028	limonene	0.12	4.87
8	1030	1,8-cineole	7.41	9.14
9	1057	γ-terpinene	0.63	0.78
10	1072	linalool oxide	1.17	—
11	1086	α-terpinolene	—	0.05
12	1094	linalool	0.04	0.17
13	1105	p-menth-2-en-1-ol	—	1.20
14	1141	trans-pinocarveol	0.09	—
15	1143	camphor	1.67	3.68
16	1148	isopulegol	0.04	0.28
17	1155	menthone	0.18	—
18	1159	β-terpineol	—	0.30
19	1164	isomenthone	12.02	1.13
20	1166	borneol	6.85	0.74
21	1178	terpinen-4-ol	0.06	2.97
22	1185	α-terpineol	1.52	1.97
23	1190	dihydrocarveol[b]	—	0.45
24	1193	neodihydrocarveol[b]	—	0.69
25	1202	carveol	—	0.83
26	1205	verbenone	0.19	—
27	1238	pulegone	54.41	1.11
28	1241	carvone	—	50.47
29	1285	bornyl acetate	0.37	0.64
30	1330	1,2,4-trimethoxybenzene[b]	0.65	—
31	1350	eugenol	—	0.27
32	1352	terpinyl acetate	0.61	0.43
33	1366	dihydroeugenol	0.18	0.18
34	1369	piperitenone oxide	3.19	0.48
35	1389	β-elemene	—	1.00
36	1390	β-cubebene	—	0.12
37	1394	cis-jasmone	0.16	1.16
38	1406	methyl eugenol	—	0.36
39	1415	β-caryophyllene	2.04	3.00
40	1432	β-gurjunene	—	0.51
41	1438	geranyl acetone	0.04	—
42	1439	α-guaiene	—	1.01
43	1450	α-himachalene	0.17	0.17
44	1454	α-humulene	2.97	0.62
45	1482	β-selinene	0.33	1.86
46	1486	α-selinene	—	0.51
47	1499	α-muurolene	0.34	—
48	1512	calamenene[b]	—	0.22
49	1513	γ-cadinene	0.31	1.12
50	1524	δ-cadinene	—	0.26
51	1534	α-cadinene	—	0.20
52	1580	β-caryophyllene oxide	0.24	0.86
53	1636	T-muurolol	0.26	0.51
54	1678	cis-α-santalol	—	0.42
55	ND	grandisol[b]	—	0.27
56	ND	dimethylfuran lactone[b]	—	0.11
		Total	99.72	99.18
		Monoterpene hydrocarbons	2.19	6.93
		Monoterpenes oxygenated	89.18	77.22
		Sesquiterpenes hydrocarbons	6.16	10.38
		Sesquiterpenes Oxygenated	0.5	1.79
		Other	1.71	2.90
		Phenolics	0.18	0.45

[a] DB-5MS nonpolar column relative to C8-C24 n-alkanes.
[b] Tentatively identified supported by good match of MS.
ND = not determined.

individually tested against a panel of microorganisms including 2 Gram-positive (*S. aureus* CIP7625, *Listeria monocytogenes* Scott A 724), 2 Gram-negative (*E. coli* ATCC10536, *Klebseilla pneumoniae* CIP8291), 2 yeasts (*S. cerevisiae* ATCC 4226 A and *C. albicans* IPA 200), and 2 fungi (*M. ramamnianus* ATCC 9314 and *A. ochraceus* NRRL 3174). The bacterial strains were cultured on nutrient broth for 48 h at 37 °C, while fungi and yeasts were propagated in CYB at 37 °C for 48 h to 3 d before use. All microorganisms were stocked at –20 °C in appropriate conditions and regenerated twice before use in the manipulations.

Well-diffusion assay

Agar well-diffusion method was employed for the determination of antimicrobial activity of essential oils (Feyza and others 2009). Briefly, 15 mL of nutrient agar and CYA, respectively, for bacterial and fungal strains were inoculated with 100 μL of microbial suspension containing 10^8 CFU/mL of the indicator strain and then poured onto a petri dish and allowed to solidify. The dish was left to cool down and to solidify at room temperature for 30 min. Wells of 10 mm dia were aseptically bored into the culture medium. A sterile cox borer was used to punch wells and 15 μL of each essential oil were added to the wells. Each plate contained only 1 essential oil. Ampicillin and nalidixic acid (30 μg/well) were used as positive reference standards to determine the sensitivity of Gram-positive and Gram-negative bacterial species, respectively. Nystatin (30 μg/well) was used as positive reference standard to determine the sensitivity of fungi and yeast species. These plates, after staying at 4 °C for 2 h to allow dispersal, were incubated for 24 h at 37 °C for bacteria and 48 h at 30 °C for yeasts and fungi. The antimicrobial activity was visually appraised as inhibition zones surrounding the wells. The diameters of the inhibition zones were measured in millimeters. Tests were carried out in triplicate.

Statistical analysis

All data were expressed as means ± standard deviations (SD) of triplicate measurements. The confidence limits were set at $P < 0.05$. Standard deviations did not exceed 5% for the majority of the values obtained.

Results and Discussion

The extraction yield of hydrodistilled *M. longifolia* essential oil was 1.3% whereas the yield of *M. viridis* oil was 0.8%. In the literature (Kokkini and others 1995; Elmasta and others 2006), yield values were between 1.2% and 3.2% for the 2 species essential oils. The yield variability in the total essential oil content within the 2 species of mint could have resulted from the variation of climatic factors (Lawrence 1989; Kokkini and others 1995).

Chemical composition

The components identified in the 2 species of mint are listed in Table 1. Thirty-five components representing 99.72% of the total volatiles were identified for *M. longifolia* essential oil. The main components were oxygenated monoterpenes (89.18%). Pulegone (54.41% of the total essential oil) was the major compound followed by isomenthone (12.02%), 1,8-cineole (7.41%), borneol (6.85%), and piperitenone oxide (3.19%). *M. longifolia* including a high percentage of pulegone is reported also by Asekun and others (2007). Several compounds such as thymol (Gullus and others 2007), carvone (Kokkini and others 1995), or piperitone oxide (Ghoulami and others 2001) previously found in *M. longifolia* essential oil, have not been found in our samples.

Forty-nine components representing 99.18% of the total *M. viridis* essential oil were identified and characterized mainly by carvone (50.47%), followed by 1,8-cineole (9.14%), limonene (4.87%), camphor (3.68%), and β-caryophyllene (3%). Oxygenated monoterpenes corresponded to the main fraction (77.22%) of the total essential oil. *M. viridis* was rich in carvone as reported by other studies (Akhila and others 1980; Kokkini and others 1995). Camphor content for *M. viridis* is reported for the 1st time.

The 2 species showed an important variation in essential oil composition due to geographical and bioclimatic factors of the region. Different essential oil chemotypes have been described for *M. viridis* (Kokkini and others 1995) and *M. longifolia* (Gulluce and others 2007).

Antioxidant activity

Free radical scavenging activity of essential oils from 2 species of mint was investigated by ABTS assay. The ABTS has not been formerly tested for *Mentha* species. A moderate antioxidant activity was obtained to scavenge the ABTS radical cation (Table 2). *M. longifolia* essential oil (476.3 mg/L) was less active than *M. viridis* (195.1 mg/L).

The DPPH radical scavenging activity of essential oils confirms the result obtained with ABTS assay. Therefore, the DPPH activity of *M. viridis* essential oil (IC_{50} of 3476.3 mg/L) was more important than for *M. longifolia* (>8000 mg/L, Table 2). IC_{50} values obtained for Tunisian *M. longifolia* essential oil (>8000 mg/L) are comparable with Turkish *M. longifolia* essential oil (10700 ± 5.01 μg/mL) (Gulluce and others 2007). A previous study showed that methanol extract of *M. longifolia* was more able to reduce the stable free radical DPPH than that of essential oil (Gulluce and others 2007).

The essential oil of *M. viridis* contains more antioxidants than *M. longifolia* essential oil. Activity might be related to its phenolic content as reported in previous studies (Mimica-Dukic and others 1999; Gulluce and others 2007). Our study confirmed this result; in effect, the percentage of phenolics in *M. viridis* was 0.45% (dihydroeugenol and eugenol) relatively superior to that in *M. longifolia* essential oil that attained a percentage of 0.18% (dihydroeugenol).

Antimicrobial activity

Antimicrobial activity of essential oils was evaluated against 2 Gram-positive bacteria, 2 Gram-negative bacteria, 2 fungi, and 2 yeast species by zone diameter (Table 3). These microorganisms are morphologically and physiologically different. Essential oils exhibited a great potential for antimicrobial activity against 2 fungi (*M. ramamnianus* and *A. ochraceus*). In fact, inhibition zone diameters for *M. ramamnianus* (35 to 40 mm) and *A. ochraceus* (32 to 43 mm) were higher (Table 3).

This is the 1st study of antimicrobial activity of *M. longifolia* and *M. viridis* essential oils against yeast species (*C. albicans* and *S. cerevisiae*). Inhibition zones were obtained by essential oils of 2 species against yeast *C. albicans* (19 to 21 mm) and *S. cerevisiae* (25 to 28 mm). These activities may be related to major compounds of the *M. longifolia* and *M. viridis* essential oils (pulegone and carvone, respectively) but there is no previous study that reported antimicrobial activity of pulegone and carvone components against yeast.

Table 2 — Antioxidant activity of *Mentha longifolia* and *Mentha viridis* using ABTS and DPPH radical scavenging assay.

Samples	ABTS IC_{50} (mg/L)	DPPH IC_{50} (mg/L)
Mentha longifolia	476.3 ± 11.7	>8000
Mentha viridis	195.1 ± 4.2	3476.3 ± 133
Vitamin C	1.9 ± 0.1	4.4 ± 0.2

Table 3 – Antimicrobial activity (diameter of the inhibition zones in mm) of *Mentha longifolia* and *Mentha viridis* essential oils.

		Essential oils		References standard		
Antimicrobials	**Microorganisms**	***Mentha longifolia***	***Mentha viridis***	**Ampicillin**	**Nalidixic acid**	**Nystatin**
Fungi	*Mucor ramamnianus* ATCC 9314	35	40	–	–	31
	Aspergillus ochraceus NRRL 3174	32	43	–	–	24
Yeast	*Candida albicans* IPA 200	19	21	–	–	25
	Saccharomyces cerevisiae ATCC 4226 A	28	25	–	–	29
Gram-positive bacteria	*Klebseilla pneumoniae* CIP8291	20	25	–	28	–
	Escherichia coli ATCC10536	–	–	–	30	–
Gram-negative bacteria	*Staphylococcus aureus* CIP7625	–	–	20	–	–
	Listeria monocytogenes Scott A 724	24	29	35	–	–

– = absence of inhibition zone detected; essential oils concentration: 15 μL/well; reference standards concentration: 30 μg/well.

The maximal inhibition zones for bacterial strains were 25 to 29 mm for *M. viridis* essential oil and 20 to 24 mm for *M. longifolia* essential oil against, respectively, *K. pneumonial* and *L. monocytogenes*. However, no antimicrobial activity of essential oils tested was noted against *E. coli* and *S. aureus*. Results obtained in this study show that mint essential oils do not have selective antimicrobial activity on the basis of the cell-wall differences of bacterial microorganisms (Gram-positive or Gram-negative bacteria) as reported previously (Cosentino and others 1999; Karaman and others 2003). Referring to the literature, the antimicrobial activity of the tested essential oils may be due to monoterpene hydrocarbons (Carlo and others 2005). *M. viridis* essential oil contains more terpene hydrocarbons (monoterpenes and sesquiterpenes) than *M. longifolia* (Table 3). This result is in agreement with the greatest diameter of inhibition obtained for about 7/8 of microorganisms tested (except *S. cerevisiae*) for *M. viridis* essential oil (Table 3). Despite the feeble percentage of the monterpene and sesquiterpene hydrocarbons for the 2 essential oils (17.31% for *M. viridis* and 8.35% for *M. longifolia*) the samples presented an interesting antimicrobial activity. Although these compounds are not abundant in essential oil, activity was important. It is necessary to signal that other compounds can contribute to improve this activity.

Conclusions

Carvone, thymol, and piperitone oxide have not been detected in Tunisian *M. longifolia*. Camphor is reported for the 1st time for *M. viridis*. Essential oils of *M. viridis* and *M. longifolia* have a medium antioxidant activity against ABTS and DPPH free radical scavenging. This activity is related to low phenolic content. Essential oils of Tunisian *M. longifolia* and *M. viridis* cultivated species exhibited a high antimicrobial activity against fungi and yeast species.

Essential oil represents a complex mixture of different chemical components. The antimicrobial effect of total essential oil cannot be reduced to major components. The results of this study showed that *M. longifolia* and *M. viridis* possess essential oils with important antimicrobial properties. Antibacterial activity could be influenced by terpene hydrocarbons (monterpenes and sesquiterpenes) but other components can contribute to this activity. *In vivo* assay is also necessary to confirm antimicrobial activity of *M. longifolia* and *M. viridis*. These studies can be used in pharmaceuticals and natural therapies of infectious diseases for humans, and management of plant diseases.

Separation of terpene hydrocarbons, responsible for the antimicrobial activity, would be an interesting study to identify the molecule generating the good efficacy.

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