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Antimycobacterial, Antifungal and Radical Scavenging Effects of Essential Oil from Moroccan *Mentha piperita*

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ABSTRACT

In order to increase the Moroccan Mentha piperita value, the antimycobacterial, antifungal and antioxidant activities of its essential oil was evaluated. The study of antimicrobial activity was performed on two mycobacterial strains Mycobacterium aurum and Mycobacterium smegmatis and two fungal strains Aspergillus niger and Penicillium expansum, using broth microdilution method. While, the antioxidant activity was studied by the 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radical scavenging assay. The Minimal Inhibitory Concentration (MIC) showed that M. piperita essential oil exhibited strong inhibitory effect against all tested microorganisms with minimum inhibitory concentrations ranging from 0.062% to 0.5% (v/v). Moreover, this essential oil presented radical scavenging power with an IC_{50} of 6,3977 mg/ml. These findings showed that the studied essential oil has both an antimicrobial and antioxidant potential that can be applied to the food and drug industries.

Keywords: Essential oil, *M. piperita*, Antimycobacterial activity, Antifungal activity, DPPH radical scavenging activity

INTRODUCTION

In a global difficult context that combines an increasing prevalence of microorganism resistance to antimicrobial agent and a disengagement of the search for new antiseptic from the pharmaceutical industry, thereby the use of synthetic antioxidants such as Butylated Hydroxyanisole (BHA) and Butylated Hydroxytoluene (BHT) in food industry for delaying lipid oxidation were found to be responsible for undesirable effects on the health of consumers [1], we witness a strong trend of "back to nature" with the introduction of "green chemistry" in which Essential Oils (E.O) extracted from aromatic plants take all their meaning. More and more numerous scientific publications associated with a popular strong interest in these new therapeutic show that a new path can be opened.

The species of the genus Mentha are among the most used aromatic and medicinal plants in the whole world thanks to their many therapeutic properties and their uses in the flavor and fragrance**.** This genus includes about 25 species, which are largely situated in the Mediterranean area [2]. In Morocco, Mentha genus occupies a very important place in traditional medicine and in Moroccan cuisine as flavoring agents. Being one of the most famous plants in Morocco, *Mentha piperita* L. is considered among the most popular herbal teas single ingredient. This mint belonging to the Lamiaceae family is a natural grown hybrid of *Mentha aquatica. (*Water Mint*)* and *Mentha spicata* L. *(*spearmint*).*

The aim of this study was to evaluate the antimycobacterial, antifungal and antioxidant activity of *M. piperita* essential oil collected in Taounate region *(Morocco*).

MATERIALS AND METHODS

Plant material

Fresh aerial part of *M. piperita* was harvested from the National Institute of the Medicinal and Aromatic Plants (NIMAP) garden in Taounate city (34°32′11″ N, 4°38′24″W, Altitude: 600 m)(Morocco). The Botanical authentication was confirmed at the NIMAP (Morocco).

Essential oils extraction

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

Chemical analysis of essential oil

The essential oil was analyzed using Gas Chromatography (GC) coupled to mass spectrometry GC/MS (Polaris Q ion trap MS). Hence, analyses were performed on a Hewlett-Packard (HP 6890) gas chromatograph (FID), equipped with a 5% phenyl methyl silicone HP-5 capillary column (30 m × 0.25 mm × film thickness 0.25 µm). 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, final hold time was 48 min. The machine was led by a computer system type "HP Chem Station", managing its functioning and allowing to follow the evolution of chromatographic analyses. Diluted samples (1/20 in methanol) of 1 µl were injected manually.

Mycobacterial strains

The essential oil of *M. piperita* was tested for its antimycobacterial activity against two reference microbial strains. *Mycobacterium aurum* A+ (*M. aurum*) is a non-pathogenic *Mycobacterium* species with a generation time of approximately 6 h. This strain was used as a model to evaluate the effect of active substances on the growth of *Mycobacterium tuberculosis*.

Mycobacterium smegmatis mc2-155 (*M. smegmatis*) is a nonpathogenic atypical mycobacterial strain with a generation time of approximately 3 h. These strains were maintained in 20% glycerol at -20°C and sub-cultured before use. The mycobacteria were cultivated at 37°C on Sauton's medium for 48-72 h [3-5]. The turbidity was adjusted at 10^6 UFC/ml (estimated by absorbance at 600 nm).

Fungal strains

Tested fungi include two isolates of *Aspergillus niger* and *Penicillium expansum*. The revivification was made by subcultures in malt extract-agar plates at 25°C for 7 days. After incubation, their spores were harvested by scraping the culture surface in sterile Tween 20 (1%) solution. Then the spore suspension was concentrated by centrifugation at 10000 g for 15 min at 4°C until a concentration of 10^6 spores/ml (counted with a hemocytometer).

Determination of MIC

The MIC values, which represent the lowest essential oil concentration that completely inhibits the growth of mycobacteria, were performed in 96-well microplate using the micro-dilution assay according to the protocol previously described by Chraibi et al. [6] with slight modifications. Due to the immiscibility of essential oils with water and thus the culture medium, each essential oil was serially diluted in Sauton broth supplemented with agar 0.15% (w/v), used as an emulsifier, in which the final concentration of the essential oil was between 8% and 0.007% (v/v) for *M. piperita*. The 12^{th} well was considered as growth control (it contained only the culture medium and strain). Then, 50 µl of bacterial inoculum was added to each well at a final concentration of 10^6 CFU/ml. After incubation at 37°C for 48-72 h, 10 µl of rezasurin were added to each well as mycobacterial growth indicator. After further incubation at 37°C for 2 h, the bacterial growth was revealed by the change of coloration from purple to pink [7]. Experiments were carried out in triplicates to minimize experimental error.

Likewise, the MIC determination against *A. niger* and *P. expansum*, was performed in 96 well-microplate according to the protocol previously described by Daouk et al. [8] was used. 50 µl of malt extract broth were added from the second to the 12^{th} well. The essential oil was diluted in Tween 20.1% (v/v) at a final concentration of 8% (v/v), then 100 µl of this solution were deposed in the first well. Afterwards, scalar dilution was made by transferring 50 µl from the first to the 11^{th} well. The 12^{th} well was considered as growth control. Thereafter 50 µl of the fungal spore suspension were added to each well to reach the final concentration of 10^6 spores/ml. The microplate was sealed and incubated for 72 h at 30°C. The lowest essential oil concentration that prevents visible fungal growth was defined as the MIC.

Determination of MBC/MFC

The Minimal Bactericide and Fungicide Concentration (MBC/MFC) were determined by inoculating 3 µl from each negative well, which were spotted on sauton plates and incubated at 37°C for 48-72 h for mycobacteria and on malt extract plates for fungal strains that were incubated at 30°C during 72 h. The MBC and MFC corresponded to the lowest concentration of the essential oil at which the incubated microorganism was completely killed [9]. Tests were performed in triplicates.

DPPH radical scavenging assay

The capacity of *M. pulegium* essential oil to scavenge free radical was evaluated using a synthetic free radical molecule 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) [10]. Briefly, 3 ml of each essential oil dilution (0.5, 1, 2.5 and 10 mg/ml) prepared in methanol was mixed with 3 ml of a methanolic solution of DPPH 0.004% (w/v). The mixtures were vortexed and kept in the dark for 30 min. The absorbance was measured at 517 nm, using Vis-UV spectrophotometer. BHT was used as positive control. The antioxidant activity was calculated as follows:

$$\text{Antioxidant activity (\%)} = \frac{\text{Abs}_{\text{control}} - \text{Abs}_{\text{sample}}}{\text{Abs}_{\text{control}}} \times 100$$

RESULTS AND DISCUSION

Chemical composition of the studied essential oil

Twenty seven compounds accounting for 99.51% of the *M. piperita* essential oil were identified. The main compounds were menthol (46.32%), menthofurane (13.18%), menthyl acetate (12.10%), menthone (7.42%) and 1.8-cineole (6.06%). Beside other compounds with relatively low levels, including limonene (3.01%), sabinene (1.38%), carvone (1.02%), isomenthyl acetate (0.82%) were also found. It is observed that this essential oil was characterized by the presence of menthol as the main component. These results are similar to several previous reports [11,12].

Antimicrobial activity

The antimicrobial activity against four strains *(M. aurum, M. smegmatis, A. niger* and *P. expansum*) was evaluated by observing their growth inhibition in contact with a sample of the essential oil of moroccan *M. piperita* at different concentrations by coloration changing from purple to pink using resazurin.

The antimicrobial activity tested *in vitro* by microdilution method showed that the essential oil displayed a remarkable power with a different MIC values and variable effect on the studied microorganisms. As can be seen in the Table 1, the essential oil of *M. piperita* exercise an

important inhibitory activity against all the studied strains; especially for fungal strains: *A. niger* and *P. expansum* 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. Also, the concentrations of 0.25% (v/v) and 0.5% (v/v) were sufficient to stop the growth of *M. aurum* and *M. smegmatis* respectively.

Table 1: 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
M. aurum	-	-	-	-	-	-	+	+	+	+	+
M. smegmatis	-	-	-	-	-	+	+	+	+	+	+
A. niger	-	-	-	-	-	-	-	-	+	+	+
P. expansum	-	-	-	-	-	-	-	+	+	+	+

Regarding the MBC values of *M. piperita* essential oil tested (Table 2), we found that MBC values could well be similar to their MIC values against *M. aurum* and *M. smegmatis* while the MFC values for fungal strains were greater than 8% (v/v).

This important activity may be related to the chemical composition of *M. piperita* essential oil, especially to the presence of menthol, menthofurane and menthone, which represents the major compounds in this essential oil. These oxygenated monoterpenes are indeed known for their antimicrobial properties [13-16,3].

Table 2: The Minimum Bactericide/Fungal Concentrations (MBC/MFC) of *Mentha piperita* essential oil against tested strains

Strains	Concentrations % (v/v)										
	8	4	2	1	0.5	0.25	0.125	0.062	0.031	0.015	0.007
M. aurum	-	-	-	-	-	-	+	+	+	+	+
M. smegmatis	-	-	-	-	-	+	+	+	+	+	+
A. niger	+	+	+	+	+	+	+	+	+	+	+
P. expansum	+	+	+	+	+	+	+	+	+	+	+

The results of this study are concordant with several previous studies and confirmed the antiseptic properties of *M. piperita* essential oil. In fact, the antiseptic effect of this oil against *C. albicans*, *Staphylococcus aureus* and *E. coli*, *Fusarium sporotrichioides*, *Haemophilus influenza*, *Proteus*, *Helicobacter pylori* and *Micrococcus luteus* [17-20] has been showed. Likewise, a remarkable antimycobacterial [21,22], antifungal, [23], antiallergic[24], antiparasitic [12] and virucidal [18] effects of this essential oil has been proved**.**

Antioxidant activity

The antioxidant activity of the studied essential oil was evaluated by the DPPH• reduction method and results were compared with the synthetic antioxidant BHT. The DPPH radical-scavenging activities of the essential oil and of standard are shown in Figure 1. As can be noted, *M. piperita* essential oil presented an important antioxidant activity with an IC_{50} of 6,3977 mg/ml. Furthermore, the BHT displayed a greater radical scavenging activity compared to this essential oil with an IC_{50} of 0.00682 mg/ml. In addition, a linear relationship was found between the antioxidant activity and the BHT concentration (R-square=0.96, P-value=0.000). Hence, the result expressed with equivalent of BHT was 1.07 µg Eq BHT/mg of essential oil. Previous works reported a wide variation in the antioxidant activity of *M. piperita* essential oil [25]. In fact, it showed significant antioxidant activity in comparison to that obtained in Libya [26], Tehran-Iran [17] and Ouargla region [19].

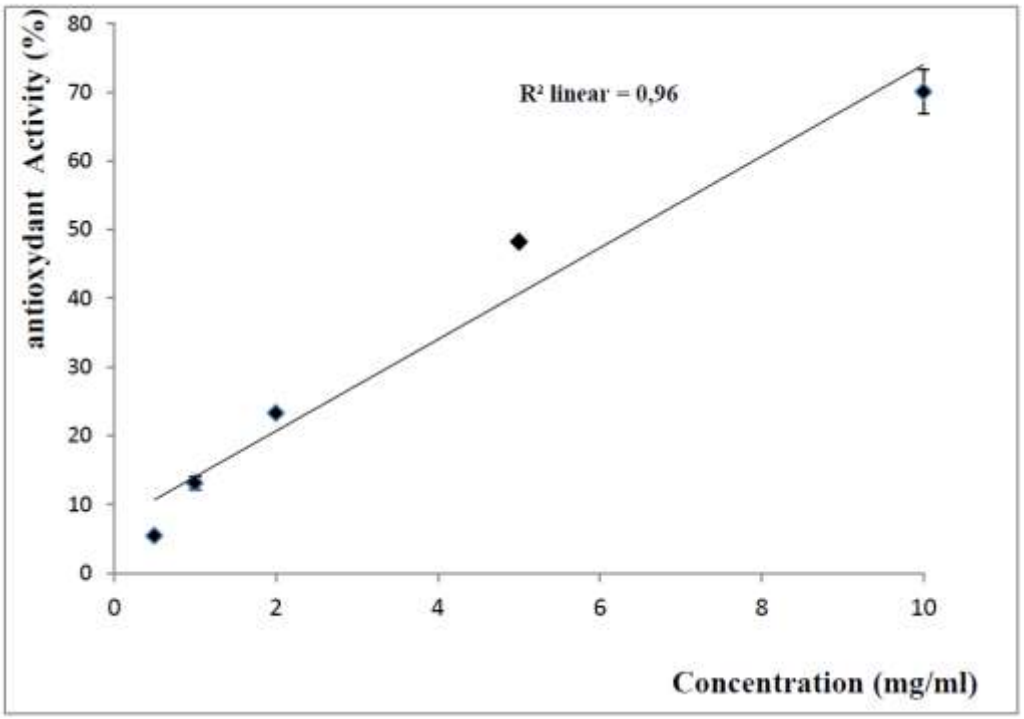

Figure 1: Linear relationship between the antioxidant activity and the concentration of BHT

This antioxidant activity of *M. piperita* essential oil is probably due to its chemical composition and to the ability of terpenes to be donor of hydrogen atoms or electrons and to capture free radicals [27]. In addition the most powerful scavenging compounds were reported to be monoterpene ketones (menthone and isomenthone) and 1, 8-cineole [25]. Also without forgetting the role of the minority compounds that can interact synergistically or antagonistically to create an effective system vis-a-vis free radicals [28].

CONCLUSION

This work aims to evaluate the antimycobacterial, antifungal and antioxidant effects of *M. piperita* essential oils. These preliminary results showed that *M. piperita* oil could act as an effective and natural drug in terms of both antimicrobial activity and ability to neutralize free radicals for preventing oxidation. These important activities were due to several major components particularly menthol, menthofurane and menthone. Further studies are also necessary to confirm in vivo the potential of this oil for use as a preservative to increase the lifetime of the raw material and processed food stuff, and as a complement in the production of pharmaceutical products enabling to struggle the infectious diseases in humans and animals, especially in developing countries.

REFERENCES

[1] K. Bohme, J. Barros-Velazquez, P. Calo-Mata, P.S. Aubourg, In: T. Villa, P. Veiga-Crespo, editors. *Antimicrob. Compd*., **2014**, 69-84.
[2] M. Gulluce, F. Sahin, M. Sokmen, H. Ozer, D. Daferera, A. Sokmen, *Food Chem*., **2007**, 103(4), 1449-1456.
[3] G. Iscan, N.E.S.E. KIrimer, M. Kürkcüoglu, H.C. Baser, F. Demirci, *J. Agric. Food Chem*., **2002**, 50, 3943.
[4]http://www.stoptb.org/wg/new_diagnostics/assets/documents/Inventory%20of%20culture%20and%20DST%20methods%20-%20July%2031%202011%20-%20final.pdf.
[5] F. Papa, M. Rivière, J.J. Fournié, G. Puzo, H. David, *J. Clin. Microbiol*., **1987**, 25(12), 2270-2273.
[6] M. Chraibi, A. Farah, S. Lebrazi, O. Elamin, M. Iraqui Houssaini, K. Fikri-Benbrahim, *Asian Pac. J. Trop. Biomed*., **2016**, 6(10), 836-840.
[7] S. Bouhdid, J. Abrini, A. Zhiri, M.J. Espuny, A. Manresa, *J. Appl. Microbiol*., **2009**, 106, 1558-1568.
[8] R.K. Daouk, S.M. Dagher, E.J. Sattout, *J. Food Prot*., **1995**, 58, 1147-1149.
[9] M. Radji, M. Kurniat, A. Kiranasar, *J. Appl. Pharm. Sci*., **2015**, 5(1), 19-22.
[10] M. Chraibi, K.F. Benbrahim, D. Ou-yahyia, M. Balouiri, A. Farah, *Int. J. Pharm. Pharm. Sci*., **2016**, 8(9).
[11] G.S. Hashimoto, F.M. Neto, M.L. Ruiz, M. Achille, E.C. Chagas, F.C.M. Chave, M.L. Martins, *Aquaculture*., **2016**, 450, 182-186.
[12] D.F. Malheiros, P.O. Maciel, M.N. Videira, M. Tavares-Dias, *Aquaculture.*, **2016**, 455, 81-86.
[13] N. Dias, M.C. Dias, C. Cavaleiro, M.C. Sousa, N. Lima, M. Machado, *Nat. Prod. Res*., **2016**, 1-5.
[14] R. Kotan, S. Kordali, A. Cakir, *Zeitschrift für Naturforschung C*., **2007**, 62(7-8), 507-513.
[15] R. Mahmoudi, F. Katiraee, H. Tajik, A. Abbas, *Int. J. Sci. Res*., **2016**, 2(1), 014.
[16] A. Ben Arfa, S. Combes, L. Preziosi-Belloy, N. Gontard, P. Chalier, *Letters Appl. Birobial*., **2006**, 43(2), 149-154.
[17] D. Yadegarinia, L. Gachkar, M.B. Rezaei, M. Taghizadeh, S.A. Astaneh, I. Rasooli, *Phytochemistry*., **2006**, 67(12), 1249-1255.
[18] J. Reichling, P. Schnitzler, U. Suschke, R. Saller, *Res. Complemen. Med*., **2009**, 16(2), 79-90.
[19] M. Mehani, L. Segni, V. Terzi, C. Morcia, R. Ghizzoni, M.B. Goudjil, S.E. Bencheikh, *Der Pharma Chemica*., **2015**, 7(12), 382-388.
[20] N. Mattazi, A. Farah, M. Fadil, M. Chraibi, K.F. Benbrahim, *Int. J. Pharm. Pharm. Sci*., **2015**, 7(9), 73-79.
[21] V.A. Shkurupii, N.V. Kazarinova, A.P. Ogirenko, S.D. Nikonov, A.V. Tkachev, K.G. Tkachenko, *Probl. Tuberk*., **2002**, (4), 36-39.
[22] S.M. Newton, C. Lau, S.S. Gurcha, G.S. Besra, C.W. Wright, *J. Ethnopharmacol*., **2002**, 79(1), 57-67.
[23] A.E. Edris, E.S. Farrag, *Nahrung*., **2003**, 47, 117-121.
[24] T. Inoue, Y. Sugimoto, H. Masuda, C. Kamei, *Biol. Pharm. Bull*., **2002**, 25, 256-259.
[25] N. Mimica-Dukic, B. Bozin, M. Sokovic, B. Mihajlovic, M. Matavuli, *Planta Med*., **2003**, 69, 413-419.
[26] R. Singh, M.A. Shushni, A. Belkheir, *Arabian J. Chem*., **2015**, 8(3), 322-328.
[27] H. Ouakouak, M. Chohra, M. Denane, *Int. Lett. Nat. Sci*., **2015**, 39, 49-55.
[28] Y. Lu, L.Y. Foo, *Food Chem*., **2001**, 75(2), 197-202.