

Original Article

**RADICAL SCAVENGING AND DISINFECTANT EFFECT OF ESSENTIAL OIL FROM MOROCCAN
*MENTHA PULEGIUM***

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ABSTRACT

Objective: As part of the valorization of medicinal and aromatic plants in Morocco, this study aims to investigate the chemical composition, the antimicrobial and the antioxidant activities of *Mentha pulegium* essential oil.

Methods: Essential oil of *M. pulegium* from Oued Laou region (Morocco) was screened for its antimicrobial activity against seven bacteria and two fungi using broth microdilution method. The radical scavenging effect was used to assess *in vitro* antioxidant capacity of this oil.

Results: The tested essential oil exhibited strong inhibitory effect against all tested microorganisms with minimum inhibitory concentrations ranging from 0.003% to 1% (v/v) except for *Pseudomonas aeruginosa*. Moreover, the studied essential oil exhibited radical scavenging power with the half maximal inhibitory concentration of 2 mg/ml.

Conclusion: These findings showed that the studied essential oil has both an antimicrobial and antioxidant potential, which is very rare. Thus, it reinforces its use as an effective, eco-friendly preservative agent in food and cosmetic field.

Keywords: Essential oil, *Mentha pulegium*, Antimicrobial, Antioxidant, Radical scavenging

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INTRODUCTION

Essential oils and other plant extracts have evoked great interest as a potential source of bioactive phytochemical compounds. They have been extensively screened for their potential use as alternatives for food protection against lipid oxidation, as well as for the research of new antimicrobials to fight multi-drug resistant microorganisms. The genus *Mentha* includes 20 species that are widely distributed and can be found in many environments. Several published papers have reported the chemical profile of essential oils from *M. pulegium* of Iran [1], Greece [2], Turkey [3] and Portugal [4]. Moreover, species of *Mentha* have been used as folk remedy for the treatment of nausea, bronchitis, flatulence, anorexia, ulcerative colitis and liver complaints, due to their anti-inflammatory, carminative, antiemetic, diaphoretic, antispasmodic, analgesic, stimulant, emmenagogue and anticatharrhal activities [5].

Mentha pulegium, known as pennyroyal in Europe, is a herbaceous perennial plant which can reach 40 cm high [6]. It grows wild in humid areas in Centre, East and West of Europe, North Africa and minor Asia [5]. In Morocco, it is very widespread, particularly between Marrakech and Azrou regions. *M. pulegium* is a fertile plant whose descendants seem fairly homogeneous. It can be distinguished from other mints species by its stretched port, highly branched stems which are spreading or lying on the soil, pink flowers arranged along the stem and branches and the closed chalice. At maturity, its glandular trichomes are responsible for the essential oil secretion. This oil has been reported by other works for its insecticidal [7], antimicrobial [8], antioxidant [9] and anti-corrosive effects [10].

The present study aims to investigate the chemical composition of Moroccan *M. pulegium* essential oil, to assess its antimicrobial activity against seven bacteria and two fungi causing food-borne illness and other health threats as well as its capacity to scavenge the DPPH free radical.

MATERIALS AND METHODS

Plant material

Fresh aerial part of *Mentha pulegium* was harvested from Oued Laou region (35°26'24" N; 5°4'48" W; 69 m altitude) in Morocco. The

botanical identification was performed, and then voucher specimen was deposited at the Herbarium of National Institute of the Medicinal and Aromatic Plants (NIMAP), Morocco.

Essential oil extraction

The fresh aerial part of *M. pulegium* (leaves and stems) were hydrodistilled for 3 h using a Clevenger-type apparatus [11]. The essential oil was 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 chromatography (FID), equipped with a 5% phenyl methyl silicone HP-5 capillary column (30m x 0.25 mm x 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 N₂ (1.8 ml/min), the split mode was used (Flow: 72.1 ml/min, ratio: 1/50), the 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.

Target strains

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 revived by subcultures in Luria-Bertani (LB) plates (Biokar, France) at 37 °C for 24 h.

As regards to the tested yeast, they include *Candida albicans* and *Candida tropicalis*. Revivification was made by subculturing each strain in malt extract agar (Biokar, France) plates at 30 °C, 48 h.

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations were determined in 96 well-microplate using the microdilution assay [12], with slight

modifications. Bacteriological agar (Biokar, France) at 0.15% (w/v) was used as an emulsifier of the essential oil in the culture medium. For bacteria, the essential oil was serially diluted in Muller Hinton broth (Biokar, France) supplemented with agar to obtain final concentrations ranging between 8% and 0.007% (v/v). The 12th well was considered as growth control (free essential oil 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 10⁶ CFU/ml. After incubation at 37 °C for 24 h, 10 µl of resazurin (Sigma-Aldrich, Germany) were added to each well as a bacterial growth indicator. After further incubation at 37 °C for 2 h, the bacterial growth was revealed by coloration change from purple to pink. Oxacillin (5 µg/ml) was used as a positive control. Experiments were carried out in triplicate.

For yeast, the microdilution was made according to the protocol previously described by CLSI [13], with slight modifications. Firstly, the essential oil was serially diluted in yeast extract-peptone-glucose broth (Biokar, France) (YPG), 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 a growth control. Then, 50 µl of inoculum were added to each well at the final concentration of 10³ CFU/ml. Finally, the microplate was incubated at 30 °C for 48 h. Experiments were carried out in triplicate.

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) (Sigma-Aldrich, Germany) [14]. 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 DISCUSSION

Antimicrobial activity

The studied essential oil has been previously subjected to the gas chromatography-mass spectrometry analysis. Eighteen compounds accounting for 98.77% of the *M. pulegium* essential oil were identified. Its major constituents were R(+)-pulegone (75.48%) (fig. 1), carvone (6.66%) and dihydrocarvone (4.64%). Beside other compounds with relatively low levels, including *p*-mentha-3,8-diene

(2.44%), limonene (1.88%), Pino Carbone (1.27%), α-peperitone (1.13%) and octanol-3 (1.86%) were found. It is observed that this essential oil was characterized by the presence of pulegone as the main component. These results are similar to the most previous reports on Moroccan *M. pulegium* [15].

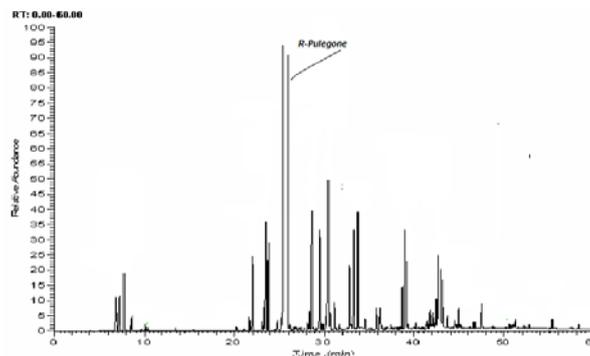


Fig. 1: Chromatogram of *Mentha pulegium* essential oil

The antimicrobial activity tested *in vitro* by microdilution method showed that the tested oil exhibited a strong antimicrobial activity against all tested strains except *P. aeruginosa* (table 1). It can also be noted that this inhibitory effect depends on the target strain. Indeed, *M. luteus* and *B. cereus* were most susceptible to the tested essential oil: they were inhibited from very low concentration (0.125% v/v). Moreover, the growth of *S. aureus* and *B. subtilis* was inhibited from the concentrations of 0.5% and 0.25% respectively. For the Gram-negative bacteria (*S. typhi* and *E. coli*), a MIC value of 0.5% (v/v) was required. It is important to note that all tested strains were resistant to oxacillin at 5 µg/ml except *Staphylococcus aureus*, *Bacillus subtilis* and *Micrococcus luteus*. The results of essential oils antimicrobial activity are in agreement with several works, which reported that Gram-negative bacteria are generally more resistant than the Gram-positive ones [16]. This finding could be due to the penetrating ability of essential oils into the cell, and more precisely to the difference of their cell wall compositions. In fact, the outer membrane of Gram-negative bacteria presents a hydrophilic barrier consisting of lipopolysaccharides molecules, which makes them protected against the effects of this kind of compounds [17]. However, Gram-positive bacteria are less protected against antibacterial agents; the peptidoglycan prevents the passage of molecules with a molecular weight more than 50 KD [18].

Table 1: The minimum inhibitory concentrations (MIC) of *M. pulegium* essential oil against bacterial strains tested

Strains	Concentrations % (v/v)											Oxacillin 5 µg/ml
	8	4	2	1	0.5	0.25	0.125	0.062	0.031	0.015	0.007	
<i>Staphylococcus aureus</i>	-	-	-	-	-	+	+	+	+	+	+	+
<i>Bacillus subtilis</i>	-	-	-	-	-	-	+	+	+	+	+	+
<i>Bacillus cereus</i>	-	-	-	-	-	-	-	+	+	+	+	-
<i>Micrococcus luteus</i>	-	-	-	-	-	-	-	+	+	+	+	+
<i>Escherichia coli</i>	-	-	-	-	+	+	+	+	+	+	+	-
<i>Salmonella typhi</i>	-	-	-	-	+	+	+	+	+	+	+	-
<i>Pseudomonas aeruginosa</i>	+	+	+	+	+	+	+	+	+	+	+	-

As regard to the fungal strains, the pennyroyal's essential oil exhibited a remarkable antifungal effect against both yeast strains tested with a MIC value of 0.031 % (v/v) (table 2).

Table 2: The minimum inhibitory concentration (MIC) of *M. pulegium* essential oil against fungal strains

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

Several studies have confirmed the antiseptic properties of *M. pulegium* essential oil. In fact, the antiseptic effect of this oil against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Aspergillus niger*, *Candida albicans*, *Listeria monocytogenes* and *Vibrio cholera* has been reported [16]. Likewise, a strong effect of this essential oil against microbial strains namely: *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumonia* has been proved [19].

The antimicrobial activity of this essential oil could be due to its chemical constituents. It is clear that our essence is characterized by the dominance of pulegone (75%), which is a ketone known for its inhibitory effect [20]. In fact, various biological properties of pulegone have been reported, including antibacterial, antifungal, antihistaminic, antipyretic, anti-treponemal, convulsant, hypercholesterolemic, cytochrome P-450 inhibitory and lysozymal enzyme inhibitory effects [21].

Being a major compound for several medicinal herbs like *M. longifolia* [22], the pulegone has been reported as an antimicrobial [23]. Also, several works demonstrated that the carvone exerts an antimicrobial power against a wider range of bacteria and fungi [24, 25]. In addition, the synergistic effect of minor compounds and between all chemical constituents of the essential oil could be responsible for its antimicrobial outcome [26].

Antioxidant activity

The antioxidant activity of the studied essential oil was evaluated by the DPPH• reduction method using butylated hydroxytoluene (BHT) as standard.

As shown in fig. 2, a linear relationship was found between the antioxidant activity and the BHT concentration ($R^2=0.99$, $P\text{-value}=0.000$). The IC_{50} of the standard was of 6.83 $\mu\text{g/ml}$. The essential oil investigated showed a scavenging effect with an IC_{50} of 2 mg/ml. Hence, the result expressed with the equivalent of BHT was 3.42 $\mu\text{g Eq BHT/mg}$ of essential oil.

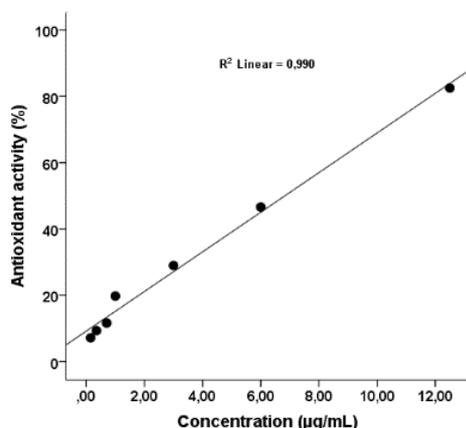


Fig. 2: Linear relationship between the antioxidant activity and the concentration of BHT

According to this finding, the whole essential oil exhibits an interesting radical scavenging effect even if the BHT was more active. However, the synthetic antioxidants have fallen out of favor because they are suspected of being carcinogenic and allergic [27].

Previous studies reported a wide variation in the antioxidant activity of *M. pulegium* essential oil. In fact, it showed significant antioxidant activity, in comparison to that obtained in Iran [28] and Portugal [4]. This antioxidant activity of *M. pulegium* essential oil is probably due to the ability of terpenes to be a donor of hydrogen atoms or electrons, and to capture free radicals [29].

CONCLUSION

To summarize, the present study revealed the interesting antimicrobial effect of *M. pulegium* essential oil with a broad spectrum. Moreover, a significant antioxidant activity of this oil assessed *in vitro* via the DPPH radical scavenging assay was found.

Hence, both activities demonstrated by the same oil shows its major interest to develop a new eco-friendly and safe preservative for the extension of the shelf-life of raw and processed foods. However, further studies should be carried out to evaluate the safety and toxicity of this essential oil.

CONFLICT OF INTERESTS

We certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript

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