Chemical composition and antimicrobial activities of the volatile oil of *Mentha- pulegium* (Labiatae)

Mohamed H.M. Abd El Azim*1, Amani M.D. El-Mesallamy1, S. Safaan1, S. Hussein2, Mohamed Abd El Maksoud1

1Department of Chemistry, Faculty of Science, Zagazig University, Zagazig, (EGYPT)
2Department of Phytochemistry and Plants systematic, Division of Pharmaceutical Chemistry, National Research Center, Dokki, Cairo, (EGYPT)
E-mail: mhmsm01213@yahoo.com

**KEYWORDS**

*Mentha pulegium*; Volatile oil; Chemical composition; GC-MS; Antibacterial and antifungal activities.

**ABSTRACT**

This study was aimed to evaluate the chemical composition and antimicrobial activities of the volatile oil of *Mentha pulegium* (Labiatae). Twenty one volatile compounds were identified by using gas chromatography (GC) and gas chromatography-mass spectroscopy (GC-MS). The results showed that the volatile oils mainly contained about 31.66 % 1-Menthol and 27.76 % 1-Menthone. Antimicrobial activities of volatile oil was studied against three bacterial strains (*E. coli.*, *Staphylococcus aureus* and *Salmonella typhi*) and four fungal species (*Fusarium*, *Aspergillus sp.*, *Penicillium sp.* and *Trichoderma sp.*) at concentration 0.1 ml and 0.3 ml (10 mg / 1 ml). The volatile oil had strong inhibitory effect for all bacterial and fungal species at concentration 0.3 ml (10 mg / 1 ml) of the volatile oil.

© 2014 Trade Science Inc. - INDIA

**INTRODUCTION**

*Mentha*; the genus of Labiatae family, includes 20 species that spread all over the world. *Mentha pulegium* L. is one of the Mentha species commonly known as pennyroyal. It is native species of Europe, North Africa and in Asia Minor and near East[3]. One of the principal causes of food quality deterioration is the oxidation of unsaturated lipids initiated by free radicals[4]. The Mentha genus is a member of this family and represents by about 6 species in the flora of Iran[7]. Mentha species are generally known under the name “na’na” and “pooneh” in Iran and commonly used as herbal tea, flavoring agent, and medicinal plant[8]. Analysis of the essential oil revealed the presence of piperitone (38.0%), pipertone (33.0%), terpineol (4.7%), and pulegone (2.3%) as the major components. The results showed a significant activity against microorganisms especially Gram-positive bacteria with inhibition zones and minimal inhibitory concentration values in the range of 8 to 21 mm and 0.25 to 4 µl/ml, respectively, whereas the least susceptible were Gram-negative bacteria especially *Escherichia coli*[8]. The dependence of *Mentha pulegium* L. (pennyroyal) essential oil composition, obtained by supercritical carbon dioxide (SC-CO2), with the following parameters: pressure, tem-
temperature, extraction time (dynamic), and modifier (methanol) was studied. The results were also compared with those obtained by conventional hydro distillation method in laboratory conditions. Regarding the percentages of menthone (30.3%) and pulegone (52.0%). The evaluation of the composition of each extract was performed by gas chromatography–mass spectrometry\textsuperscript{11}. The chemical composition of European pennyroyal (\textit{Mentha-pulegium}) essential oil and to characterize the in vitro antioxidant and antimicrobial activities of its water (hot and cold) and ethnologic extracts and of the essential oil. The essential oil revealed menthone, pulegone and neo-menthol as the main constituents, comprising 35.9, 23.2 and 9.2% of the essential oil, respectively\textsuperscript{9}. The aim of this study was to identify the chemical composition and evaluate the antimicrobial activity of Egyptian \textit{Mentha pulegium} \textit{L}. oil against different microorganisms in order to validate its traditionally used.

\textbf{MATERIALS AND METHODS}

\textbf{Isolation of the volatile oils}

Air-dried aerial parts were cut in small pieces and subjected to steam distillation for three hours using the method described by Marcus and Lichtenstein\textsuperscript{6}, and Weaver\textsuperscript{11}. The volatile oils were dried over anhydrous sodium sulfate and stored under \textit{N}2 atmosphere in amber vials at 4 \textdegree C until they were analyzed.

\textbf{GC-MS analysis conditions}

For qualification, the essential oil was analyzed on Gas Chromatography Mass Spectrometry HP 6890 Series A (Agilent) by using A Thermo Scientific (TR-5MS), (5% Phenyl Polysil Phenylene Siloxane) capillary column (30 m x 0.25 mm i.d.; 0.25 \mu m film thickness). Helium (He), having a flow rate of 1.00 ml/min, was used as carrier gas. The GC oven temperature was kept at 50 \textdegree C for 5 minutes and programmed to 250 \textdegree C. The injector temperature was 250 \textdegree C. The amount of injection was 1 \mu L.

\textbf{Identification of components}

Retention indices for all compounds were determined according to the Van Den Dool method\textsuperscript{9}. While identification of the components was based on comparison of their mass spectra with those of internal (computer) library, Wiley7n.1 and PMW-Tox3.1 libraries and some reference compounds.

\textbf{Antimicrobial activities of volatile oils}

\textbf{1. Microbial strains}

The bacterial and fungal strains were obtained from the microbiology Lab., Botany Department, Faculty of Science, Zagazig University. Bacterial species tested were \textit{E. coli.}, \textit{Staphylococcus aureus} and \textit{Salmonella typhi} and fungal species were \textit{Fusarium}, \textit{Aspergillus sp.}, \textit{penicillium sp.} and \textit{Trichoderma sp.} The oil was dissolved in dimethylformamide (DMF) for antimicrobial investigation at the final concentration of (10 mg / 1 ml).

\textbf{2. Antibacterial activity}

Antibacterial activities of volatile oil were tested using pour plate technique at two concentrations 0.1 ml and 0.3 ml (10 mg / 1 ml). Culturing and incubated of different bacterial species were carried out at 37 \textdegree C for 24 hours. After the elapse of incubation periods, the diameter of inhibition zones was measured. The inhibition zone formed by the volatile oil against the particular test bacterial strain determined as the antibacterial activities\textsuperscript{10}.

\textbf{3. Antifungal activity}

Czepak Dox media used for cultivation of fungal species. The medium was seeded with different fungal species. After solidification of media on plates, make pores in agar with cup pores (15 mm) diameter. Two concentrations 0.1 ml and 0.3 ml (10 mg / 1 ml) of the volatile oil were transferred into the well. Dimethyl formamide (DMF) was used only as a control. The plates were incubated for 7 days at 30 \textdegree C. The inhibition zone formed by the extract against the particular test fungal strain determined as the antifungal activities of the extract.

\textbf{RESULTS AND DISCUSSION}

\textbf{Chemical composition of the volatile oils}

The results obtained from the gas liquid chromatogram were reported in TABLE 1, we can see from these results that we have twenty one compounds were characterized. The major components are as follows: 30.85 \% 1-Menthol and 27.76 \% 1-Menthone.
An Indian Journal  

Natural Products  

Antimicrobial screening  

The antimicrobial activities of Mentha pulegium volatile oil were evaluated by a pour plate technique method against bacterial species (E. coli, Staphylococcus aureus and Salmonella typhi) and fungal species (Fusarium, Aspergillus sp., penicillium sp. and Trichoderma sp.). Volatile oils strongly exhibited antimicrobial activity against the tested strains at all concentration.

**Antibacterial activity**
TABLE 2: Antibacterial activity of volatile oil of Mentha-pulegium

<table>
<thead>
<tr>
<th>Bacterial species</th>
<th>Inhibition zone diameter in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Escherichia-coli</td>
<td>7</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>-</td>
</tr>
<tr>
<td>Salmonella typhi</td>
<td>13</td>
</tr>
</tbody>
</table>

Data in TABLE 2 and in Figure 2 evaluate that the maximum inhibitory responses are indicated after the treatment of *E. coli.*, *Staphylococcus aureus* and *Salmonella typhi* with highest concentration of the oil (0.3 ml), while the moderating inhibitory response after the treatment of *E. coli.*, and *Salmonella typhi* with normal concentration of the oil (0.1 ml). The result was showed that *Staphylococcus aureus* had is the highest resistance species to the oil at 0.1 ml concentration.

Antifungal activity

TABLE 3: Antifungal activity of the volatile oil of Mentha-pulegium

<table>
<thead>
<tr>
<th>Fungal strains</th>
<th>Inhibition zone diameter in mm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1 ml</td>
</tr>
<tr>
<td>Fusarium oxysporum</td>
<td>35.0</td>
</tr>
<tr>
<td>Aspergillus sp.</td>
<td>28.0</td>
</tr>
<tr>
<td>Penicillium sp.</td>
<td>38.0</td>
</tr>
<tr>
<td>Trichoderma sp.</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Antifungal activities of the volatile oil of *Mentha-pulegium* were evaluated against the tested fungal
strains. The oil showed strongly inhibitory activity against all species at 0.1 and 0.3 ml concentrations as shown in TABLE 3 and Figure 3.

CONCLUSIONS

The chemical composition of Egyptian Mentha-pulegium oil was investigated. Volatile oil from Mentha pulegium, was obtained by steam-distillation method, and its chemical composition was determined by GC-MS. The results indicated that the volatile oils mainly had about 30.85 % 1-Menthol and 27.76 % 1-Menthone.

The results showed that volatile oils of Mentha-pulegium have strong antibacterial activities against (E. coli. and Salmonella typhi at concentration 0.1 ml and 0.3 ml (10 mg / 1 ml)) and antifungal activities against (Fusarium, Aspergillus sp., penicillium sp. and Trichoderma sp. at the same concentrations.

REFERENCES