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Comparative Essential oil Composition of Leaves of *Mentha rotundifolia* and *Mentha pulegium* a Traditional Herbal Medicine in Morocco

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ABSTRACT

The essential oil of the leaves of *Mentha rotundifolia* and *Mentha pulegium* grown in Morocco was determined by hydro-distillation method and analysed by gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC-MS). The identified components constituting 90.40% and 97.34% of the total oil of *Mentha rotundifolia* and *Mentha pulegium* respectively. Thirty two volatile constituents were identified in leaves oil of *Mentha rotundifolia* and the main constituents of the essential oil were menthol (40.50%), other predominant constituents were: menthone (5.0%), menthyl acetate (4.50%), menthofuran (4.20%), oxyde de piperitone (3.80%), linalyl acetate (3.50%), isomenthone (2.50%), 1,8-cineole (2.40%), linalool (2.0%), limonene (1.80%), geraniol (1.70%), myrcene (1.60%), geranyl acetate (1.50%) and trans-Sabinene hydrate (1.40%). Twenty eight compounds were identified in leaves oil of *Mentha pulegium* and the major component was piperitone (35.56%), other predominant constituents were: piperitenone (21.18%), α-terpineol (10.89%), pulegone (6.452%), pipéritone oxide (4.02%), menthol (3.28%), menthone (3.09%), neomenthol (2.80%), menthofuran (2.15%), isomenthone (1.56%), carvone (1.13%), geranyl acétate (1.06%), germacrène D (1.03%) and limonène (1.02%).

**Key words:** *Mentha rotundifolia*, *Mentha pulegium*, Essential oil composition, GC/MS

Introduction

Aromatic and Medicinal plants were used for centuries as remedies for human diseases because they contain chemical components of therapeutic value (Nostro et al., 2000). The family of Lamiaceae contains an extremely wide variety of aromatic plants mainly in temperate countries. Among this rich array of plants yielding essential oils, the genus of *Mentha*, includes 20 species that spread all over the world.

Essential oils were used in ancient Rome, Greece and Egypt and throughout the Middle and Far East as perfumes, food flavours, deodorants and pharmaceuticals (Baris and al., 2006). Lamiaceae family is recognized for their medicinal uses, because of their vital oils content, antimicrobial activity of different species of this family was demonstrated (Skaltsa et al., 2003). It was reported that *Mentha* spp. exhibit antimicrobial activity against *Escherichia coli*, *Bacillus subtilis*, *Salmonella enteritidis* and *Staphylococcus aureus* (Sivropoulou et al., 1995; Tassou et al., 2000). For thousands of years, plant products and their modified derivatives have been rich sources for clinically useful drugs. Even today, about 80% of the world’s population relies predominantly on plants and plant extracts for health care (Jennifer et al., 2007). Plant essential oils and their components have been known to exhibit biological activities, especially antimicrobial, since ancient time. With the growing interest of the use of either essential oils or plant extracts in the food and pharmaceutical industries, screening

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of plant extracts for these properties has become of increasing importance (Amvam et al., 1998). Mentha rotundifolia is an hybrid between Mentha longifolia and Mentha suaveolens Ehrh., whose essential oil has been the object of several studies (Raya et al., 1990; Nagell and Hefendehel., 1974; Hendriks et al., 1976; Umemoto., 1998 and Daniel et al., 2002), and different chemotypes have been characterised.

Some authors have considered Mentha rotundifolia as a synonym of Mentha suaveolens (Hendriks and Van Os., 1976). Morocco is blessed with a rich source of aromatic plants, many of which have not been previously investigated for their chemical constituents and biological potentials. Mentha rotundifolia is a plant belongs to the family Lamiaceae, which grows in Morocco region and is a potential source of essential oils. It is very widely distributed around the Mediterranean basin, in America and in occidental Asia (Mailhebiau P, 1994; Bezanger B et al., 1980). She has been used for their flavours in cooking, in folk medicine as antiseptic and as antimicrobial agents (Bremnes L., 2002). In Morocco and northern Africa, this aromatic plant is well known such as "tmarrsad. Multiple studies have been reported on the chemical composition of the essential oils of Mentha rotundifolia belonging to different regions in the world (Brada et al., 2006; Kokkini et al., 1988 and El Arch et al., 2003) have been reported and related chemotypes have been defined. One of them is particularly rich in piperitenone oxide, an oxygenated monoterpene whose biological effects (cardiovascular effects, activity antibacterial and antifungal, toxic, repellent and reproduction retardant toward malarial vector Anopheles stephensi) have been investigated (Damien et al., 2003 and Tripathi et al., 2004).

In this work, we studied and compared the Chemical composition of essential oils of Mentha rotundifolia and Mentha pulegium collected in Atlas mean a mountainous region from Morocco where people frequently use this plant in traditional medicine.

Materials and methods

- Plant material and extraction of essential oil

The leaves of Mentha rotundifolia and Mentha pulegium were collected in Mai 2009 at Skoura near Boulmane (90 km in the south east of Fez. The coordinates: latitude: 35 ° 42'21" longitude: 4 ° 32'31"; altitude: 3200 m). The climate is semi-humid with strong continental influence with an annual average temperature of 20°C. The plants were then isolated from the other specimen and conserved for extraction.

The leaves of Mentha rotundifolia and Mentha pulegium were shade dried (25 days) and immediately hydro-distilled (100g) for 2.5 h using a modified Cleveenger-type apparatus. The oil was extracted from the distillate with hexane and then dried over anhydrous sodium sulfate. After filtration, the solvent was removed by distillation under reduced pressure in a rotary evaporator and the pure oil kept at 4°C in the dark, until the moment of analysis.

- Gas chromatography (GC-FID) analysis

The isolated oil was diluted with hexane, and 1µL was sampled for the gas chromatographic analysis. Trace GC (ULTRA S/N 20062969, Thermo Fischer), gas chromatograph equipped with HP-5MS non polar fused silica capillary column (60 m x 0.32 mm, film thickness 0.25 µm) was used. Operating conditions: oven temperature program from 40°C (6 min) to 280°C at 5°C/min and the final temperature kept for 20 min; "split mode” ratio 1:20; carrier gas Azoth (N2), flow rate 1 ml/min; injector and flam ionization detector (FID) were fixed at 260°C and 280°C, respectively.

- Gas chromatography-mass spectrometry (GC/MS) analysis

GC/MS analyses were performed on a Thermo Fischer capillary gas chromatograph directly coupled to the mass spectrometer system (model GC ULTRA S/N 20062969; POLARISQ S/N 210729). HP-5MS non polar fused silica capillary column (60 m x 0.32 mm, 0.25 µm film thickness) was used under the following conditions: oven temperature program from 40°C (6 min) to 260°C at 3°C/min and the final temperature kept for 10 min; injector temperature, 240°C; carrier gas He, flow rate 1 mL/min; the volume of injected was of 1µl of diluted oil in hexane; splitless injection technique; ionization energy 70 eV, in the electronic ionization mode; ion source temperature 200°C; scan mass range of m/z 30-650 and interface temperature was 300°C. The constituents of essential oils were identified in comparison with their retention index, calculated in relation to the retention time of a series of linear alkanes (C4-C28) with those of reference products and in comparison with their retention index those of the chemical components gathered by Adams (2001) and in comparison with their spectra of mass with those gathered in a library of (NIST-MS) type.
Results and discussion

The chemical composition of essential oils of Mentha rotundifolia and Mentha pulegium are presented in Table 1.

Table 1: Chemical composition of the essential oil from leaves of Mentha rotundifolia and Mentha pulegium

<table>
<thead>
<tr>
<th>RI</th>
<th><strong>Largest peaks (m/z)</strong></th>
<th><strong>Method of identification</strong></th>
<th><strong>Constituents</strong></th>
<th><strong>Air (%)</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>1061</td>
<td>(152),110,81,95,67,68,64,69,109,55,70</td>
<td>RI, GC/MS</td>
<td>α-Thujone</td>
<td>0.10</td>
</tr>
<tr>
<td>947</td>
<td>(136),93,91,39,121,77,92,79,43,41,105</td>
<td>RI, GC/MS</td>
<td>α-Pinene</td>
<td>0.40</td>
</tr>
<tr>
<td>933</td>
<td>(136),93,91,69,39,72,79,53,41,27</td>
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<td>0.10</td>
</tr>
<tr>
<td>1017</td>
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<td>RI, GC/MS</td>
<td>Limonene</td>
<td>1.80</td>
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<tr>
<td>942</td>
<td>(136),93,91,77,41,121,67,27,107,39</td>
<td>RI, GC/MS</td>
<td>Camphene</td>
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<td>2545</td>
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<td>Isomenthone</td>
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<tr>
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<tr>
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<tr>
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<td>Neomenthol</td>
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<td>RI, GC/MS</td>
<td>Menthofuran</td>
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<td>RI, GC/MS</td>
<td>Piperitone</td>
<td>3.10</td>
</tr>
<tr>
<td>1223</td>
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<td>RI, GC/MS</td>
<td>Piperitone oxide</td>
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<td>RI, GC/MS</td>
<td>Piperitone</td>
<td>3.10</td>
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<td>Piperitone</td>
<td>3.10</td>
</tr>
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<td>RI, GC/MS</td>
<td>Borneol</td>
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<td>1227</td>
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<td>RI, GC/MS</td>
<td>Geranial</td>
<td>3.00</td>
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<td>RI, GC/MS</td>
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</tr>
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<td>1276</td>
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<td>RI, GC/MS</td>
<td>Linalool</td>
<td>3.00</td>
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<tr>
<td>997</td>
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<td>RI, GC/MS</td>
<td>α-Terpineol</td>
<td>1.10</td>
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<td>1271</td>
<td>(96),43,41,69,80,121,68,55,71,79</td>
<td>RI, GC/MS</td>
<td>Linalyl acetate</td>
<td>3.50</td>
</tr>
<tr>
<td>1051</td>
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<td>RI, GC/MS</td>
<td>β-Terpineol</td>
<td>0.30</td>
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<td>1157</td>
<td>(152),91,77,93,41,27,95,137,109,54,152</td>
<td>RI, GC/MS</td>
<td>Piperitone</td>
<td>3.10</td>
</tr>
<tr>
<td>1137</td>
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<td>RI, GC/MS</td>
<td>Borneol</td>
<td>0.50</td>
</tr>
<tr>
<td>1136</td>
<td>(154),71,111,93,43,86,41,69,55,68,154</td>
<td>RI, GC/MS</td>
<td>Terpinen-4-ol</td>
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</tr>
<tr>
<td>1058</td>
<td>(154),43,93,81,71,69,84,68,108,41,55</td>
<td>RI, GC/MS</td>
<td>1,8-Cineole</td>
<td>2.40</td>
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<td>1227</td>
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<td>RI, GC/MS</td>
<td>Geranial</td>
<td>1.70</td>
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<td>RI, GC/MS</td>
<td>Linalool</td>
<td>2.00</td>
</tr>
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<td>RI, GC/MS</td>
<td>Bornyl acetate</td>
<td>0.20</td>
</tr>
<tr>
<td>1261</td>
<td>(150),135,150,91,136,77,107,117,115,79,105</td>
<td>RI, GC/MS</td>
<td>Carvacrol</td>
<td>0.30</td>
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<tr>
<td>1578</td>
<td>(204),93,80,121,92,43,55,67,91,147</td>
<td>RI, GC/MS</td>
<td>β-Humulene</td>
<td>0.10</td>
</tr>
<tr>
<td>1175</td>
<td>(154),93,91,136,131,43,68,95,67,41</td>
<td>RI, GC/MS</td>
<td>α-Terpineol</td>
<td>0.30</td>
</tr>
<tr>
<td>1222</td>
<td>(152),152,81,67,109,82,41,137,69,95,55</td>
<td>RI, GC/MS</td>
<td>pulegone</td>
<td>6.42</td>
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<tr>
<td>1223</td>
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<td>RI, GC/MS</td>
<td>Piperitone</td>
<td>21.12</td>
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<tr>
<td>1182</td>
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<td>RI, GC/MS</td>
<td>carvone</td>
<td>1.13</td>
</tr>
</tbody>
</table>

Total Identified Constituents 90.40 97.34

Yields (%) 1.54 1.66

RI: Retention Index was determined by GC-FID on a HP-5MS column.
**Largest peaks (m/z) were determined by mass spectrometry (PlarisQ).**
**Air (%) was determined by mass spectrometry (PlarisQ).**

The compounds of mentha rotundifolia leaf oil from Morocco are listed in order of their elution on the HP-5MS column (Figure 1). The essential oil yields of the studies were 1.54%. It is relatively higher than other plants industrially exploited as a source of essential oils: Artemisia herba-alba (0.59%), Artemisia absinthium (0.57%) and Artemisia pontica (0.31%) (Derwich et al., 2009), lavender (0.8-1.8%), menthe (0.5-1%), neroli (0.5-1%), Laurel (0.1-0.35%) (Edward et al., 1987), Lippia rotundifolia (0.01%) (Suzana et al., 2008). Contrary to the Mentha rotundifolia essential oils yield of Morocco which is very high level (4.33%) (Benayad, 2004). In this study the yield is low to those of Laurus Nobilis essential oils analyzed in Morocco by Derwich et al. (2009), lavender (0.8-1.8%), menthe (0.5-1%), neroli (0.5-1%), Laurel (0.1-0.35%) (Edward et al., 1987), Lippia rotundifolia (0.01%) (Suzana et al., 2008). Also it’s similar to the composition of essential oil of leaves of Mentha pulegium study in Tunisia which the major component is menthol (Marzouk et al., 2008).
The total essential oil in this study (90.40%) is different to those found in Mentha rotundifolia oil in Uruguay by Daniel L. et al., (2002) which is of 93.5% and its major component is of piperitenone oxide (80.8%) and in Tunisia which is 96.66% with the major component is pulegone (47.15%) (Hajlaoui et al., 2009). Intense study on the essential oil of Mentha rotundifolia revealed the existence of different principal constituent: piperitenone oxide (Kokkini et al., 1988; Hendriks and Van Os., 1976), methyl acetate (Kokkini et al., 1988), dihydrocarvone (Hendriks and Van Os., 1976), carvone (Marina et al., 2009) and piperitenone (Benayad, 2004).

The essential oil content shows variations in plants of different geographical origin and also in different part of the tree: Stoyanova et al., (2005); studied the composition of Mentha pulegium oil collected from the Batak in the Rhodope Mountains (Bulgaria), they reported that the yields of oil obtained by water and steam distillation were 1.54% and 1.48%, respectively and the composition is characterized by a high content of pulegone (42.9-45.4%), piperitenone (21.7-23.1%) and isomenthone(11.3-12.8%). In our previous studies on the chemistry of Brazil Lippia rotundifolia (Suzana et al., 2008), considerable differences were observed in the essential oil composition between leaves and flowers: α-pinene (8.7% and 1.8%), myrcene (5.1% and 3.6%), limonene (26.0% and 7.9 %), cis-pinocamphone (4.5% and 3.1%) and myrtenal (22.3% and 16.7%), respectively. Furthermore, the essential oils, obtained from leaves and stems from menth in Tunisia contained: menthol (46.60-49.86% and 40.57-51.61%), 1, 8 cineole (13.53-17.31% and 11.10-18.46%), menthone (11.13-12.34% and 7.32-20.04%) (Marzouk et al., 2008). Also the main constituents of the essential oil of flower, leaves and stem oils, from basil (Ocimum basilicum L.) in Turkey, were estragole (58.26%, 52.60% and 15.91%) and limonene (19.41%, 13.64% and 2.40%) and p-cymene (0.38%, 2.32% and 2.40%) respectively (Jean-Claude et al., 2008). In our previous studies on the chemistry of Uruguay (Daniel et al., 2002), considerable differences were observed in the essential oil composition between Mentha rotundifolia and Mentha pulegium: Piperitenone (80.8%) and Pulegone (73.4%) and the total constituents identified is 93.5% and 99.3% respectively.

The constituents of leaves essential oil of Mentha pulegium from Morocco are listed in order of their elution on the HP- 5MS non polar column (Figure 2). The essential oil yields of the studies were 1.66%. It is relatively higher than other plants industrially exploited as a source of essential oils: Eucalyptus globulus (1.21%) (Derwich et al., 2009), Lippia rotundifolia (0.01%) (Suzana et al., 2008) and Juniperus phoenicea (1.62%) (Derwich et al., 2010). Contrary to the Mentha pulegium essential oils yield of Morocco, which is very high level (2.33%) (Benayad, 2004).

In this study, twenty eight compounds were identified in the leaf essential oil of Mentha pulegium, which made up 97.34% of the total oil (Table 1). This total oil was globally similar to that of Mentha pulegium oil.
Mentha rotundifolia oil in Uruguay by Daniel et al (2002) which is 93.5%.

The results obtained in the chemical composition study of the essential oils are shown on Table 1, which the major component was piperitone (35.56%), other components present in appreciable contents were: piperitenone (21.18%), alpha-terpineol (10.89%), pulegone (6.452%), pipérítone oxide (4.02%), menthol (3.28%), menthone (3.09%), neomenthol (2.80%), menthofuran (2.15%), isomenthone (1.56%), carvone (1.13%), geranyl acetate (1.06%), germacrène D (1.03%) and limonène (1.02%).

The essential oils composition showed a relatively similar pattern to those published for other geographical regions: Piperitone (38.00%), piperitenone (33.0%), α-terpineol (4.7%) and pulegone (2.3%), were reported as the major component in the essential oil from Iran (Mahboubi and Haghi, 2008), menthone, isomenthone, isomenthol, menthol and pulegone from Uruguay (Daniel et al., 2002), pulegone, piperitenone and isomenthone from Bulgaria (Stoyanova et al., 2005) and pulegone (43.3- 87.3%), menthone (6.7%), isomenthone (22.6%), piperitone (2.13%) and piperitenone (26.73%) from Algerie (Beghidja et al., 2007) and piperitone (1.3-3.2%), pulegone (65.9-83.1%), menthone (83.8-7.7%), isomenthone (3.8-4.0%), neo-menthol (0.7-1.3%), pulegol acetate (0.1-1.2%), γ-terpinene (0.9-1.2%), β-caryophyllene (0.1-0.9%) and β-caryophyllene oxide (0.3-1.9%) from India (Agnihotri et al., 2005). Also it’s different to the composition of essential oil of leaves of Mentha pulegium study in Tunisia and Morocco which the major component were menthol (48.56%) (Marzouk et al., 2008), and pulegone (73.33%) respectively (Benayad, 2004) and it are different to those found in Mentha rotundifolia oil in Uruguay by Daniel et al (2005) which the major component was of piperitenone oxide (80.8%).

Daniel et al (2002), studied the composition of Mentha pulegium oil collected from the Uruguay, they reported that the composition is characterized by a high content of pulegone (73.4%) and isomenthone (12.9%); from Egypt: pulegone (43.5%) and piperitone (12.2%) (El-Ghorab, 2006); from Tunisia: pulegone (41.8%) and isomenthone (11.3%) (Mkaddem et al., 2007). The chemical composition, obtained from Foeniculum vulgare var. vulgare Mill and Foeniculum vulgare var. azoricum Mill, in Egypt by Osman (2009), contained anethole (Anisole) (64.67-71.49%) and (48.51-62.72%) respectively. Abd El-Wahab (2009), studied the productivity of Mentha spicata in four locations in Upper Egypt: Beni-Suef, Sohag, Qena and Aswan Governorates, the highest content of carvone was obtained from Qena (53.09%), Aswan (53.32%) and Beni-Suef (46.45%).

Conclusion

The present study has been concerned with determining and comparing the chemical composition characteristics of essential oils extracted from Mentha rotundifolia and Mentha pulegium, collected in the Atlas region of Boulmane (Morocco). The leaf oil obtained from Mentha rotundifolia and Mentha pulegium grown of Tunisia which is (96.13%) (Hajlaoui et al., 2009) and is (92.26%) (Marzouk et al., 2008) and of northwestern Himalayas India which is between (87-98%) (Agnihotri et al., 2005), contrary it’s different to those found in Mentha rotundifolia oil in Uruguay by Daniel et al (2002) which is 93.5%.
in Morocco was characterized by GC-MS, GC-FID. Thirty two constituents were identified in leaves oil of *Mentha rotundifolia* representing 90.40% of the total oil composition. The yield was 1.54% and the major constituent in leaves was menthol. Twenty eight constituents were identified in leaves oil of *Mentha pulegium* representing 97.34% of the total oil and the yield was 1.66%. The leaves oil was characterised by high contents of piperitone (35.56%). In this work the yield and the total oil of the plants essential oil extracted from *Juniperus phoenicea* and *Juniperus oxycedrus* are relatively similar.

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**References**


