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## Chemical composition of essential oils and floral waters of *Mentha longifolia* (L.) Huds. from Senegal

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#### Abstract

Essential oils of *Mentha longifolia* from Senegal were extracted from fresh plants and dried plants in the shade for 7 days. The yields were of 0.39 and 0.32% in the fresh and dried plants respectively. Analysis of the essential oils, floral waters and the assay of pulegone were carried out by GC/FID and GC/MS. The major compounds identified in the oils were pulegone (52.0 and 42.4%), menthone (14.3 and 21.2%), 1, 8-cineole (13.1 and 11.4%) and isomenthone (9.0 and 13.2%) in the fresh and dried plants, respectively. Analyses revealed that floral waters were also characterized by the same major compounds as essential oils but at different rates. In these floral waters, pulegone constituted 60.2 and 47.0%, 1,8-cineole 7.9 and 19.6%, isomenthone 7.2 and 10.7%, menthone 6.4 and 9.2%, chrysanthenone 6.4 and 3.2% and  $\alpha$ -terpineol 3.0 and 2.7% in the fresh and dried plants, respectively. The assay results of pulegone, a hepatotoxic compound, have shown very high levels (444.6 and 393.3mg/g) in both fresh and dried plants.

**Keywords:** *Mentha longifolia*, essential oils, floral waters, pulegone

#### 1. Introduction

The Senegalese industry uses several varieties of essential oils. However, the country does not produce essential oils and import all of them for food and cosmetic industries [1]. In anticipation of the establishment of optimum industrial operating conditions, we proposed to study the plants that produce essential oils in Senegal as mint species.

In Senegal, several species of mint such as *Mentha longifolia* L., *Mentha x piperita* L. and *Mentha spicata* L. are commonly added as a flavoring in drinks and consumed as tea. Essential oils of mints are often used for flavoring food products such as candies and chewing gums. They show also very interesting biological properties such as antimutagenic potency [2] and the treatment of secondary amenorrhea and oligomenorrhea [3]. The chemical composition of *M. longifolia* essential oils has been the subject of several studies [3-5] and its composition may change with the drying methods [4]. According to the study of Asekun *et al.* (2007) [4], *M. longifolia* fresh plants give: pulegone 35.0%, menthone 31.1% and 1,8-cineole 13.0% and the air-dried plants at room temperature give: menthone 47.6%, pulegone 18.4% and 1,8-cineole 16.4%. The sun-dried plants reveal: menthone 38.3%, pulegone 20.2% and 1, 8-cineole 16.6% and the oven-dried plants (40 °C): limonene 40.8% and  $\alpha$ -pinene 15.0% [4]. In most *M. longifolia* chemotypes, ketone compounds such as pulegone, menthone and isomenthone are major constituents [3-5]. Ketone compounds often cause adverse effects and their use should be controlled [6]. Other *M. longifolia* oils rich in piperitone oxide and piperitenone oxide [7], menthol and menthyl acetate [8] have also been considered. In the literature, floral waters are less studied than essential oils. Floral waters of *M. suaveolens* ssp. *insularis* from Corsica revealed *cis*, *cis-p*-menthenolide (67.3%) and pulegone (14.8%) as major components in floral waters [9]. The oils as floral waters were also rich in pulegone (44.4%) and *cis*, *cis-p*-menthenolide (27.3%) [9].

The objective of this work is to study the variation of the chemical composition of the essential oils and floral waters of *M. longifolia* from Senegal according to the drying in the shade. Pulegone (a common product in several mints which presents potential hepatotoxicity in rats at 400 mg/kg [10]) under European Union regulation (restriction to 20 mg/Kg in non-alcoholic beverages containing mint or peppermint [6]), is identified in the oils of *M. longifolia*. This compound was measured according to AFNOR method NF T 75-418 [11].

## 2. Materials and methods

### 2.1 Plant material and extraction of essential oils

*M. longifolia* plants were harvested in the micro-garden of the traffic circle of “Liberté 6 in Dakar”. A voucher specimen was deposited in the herbarium of the “Institut Fondamental d’Afrique Noire de l’Université Cheikh Anta Diop de Dakar”. Fresh plants and shade-dried plants (let at room temperature for 7 days) were used for the extractions. About 100 g of each the fresh and dried plants were separately subjected to steam distillation for 45 min using a Clevenger-type apparatus. Essential oils and floral waters obtained were stored in the refrigerator (at 4 °C) in amber vials until analysis. Quantification and identification of the compounds were performed by GC/FID (gas chromatograph coupled to a flame ionization detector) and GC/MS (gas chromatograph coupled to a mass spectrometry detector) respectively.

### 2.2 Gas Chromatography

In FID as in GC-MS, the temperature conditions were as follows: isotherm at 40 °C for 5 min then increased to 280 °C by 8 °C/min and final hold at 280°C for 5 min. Detector temperature was at 290 °C and the injector was operating in splitless mode at 290 °C. The carrier gas was helium at a constant flow rate fixed at 1.5 ml/min. The capillary column used was an Optima-5-accnt (Macherey-Nagel, Germany), 5% phenyl-95% methylsiloxane: length 30 m, internal diameter 0.25 mm and film thickness of 0.25 µm.

GC/FID - The gas chromatograph used was an HP 6890N GC fitted with a flame ionization detector, the air-flow was 350 ml/min and the hydrogen-flow 35 ml/min. For each analysis, 1 µl of prepared solutions (10 mg / 20 ml in *n*-hexane) was injected.

GC/MS - The mass spectrometer was an Agilent 5973 Quadrupole coupled to a gas chromatograph Agilent 6890 (GC/MS). 1 µl of essential oil solutions (10 mg / 20 ml in *n*-hexane) was injected in the splitless mode.

The retention times of the interpreted peaks were between 4 and 40 minutes and the mass range scanned (EI at 70 eV) was from  $m/z = 35$  to  $m/z = 350$ . The identification of the compounds was carried out by comparing the mass spectra with those available in the computerized database Wiley 275 L. and by measuring the retention indices compared to those given in the literature [12, 13]. Pure references were also injected to confirm the identification of the compounds.

### 2.3 Pulegone assay and analysis of floral waters

The assay of pulegone in the oils was performed by GC-FID according to AFNOR method NF T 75-418 [11] on a polar column, VF-WAX (Agilent-The Netherlands), length 30 m, internal diameter 0.25 mm and film thickness of 0.25 µm. The temperature conditions were: 50 °C (00 min) at 250 °C by 8 °C/min and isotherm at 250 °C for 15 min. Detector temperature was 260 °C and injections were done in the splitless mode. The carrier gas was helium at 1 ml/min. In FID, the air-flow was 400 ml/min and the hydrogen-flow 30ml/min. The GC/FID HP 6890 Series was from Agilent. The internal standard used was methyl octanoate.

Floral waters were also analyzed on a polar column according to the fore mentioned method. They were initially extracted by liquid-liquid extraction with *n*-hexane (10/2, v/v) to recover the products of interest from the residual floral waters.

Identification was performed according to the method described in §2.2 and by comparison retention indices calculated in the analytical conditions [14] with those given in the literature.

## 3. Results and Discussion

### 3.1 Results

Oil yields obtained in the fresh and dried plants are 0.39 and 0.32%, respectively. Chromatographic study revealed 28 constituents in the fresh plants and 24 in the dried plants. The oils are rich in oxygenated monoterpenes with 95.3% for the fresh plants and 93.4% for the dried plants. The monoterpene hydrocarbons increase from 3.4% in the fresh plants to 4.7% after drying. Table 1 shows the chemical composition of essential oils of *M. longifolia* from Senegal.

**Table 1:** Chemical composition of essential oils of *Mentha longifolia* from Senegal according to the drying

| Compounds                                     | Retention indices | % composition |                  |
|---|-------------------|---------------|------------------|
|   |                   | Fresh plants  | 7 days of drying |
| $\alpha$ -Pinene                              | 933               | 0.4           | 0.6              |
| Sabinene                                      | 974               | 0.7           | 0.9              |
| $\beta$ -Pinene                               | 978               | 1.0           | 1.5              |
| Myrcene                                       | 990               | 0.6           | 0.8              |
| Limonene                                      | 1030              | 0.7           | 0.9              |
| 1,8-Cineole                                   | 1034              | 13.1          | 11.4             |
| <i>cis</i> -Sabinene hydrate                  | 1072              | 0.1           | 0.1              |
| <i>cis</i> -Sabinol                           | 1143              | 0.3           | 0.4              |
| <i>trans</i> -Verbenol                        | 1149              | 0.2           | 0.2              |
| Menthone                                      | 1159              | 14.3          | 21.2             |
| Isomenthone                                   | 1169              | 9.0           | 13.2             |
| $\delta$ -Terpineol                           | 1173              | 0.9           | 0.7              |
| <i>cis</i> -Isopulegone                       | 1178              | 0.9           | 0.5              |
| Menthol                                       | 1180              | 0.6           | 0.1              |
| terpinen-4-ol                                 | 1184              | T             | -                |
| $\alpha$ -Terpineol                           | 1197              | 1.8           | 1.4              |
| <i>trans</i> -Carveol                         | 1221              | T             | -                |
| Pulegone                                      | 1243              | 52.0          | 42.4             |
| Piperitone                                    | 1259              | 0.4           | 0.5              |
| Neomenthyl acetate                            | 1274              | T             | -                |
| 8-Hydroxy-delta-4(5)- <i>p</i> -menthen-3-one | 1292              | 0.3           | 0.2              |
| Piperitenone                                  | 1344              | 1.4           | 1.1              |
| ( <i>E</i> )- $\beta$ -caryophyllene          | 1426              | 0.4           | 0.8              |
| Not identified                                | 1448              | 0.1           | 0.2              |
| Germacrene D                                  | 1487              | 0.1           | 0.2              |
| $\gamma$ -Cadinene                            | 1518              | 0.1           | 0.2              |
| Caryophyllene oxide                           | 1591              | 0.1           | 0.1              |
| Globulol                                      | 1594              | 0.1           | -                |
| <i>epi</i> - $\alpha$ -Cadinol                | 1648              | 0.3           | 0.4              |
| Monoterpene hydrocarbons                      |                   | 3.4           | 4.7              |
| Oxygenated monoterpenes                       |                   | 95.4          | 93.4             |
| Sesquiterpene hydrocarbons                    |                   | 0.6           | 1.2              |
| Oxygenated sesquiterpenes                     |                   | 0.5           | 0.5              |
| Not identified                                |                   | 0.1           | 0.2              |

t = trace (<0.1%).

Oils are dominated by four compounds: pulegone, 1,8-cineole, menthone and isomenthone. The major compound, pulegone decreases with drying, 52.0% in the fresh plants against 42.4% after drying. 1,8-cineole also decreases from 13.1% in the fresh plants to 11.4% in the dried plants. However, menthone increases from 14.3 to 21.2% and isomenthone from 9.0 to 13.2% in the fresh and dried plants, respectively.  $\alpha$ -Terpineol represents 1.8 and 1.4%, piperitenone 1.4 and 1.1% and  $\beta$ -pinene 1.0 and 1.5% in the fresh and dried plants, respectively. The concentrations of pulegone according to AFNOR method NF T 75-418 [11] are given in Table 2.

**Table 2:** Pulegone content of essential oils of *Mentha longifolia* from Senegal

| Time of drying          | Fresh plants | 7 days of drying |
|-------------------------|--------------|------------------|
| Pulegone content (mg/g) | 444.6        | 393.3            |

Pulegone content decreases with drying, from 444.6 mg/g in the fresh to 393.3 mg/g after drying.

Sixteen compounds were identified in the floral waters. Table 3 shows the chemical composition of floral waters of *M. longifolia* from Senegal.

**Table 3:** Chemical composition of floral waters of *Mentha longifolia* from Senegal according to the drying

| Compounds  | Retention indices | % composition |                  |
|--|-------------------|---------------|------------------|
|  |                   | Fresh plants  | 7 days of drying |
| 1,8-Cineole  | 1211              | 7.9           | 19.6             |
| <i>trans</i> -Sabinene hydrate                     | 1459              | -             | 0.2              |
| Menthone   | 1464              | 6.4           | 9.2              |
| Isomenthone  | 1491              | 7.2           | 10.7             |
| Not identified                                     | 1569              | 0.4           | 0.4              |
| Not identified                                     | 1581              | 0.7           | 0.8              |
| Terpinen-4-ol                                      | 1595              | 0.3           | 0.1              |
| Isomenthol   | 1633              | 0.6           | 0.5              |
| Pulegone   | 1647              | 60.2          | 47.0             |
| $\delta$ -Terpineol                                | 1664              | 1.3           | 1.3              |
| <i>trans</i> -Verbenol                             | 1671              | 0.7           | 0.6              |
| $\alpha$ -Terpineol                                | 1689              | 3.0           | 2.7              |
| Not identified                                     | 1696              | 0.6           | 0.5              |
| Not identified                                     | 1715              | 0.3           | 0.2              |
| Piperitone   | 1722              | 1.0           | 1.2              |
| Not identified                                     | 1746              | -             | 0.1              |
| (1R,4S)-8-Hydroxy- <i>p</i> -menthan-3-one         | 1755              | 0.4           | 0.2              |
| Not identified                                     | 1802              | 0.2           | 0.1              |
| 2-Hydroxy-2-isopropyl-5-methylcyclohexanone        | 1821              | 0.2           | 0.2              |
| Not identified                                     | 1834              | 0.3           | 0.2              |
| 8-Hydroxy- $\delta$ -4(5)- <i>p</i> -menthen-3-one | 1895              | 1.0           | 0.6              |
| Chrysanthenone                                     | 1917              | 6.4           | 3.2              |
| Globulol   | 2067              | 0.6           | -                |
| Not identified                                     | 2071              | 0.3           | 0.2              |
| Not identified                                     | 2160              | -             | 0.2              |
| Oxygenated compounds                               |                   | 97.2          | 97.3             |
| Not identified                                     |                   | 2.8           | 2.7              |

All compounds identified in floral waters are oxygenated monoterpenes. They constitute 97.2 and 97.3% in the fresh and dried plants respectively. In addition to the major compounds identified in the essential oils (pulegone, 1,8-cineole, menthone and isomenthone), other compounds are present in representative quantities in floral waters:  $\delta$ -terpineol,  $\alpha$ -terpineol, piperitone and chrysanthenone. Pulegone remains the major compound with percentages greater than those obtained in the essential oils. It decreases with drying as in oils with 60.2% in the fresh plants and 47.0% after drying. It is followed by 1,8-cineole with 7.9 and 19.6%, isomenthone 7.2 and 10.7%, menthone 6.4 and 9.2%, chrysanthenone 6.4 and 3.2% and  $\alpha$ -terpineol 3.0 and 2.7% in the fresh and dried plants, respectively.  $\delta$ -Terpineol and piperitone represent 1.3 and 1.0% in the fresh plants, 1.3 and 1.2% after drying respectively.

### 3.2 Discussion

Due to the drying process, the results revealed that the fresh plants are richer in essential oils than the dried plants. This is

explained by the fact that the oils are volatile and may evaporate during the drying process [15]. Essential oils and floral waters studied are characterized by the same major compounds: pulegone, 1,8-cineole, menthone and isomenthone. This corroborates the results of Sutour (2010) [9] which found that pulegone and *cis*, *cis-p*-menthenolide were also major both in the essential oils and floral waters of *M. suaveolens* ssp. *insularis* from Corsica. Drying also influences the evolution of prominent compounds of essential oils and floral waters of *M. longifolia* from Senegal. Furthermore, pulegone rate decreases in the oils and floral waters of the dried plants. Asekun *et al.* (2007) [4] have also found that pulegone decreased in the oils of *M. longifolia* (35.0% in the fresh plants to 18.4% in the air-dried plants). The pulegone rate obtained in the oils of *M. longifolia* from Senegal is higher than that obtained by Gulluce *et al.* (2007) [5], 15.5% after drying in the shade. Menthone and isomenthone increase with drying in oils and floral waters. According to Asekun *et al.* (2007) [4], menthone increases with drying, 1,8-cineole decreases in essential oils and increases in Senegalese floral waters. In essential oils, pulegone, 1,8-cineole, menthone and isomenthone constitute 88.4% of the fresh plants and 88.2% of the dried plants. Similarly in floral waters, pulegone, 1,8-cineole, menthone, isomenthone, chrysanthenone and  $\alpha$ -terpineol represent 91.1% of the composition of the fresh plants and 91.9% of the dried plants. Except chrysanthenone, all major compounds identified in the essential oils and floral waters of *M. longifolia* from Senegal contain the same carbon skeleton *p*-menthan. So the decrease of pulegone and 1,8-cineole and the increase of menthone and isomenthone in the essential oils may be caused by simple conversions of major molecules that decrease or increase. This hypothesis could also explain the decrease of pulegone,  $\alpha$ -terpineol and chrysanthenone and the increase of 1,8-cineole, menthone and isomenthone in the floral waters [16]. According to Zrira *et al.* (1991) [17], the biosynthesis of essential oil components in the plant may continue after harvest; which would explain why some compounds increase after drying. The main difference between essential oils and floral waters of *M. longifolia* from Senegal is chrysanthenone, compound present in floral waters and missing in oils. This molecule was already identified in extracts of *M. pulegium* [18]. Other representative compounds identified in essential oils are:  $\alpha$ -terpineol, piperitone and  $\beta$ -pinene and those obtained in floral waters are:  $\alpha$ -terpineol,  $\delta$ -terpineol and piperitone. These compounds are in almost constant proportions.

The pulegone concentrations in the essential oils according to AFNOR method NF-T-75-418 [11] show lower levels in the dried plants than in the fresh plants. These levels nevertheless remain very high according to the regulations of the European Union [6] which sets rates of pulegone restriction in food products between 20 mg/kg (in non-alcoholic beverages containing mint or peppermint) and 2000 mg/kg (in confectionary for freshening breath).

### 4. Conclusion

The present study is the first conducted on *Mentha longifolia* from Senegal. Oils and floral waters studied have the same major compounds: pulegone, 1,8-cineole, menthone and isomenthone but at different rates. The percentages of these prominent compounds vary also with drying. Pulegone, a hepatotoxic compound, decreased with drying. These results indicate that drying has an important role in the quality of essential oils. Ongoing studies focus on the determination of essential oil composition of different wild mints from Senegal.

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