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Research Article

Identification of two chemotypes for essential oils and floral waters of *Mentha spicata* L. from two localities of Senegal

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Abstract

The aim of the study is to determine the chemical composition of essential oils (EOs) and floral waters (FWs) in *Mentha spicata* L. from Senegal. For this purpose, extractions were conducted by steam distillation from both fresh (F) and dried (D) plants harvested in Dakar and Kaolack regions, Senegal. Essential oils and floral waters obtained were analyzed by gas chromatography GC/FID-MS Results showed that EOs and FWs were characterized by the same prominent components. In the oils from Dakar, it is noted an abundance of carvone which represented 64.4 (F) and 59.8% (D), limonene 11.7 (F) and 11.9% (D) and 1.8-cineole 4.4 (F) and 4.4% (D). This composition is typical for *M. spicata*. The corresponding FWs also contained mainly carvone 81.1 (F) and 80.2% (D). For Kaolack, EOs were dominated by pulegone 35.3 (F) and 35.8% (D), piperitenone 24.5 (F) and 24.6% (D), 1.8-cineole 11.5 (F) 11.0% (D) and limonene 5.5 (F) and 6.6 % (D). In our best knowledge, this chemotype was not reported for *M. spicata*. Their FWs contained: 66.1 (F) and 67.2% (D) of piperitenone, (19.2 (F) and 18.8% (D) of pulegone. Futhermore, chiral chromatography revealed the presence of pure enantiomers: (S)-(-)-limonene, (R)-(-)-carvone and (R)-(+)-pulegone in the oils.

1. Introduction

Senegal has a wide variety of aromatic plants, mainly used in phytotherapy and food. However, the use of these aromatic and perfume plants is often carried out with a weak prior knowledge of their biochemical constituents. However, if the substances contained in these plants were correctly identified and their properties studied, it would be possible to add value to them, especially at the industrial level, in order to reduce imports. In recent years, interest in essential oils has grown steadily. In West Africa, the essential Oil industries are booming in Benin, Ghana and Togo. However, despite the availability of resources, Senegal has yet to produce any essential oils (EOs). Therefore, the industry depends on imports for its needs.

This work is part of an extensive characterization study of essential oils from aromatic plants in Senegal. It was initiated as part of a project to set up an essential oil production unit in northern Senegal to offset the massive imports of essential oils. This study has already led to the characterization of several essential oils. Among them, we may cite: *Apium graveolens* L., *Citrus sinensis* L., *Cymbopogon citratus* DC, *Eucalyptus camaldulensis*, *Mentha arvensis* L., *M. longifolia* L., *Ocimum basilicum* L., *Xylopia aethiopica* (Dunal) A. Rich. [1-8]. To make this study more exhaustive, other plants such as *Mentha spicata* L. are also being investigated. This mint was chosen due to its widespread adoption by horticulturalists, its widespread use by local populations and, above all, the lack of scientific data on the constituents of its oil. Indeed, Senegal produces a large number of mints, which are mainly used as flavoring for tea and hibiscus juice. It is also used as an herbal tea to fight headaches. Two varieties of *M. spicata* are grown in Senegal. They are locally known as "nahnah fass" and "nahnah ordinaire". The variety studied in this work was *M. spicata* L. var. "nahnah fass".

In the literature, carvone and limonene have been reported as the main constituents of *M. spicata* essential oils [9-11]. The present work aimed to study the chemical composition of essential oils and floral waters (FWs) of *Mentha spicata* L. from Senegal in order to improve the knowledge with a view to valorization.

2. Materials and methods

2.1 Plant material and essential oil extraction

A study was conducted on *Mentha spicata* L., locally named "nahnah fass". Plants were collected in both Dakar (14° 45' N, 17° 20' W) and Kaolack (14° 09' N, 14° 30' W) regions in Senegal. After identification, two specimens CM_1 and KL_2 corresponding to plants from Dakar and Kaolack, respectively were deposited in the herbarium of the "Institut Fondamental d'Afrique Noire" of Cheikh Anta Diop University of Dakar, Senegal. Essential oils and floral waters were obtained by submitting separately 100 g of both fresh (F) and dried (D) plants (left at room temperature, 18-27 °C for 7 days in the shade) to steam distillation for 30 min using a Clevenger-type apparatus. EOs yields were of 0.10 (F) and 0.10% (D) for Dakar; 0.20 (F) and 0.18% (D) for Kaolack. Oils and floral waters obtained were stored in the refrigerator (at 4 °C) in amber vials until analysis.

2.2 Essential oils and floral water characterization

Essential oil and floral water samples were subjected to gas chromatography. Essential oil solutions: 10 mg/10 mL (EOs/*n*-hexane) were prepared and diluted four times before analysis. Organic substances from floral waters were extracted by liquid-liquid with *n*-hexane (10/2, $V_{FWs}/V_{n\text{-hexane}}$). 1 µL of these solutions were injected by analysis. The following chromatographic conditions were used: injector (Splitless mode) and detector temperatures: 280 °C and 290 °C, respectively; oven: initial temperature 40 °C (5 min), ramp of 8 °C/min until final temperature 280 °C (5 min); carrier gas, helium at a constant rate set at 1.5 mL/min; air and hydrogen flows: 350 mL/min and 35 mL/min, respectively. The column used was a fused silica capillary, Optima-5-MS-Accent (Macherey-Nagel, Düren-Germany), 5%phenyl-95%methylsiloxane (30 m x 0.25 mm, 0.25 µm film thickness).

GC/FID-A Trace Ultra GC from Thermo Electron Corporation (Interscience Louvain- La-Neuve, Belgium) fitted with a flame ionization detector was used for the determination of the proportions (%) of EOs and FWs constituents. The percentage of each constituent was calculated as the ratio of peak area and the total of GC peak areas.

GC/MS-Identification of components was carried out on a mass spectrometer from Agilent 5973 Network Mass Selective Detector Quadrupole coupled to a gas chromatograph Agilent 6890N (G1530N), USA. Mass spectra were recorded at 70 eV and the mass scanned range was from 50 to 550 amu. The mass spectra were compared to those from a computerized database (Wiley 275 L) and those given in the literature [12, 13]. Pure compounds (from SIGMA ALDRICH Boornem, Belgium) confirmed the identification of the major constituents and the tolerance limit was ± 0.1 min.

2.3 Chiral chromatography

Identification of enantiomers of some chiral components obtained in *M. spicata* oils was carried out by injecting under the same conditions on a chiral column: essential oils, racemates and pure enantiomers from SIGMA ALDRICH Boornem, Belgium. 1µl (50µg/10mL, EOs/*n*-hexane) was injected into the GC/FID described above. The chiral column was a β-DEX 110 (30 m x 0.25 mm, 0.25 µm film thickness) from Supelco, USA. The oven temperature was programmed as follows: isotherm at 40 °C for 2 min then increased by 2°C/min to 220 °C, where it was maintained for 4 min. Detector temperature was at 230 °C and the injector was operating in splitless mode at 220 °C with a split flow of 30mL/min. The carrier gas was helium with a constant flow of 1.5 mL/min. The air and hydrogen flows were of 350 mL/min and 35 mL/min, respectively. The enantiomers present in oils have been identified by comparing their retention data with those of pure enantiomers.

3. Results

3.1 Chemical composition of essential oils from Mentha spicata *L.*

Chromatographic analyses showed that essential oils of *M. spicata* were dominated by oxygenated monoterpenes. The latter represented 77.1 (F) and 75.2% (D) in Dakar, 87.8 (F) and 84.7% (D) in Kaolack. Monoterpene hydrocarbons were 13.8 (F) and 14.2% (D) in the EOs from Dakar, 5.8 (F) and 9.3% (D) in those from Kaolack.

The prominent compounds identified in oils from Dakar were carvone (64.4 and 59.8%), limonene (11.7 and 11.9%), 1.8-cineole (4.4 and 4.4%) and *cis*-sabinene hydrate (1.7 and 2.1%) in the oils from fresh and dried plants, respectively. Sesquiterpenes which represented 8.7 (F) and 10.5% (D) were dominated by β-caryophyllene (3.4 and 4.0%), β-elemene (1.8 and 1.7%), germacrene D (1.6 and 1.8%) and β-bourbonene (0.9 and 1.4%) (Table 1).

In Kaolack, it was mainly identified pulegone (35.3 and 35.8%), piperitenone (24.5 and 24.6%), 1.8-cineole (11.5 and 11.0%) and limonene (5.5 and 6.6 %) in the oils from fresh and dried plants, respectively. Other significant components were also identified in these oils: menthol (4.9 and 3.9%), *cis*-isopulegone (3.2 and 2.7%), isopiperitenone (2.1 and 2.4%), 3-methylcyclohexanone (2.7 and 0.5%) and piperitenone oxide (1.1 and 1.1%). The main sesquiterpene of the oils from Kaolack was caryophyllene oxide (1.6 and 1.3%).

3.2 Chemical composition of floral waters from Mentha spicata *L.*

Floral waters were dominated by oxygenated monoterpenes which represented 96.2 (F) and 95.5% (D) in Dakar, 98.6 (F) and 98.5% (D) in Kaolack (Table 2). Carvone (81.1 and 80.2%), *cis*-dihydrocarveol (5.0 and 5.2%), 1.8-cineole (2.7 and 3.5%) and *cis*-sabinene hydrate (2.1 and 3.2%) were the major constituents identified in the FWs of fresh and dried plants from Dakar, respectively. Piperitenone which constituted 66.1 (F) and 67.2% (D) was the major component of floral waters from Kaolack. Other representative compounds identified in the FWs from Kaolack were: pulegone (19.2 and 18.8%) and isopiperitenone (6.1 and 4.6%) in the FWs from fresh and dried plants, respectively. 1.7 and 2.1% of 1.8-cineole, 1.3 and 1.6% of *cis*-sabinne hydrate were also obtained in the FWs from Kaolack.

3.3 *Chiral constituents*

Chiral chromatography showed the presence of pure enantiomers in the oils (Figs. 1-3). (S)-(-)-Limonene and (R)-(-)-carvone were identified in EOs from Dakar whereas EOs from Kaolack contained (S)-(-)-limonene and (R)-(+)-pulegone (Fig. 4).

4. Discussion

A study of the chemical composition of essential oils and floral waters of *M. spicata* from Senegal showed two different chemotypes. Oils from Dakar were dominated by carvone and limonene, a typical composition of *M. spicata* [9-11, 14, 15]. Shahbazi (2015) [9] reported in Iran 78.8 % of carvone and 11.5% of limonene whereas Snoussi et al. (2015) [10] obtained carvone (40.8 %) and limonene (20.8%) in Tunisia. Oils from Kaolack were dominated by pulegone and piperitenone. Telci et al. (2010) [16] characterized in Turkey, pulegone (26.7-29.6%) and piperitone (22.2-28.2%) as major compounds of *M. spicata* oils. However, to our knowledge, piperitenone has never been identified as a major constituent in *M. spicata* oils. Thus, *Mentha spicata* from Kaolack could constitute a new chemotype.

Essential oils and floral waters have been characterized by the same major compounds. It is also noted that in FWs, polar components increase by 19% for Dakar and 5% for Kaolack, compared to EOs. As an example, carvone increased from 16 to 20% (Dakar) and piperitenone over 40% (Kaolack) in the FWs. This abundance of polar compounds in the FWs is quite logical, because the FWs constitute the aqueous phase of the extracts. In contrary, the apolar compounds decreased in the FWs: limonene that represented over 11% (Dakar) and 5% (Kaolack) in oils was not identified in the floral waters.

Pulegone and piperitenone, the main compounds of *M. spicata* extracts from Kaolack were reported as toxic against humans [17, 18]. They represented 60 and 85% of EOs and FWs from Kaolack, respectively. The use of these oils and floral waters in food should

Table 1. Chemical composition of essential oils of *Mentha spicata* L. from Senegal

Compounds	Retention indices	Proportions (%)			
		Dakar		Kaolack	
		Fresh plants	Dried plants	Fresh plants	Dried plants
α-Pinene	937	0.3	0.3	-	0.3
3-Methylcyclohexanone	955	-	-	**2.7**	0.5
Sabinene	976	0.5	0.4	-	0.7
β-Pinene	982	0.6	0.7	-	**1.0**
Myrcene	990	0.7	0.7	0.3	0.6
Octan-3-ol	995	0.8	0.6	**1.0**	**0.9**
***para*-Cymene**	1028	-	-	0.9	0.7
(S)-(-)-Limonene	1033	**11.7**	**11.9**	**5.5**	**6.6**
(Z)-β-Ocimene	1036	-	0.1	-	-
1,8-Cineole	1037	**4.4**	**4.4**	**11.5**	**11.0**
γ-Terpinene	1062	-	0.1	-	-
***cis*-Sabinene hydrate**	1075	**1.7**	**2.1**	-	-
Linalol	1100	-	0.2	-	-
Octan-3-yl acetate	1117	-	0.1	-	-
allo-Ocimene	1128	-	-	-	0.1
Isopulegol	1155	-	-	0.8	0.9
Menthone	1162	-	0.5	-	-
Menthofurane	1172	-	0.4	-	-
Isomenthone	1175	-	0.1	-	-
δ-Terpineol	1176	0.3	0.3	0.4	0.3
Borneol	1181	-	0.1	-	-
(1R,2S,5R)-(-)-Menthol	1184	-	0.2	**4.9**	**3.9**
***cis*-Isopulegone**	1187	-	-	**3.2**	**2.7**
Terpinen-4-ol	1188	0.6	0.2	-	-
α-Terpineol	1200	0.5	0.4	-	0.1
***cis*-Dihydrocarvone**	1204	**1.9**	**2.3**	-	-
Not identified	1221	-	-	0.4	0.4
trans-Carveol	1224	1.0	0.7	-	0.3
cis-Carveol	1234	0.8	**1.6**	-	-
cis-Hex-3-enyl isovalerate	1234	-	-	-	0.2
(R)-(+)-Pulegone	1246	0.5	0.5	**35.3**	**35.8**
(R)-(-)-Carvone	1251	**64.4**	**59.8**	-	-
Geranial	1270	-	-	0.3	-
Isopiperitenone	1277	-	-	**2.1**	**2.4**
trans-Carvone oxide	1279	-	0.1	-	-
Bornyl acetate	1287	-	0.1	-	-
Not identified	1314	-	-	0.7	0.8
Dihydrocarvyl acetate	1327	0.2	0.3	-	-
δ-Elemene	1345	-	0.1	-	-
Piperitenone	1350	-	-	**24.5**	**24.6**
α-Cubebene	1360	-	-	0.7	0.4
cis-Carvyl acetate	1362	-	0.2	-	-
Not identified	1365	-	-	0.2	-
Piperitenone oxide	1370	-	-	**1.1**	**1.1**
β-Bourbonene	1400	**0.9**	**1.4**	0.6	0.8
β-Elemene	1407	**1.8**	**1.7**	-	-
cis-Jasmone	1413	-	-	-	0.1
Not identified	1430	-	-	0.3	-
(*E*)-β-Caryophyllene	1437	**3.4**	**4.0**	-	1.2

Table 1. *(Continued)*

Compounds	Retention indices	Proportions (%)			
		Dakar		Kaolack	
		Fresh plants	Dried plants	Fresh plants	Dried plants
α-Guaiene	1448	-	-	0.3	0.2
ε-Muurolene	1451	0.3	0.3	-	-
β-Gurjunene	1456	-	-	0.3	-
α-Humulene	1463	-	0.2	-	-
Germacrene D	1496	**1.6**	**1.8**	-	-
Bicyclogermacrene	1508	0.4	0.3	-	-
γ-Cadinene	1515	-	0.1	-	-
cis-Calamenene	1533	-	0.2	-	-
Elemol	1563	-	-	0.4	-
Germacrene D-4-ol	1584	-	0.1	-	-
Spathulenol	1596	-	-	-	0.1
Caryophyllene oxide	1600	0.3	0.2	**1.6**	**1.3**
1,10-di-*epi*-Cubenol	1622	-	0.1	-	-
Not identified	1662	0.4	0.1	**-**	-
Compound Classes					
Oxygenated monoterpenes		*77.1*	*75.2*	*87.8*	*84.7*
Monoterpenic hydrocarbons		*13.8*	*14.2*	*5.8*	*9.3*
Oxygenated sesquiterpenes		*0.3*	*0.4*	*2.0*	*1.5*
Sesquiterpenic hydrocarbons		*8.4*	*10.1*	*1.9*	*2.6*
Aromatic compounds		*0.0*	*0.0*	*0.9*	*0.7*
Not identified		*0.4*	*0.1*	*1.6*	*1.2*

Table 2. Chemical composition of floral waters of *Mentha spicata* L. from Senegal

Compounds	Retention indices	Proportions (%)			
		Dakar		Kaolack	
		Fresh plants	Dried plants	Fresh plants	Dried plants
3-Methylcyclohexanone	955	-	-	-	0.3
Benzaldehyde	964	-	0.2	-	0.2
Octan-3-ol	995	**0.9**	**1.0**	0.2	0.3
α-Phellandrene	1008	0.1	0.1	-	-
1,8-Cineole	1037	**2.7**	**3.5**	**1.7**	**2.1**
***cis*-Sabinene hydrate**	1075	**2.1**	**3.2**	**1.3**	**1.6**
n-Nonanal	1105	0.1	0.2	0.1	0.2
Isopulegol	1155	-	-	0.5	0.6
δ-Terpineol	1176	0.4	0.5	0.3	0.3
Borneol	1181	0.4	0.3	1.0	0.8
Terpinen-4-ol	1188	0.8	0.4	0.7	0.5
α-Terpineol	1200	0.2	0.2	0.1	0.3
***cis*-Dihydrocarveol**	1204	**5.0**	**5.2**	-	-
trans-Dihydrocarvone	1207	0.1	0.1	-	-
4,7-Dimethylbenzofurane	1214	-	-	0.6	0.2
***trans*-Carveol**	1224	**2.1**	0.6	-	-
Not identified	1227	-	-	0.3	0.4
Not identified	1242	**3.2**	**3.6**	**-**	**-**
(R)-(+)-Pulegone	1246	0.2	-	**19.2**	**18.8**
(R)-(-)-Carvone	1251	**81.1**	**80.2**	-	-
Isopiperitenone	1277	-	-	**6.1**	**4.6**
Not identified	1282	0.2	0.2	**-**	**-**

Table 2. *(Continued)*

Compounds	Retention indices	Proportions (%)			
		Dakar		Kaolack	
		Fresh plants	Dried plants	Fresh plants	Dried plants
para-Methoxyacetophenone	1295	-	-	0.1	0.2
4-Methyl-2-(3-methyl-2-butenyl)-furane	1306	-	-	-	0.3
Not identified	1324	0.1	0.1	**-**	**-**
Piperitenone	1350	-	-	**66.1**	**67.2**
Piperitenone oxide	1373	0.1	0.1	**1.3**	0.6
β-Bourbonene	1400	0.2	0.2	0.4	0.4
cis-Jasmone	1413	-	0.1	-	0.1
Compound Classes					
Oxygenated monoterpenes		*96.2*	*95.5*	*98.6*	*98.5*
Monoterpenic hydrocarbons		*0.1*	*0.1*	*0.0*	*0.0*
Oxygenated sesquiterpenes		*0.0*	*0.1*	*0.0*	*0.1*
Sesquiterpenic hydrocarbons		*0.2*	*0.2*	*0.4*	*0.4*
Aromatic Derivatives		*0.0*	*0.2*	*0.7*	*0.6*
Not identified		*3.5*	*3.9*	*0.3*	*0.4*

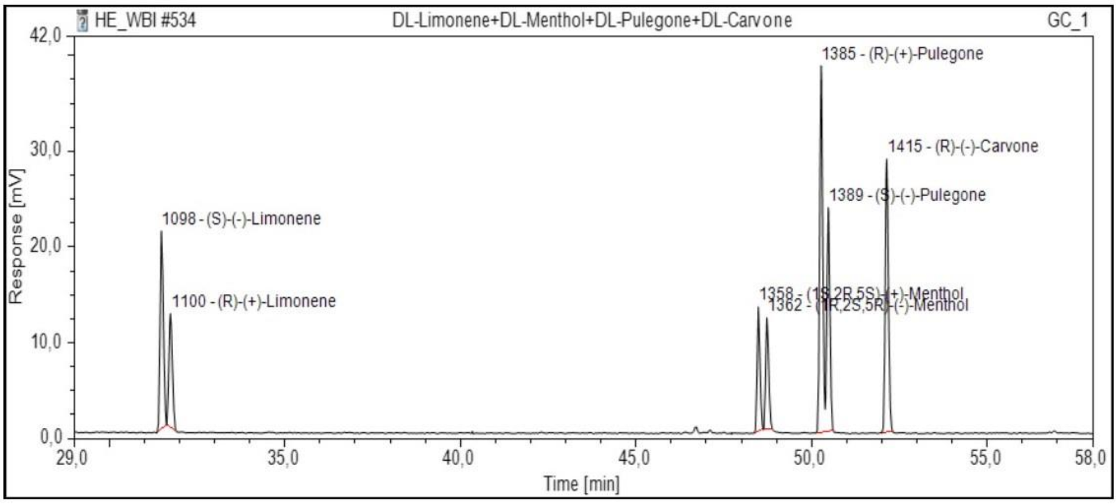

Figure 1. Chromatogram of enantiomeric standards on chiral column

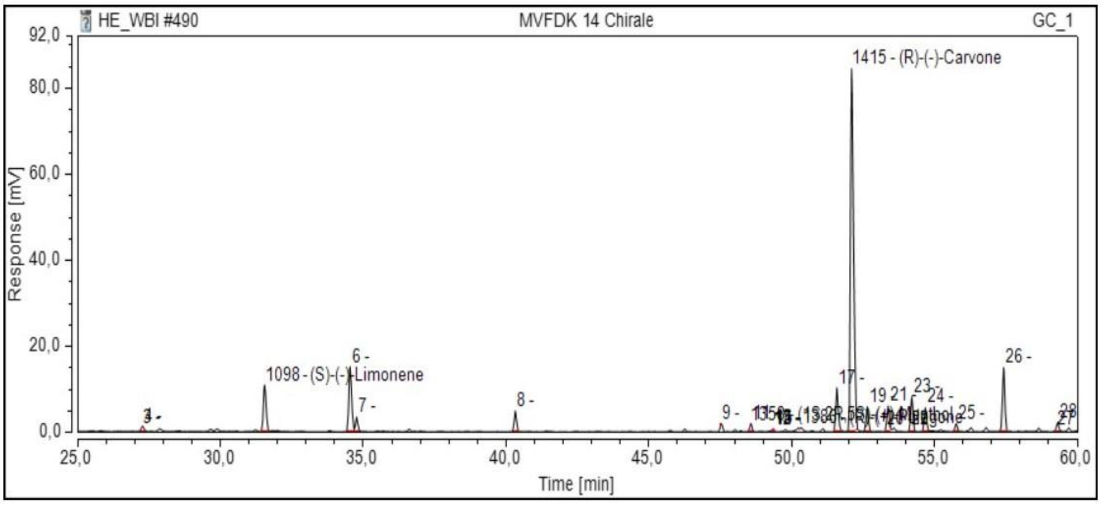

Figure 2. Chromatogram of *Mentha spicata* L. essential oil from Dakar on chiral column

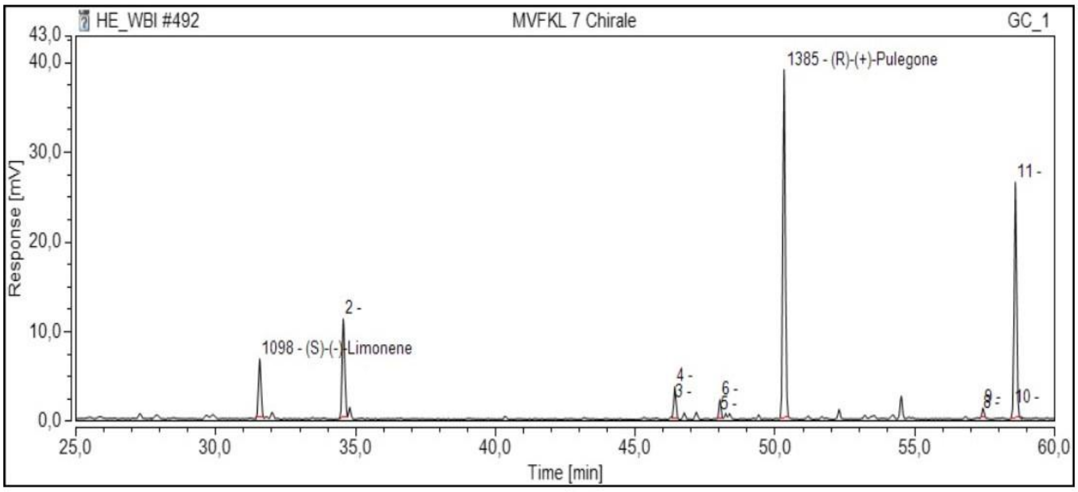

Figure 3. Chromatogram of *Mentha spicata* L. essential oil from Kaolack on chiral column

be avoided.

Pure enantiomers were identified in EOs. (S)-(-)-Limonene and (R)-(-)-carvone have already been reported for *M. spicata* oils [19]. (R)-(+)-Pulegone present in Kaolack oils was identified by Lorenzo et al. (2002) [20] in *M. pulegium* oils. These enantiomers could be used as auxiliaries in asymmetric synthesis and for racemate splitting.

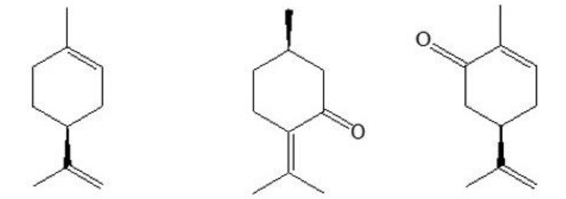

Figure 4. Structures of chiral constituents identified in *Mentha spicata* L. essential oils from Senegal

5. Conclusions

This study allowed us to determine the chemical composition of the essential oils and floral waters of *M. spicata* from Senegal. According to the origin of plants, two different compositions have been obtained. A typical composition characterized by carvone and limonene was identified in Dakar whereas oils from Kaolack have been dominated by pulegone and piperitenone, corresponding to a new chemotype to our knowledge. To validate this hypothesis, a more complete series of analyses on a large series of EOs samples from different parts of the country is required. It was also noted that EOs and their FWs were characterized by the same major components. In addition, pure enantiomers which could be used as auxiliaries in asymmetric synthesis were detected in the oils.

Authors' contributions

Conceptualization, S.M.D. and M.T.G.; Methodology, S.M.D., M.G., M.T.G. and M.L.F.; Essential oils extractions, S.M.D., E.H.B.N. and A.T.; Chemical analysis, S.M.D., E.H.B.N. M.G., and M.L.F.; Writing – original draft, S.M.D.; Writing – Review & Editing, S.M.D. and M.T.G.; Supervision, M.T.G.; Project administration, M.T.G. and M.L.F.; Funding acquisition, M.T.G. and M.L.F.

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Availability of data and materials

All data will be made available on request according to the journal policy.

Conflicts of interest

The authors declare no conflict of interest.

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