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Chemical Characterization of Essential Oils of Mints from Senegal

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Mints from Senegal were extracted separately from fresh (F) and shade-dried (D) plants by steam distillation. Yields were of 0.28 and 0.21% for *M. citrata* L., 0.21 and 0.18% for *M. x piperita* L. and 0.10 and 0.19% for *M. spicata* L. in the fresh and dried plants, respectively. GC/FID and GC/MS analysis revealed that many of the major compounds of essential oils decreased with drying. The prominent components of *M. citrata* oils were linalool that constituted 45.8% (F) and 42.0% (D) and linalyl acetate 42.7 (F) and 38.5% (D). *Mentha x piperita* was characterized by menthofuran with 30.7% (F) and 28.1% (D), menthol 15.9(F) and 16.4% (D), menthone 13.0 (F) and 14.2% (D), pulegone 17.6%(F) and 13.8%(D) and 1.8-cineole 3.7%(F) and 3.4% (D). *Mentha spicata* contained mainly carvone 67.8 and 74.7% and limonene 18.1 and 12.5% in the fresh and dried plants respectively.

Keywords: *Mentha citrata*, *Mentha x piperita*, *Mentha spicata*, Essential oils, Chemical composition.

Since some years, interest on essential oils increases strongly in Senegal where grow many aromatic plants, including those of *Mentha* genus. This genus contains several species such as *M. arvensis* L., *M. longifolia* L., *M. x piperita* L., *M. rotundifolia* L. and *M. spicata* L. Mints are traditionally and widely used to flavor tea and hibiscus juice. Their essential oils are also used in syrups, chewing-gums, etc.

The properties of essential oils are very dependent of their chemical composition that can vary in the same species. Among factors, we can mention the growing conditions [1], the phenologic stage of plants [2], the drying method [3], etc. In the literature, many chemotypes of essential oils are reported.

For *Mentha x piperita*, two types of essential oil are generally identified. The essential oils characterized by linalool and its acetate belong to bergamot mint (*Mentha x piperita* var. *citrata*). Garlet et al. (2013) obtained in the oils of *M. x piperita* var. *citrata* linalool (53.5 to 45.0%) and linalyl acetate (34.0 to 28.1%) [4]. Padalia et al. (2013) identified in the oils from fresh plants of *Mentha citrata* linalool (59.7%) and linalyl acetate (18.4%) [5]. Oils characterized by menthol and its derivatives: menthone, menthyl acetate, menthofuran, etc. are connected to peppermint (*Mentha x piperita* var. *piperita*). In Senegal, Koyalta (1993) obtained 32.46-40.93% menthol, 15.29-24.99% menthone, 2.25-1288% isomenthone, 3.13-4.40% menthofuran, 267-6.84% neomenthol and 2.49-27.22% menthyl acetate in the fresh plants of peppermint [6a]. Lazutka et al. (2001) obtained mainly menthol 59.17%, menthone 18.78% and limonene 5.16% [7]. Verma et al. (2010) identified in oils extracted from plants at different stages of growth 22.56-42.83% menthol, 0-17.87% menthofuran, 0.77-33.77% menthone and 0.55-30.70% menthyl acetate [2]. Zheljazkova et al. (2012), reported 36.8 to 45.5% menthol, 10.2 to 17.3% menthone, 12.6 to 22.3% menthofuran and 5.8 to 9.8% menthyl acetate in their oils [8]. Curutchet et al. (2014) obtained 46.8-48.7 % menthone and 21.6-23.5% menthol in the fresh and dried plants respectively [3]. *M. spicata* (spearmint) oils are principally rich in carvone and limonene. Koyalta (1990) identified 74.5% carvone and 11.6% limonene in the fresh plants from Senegalese spearmint [6b]. Chauhan et al. (2009) obtained in the fresh plants 49.6 to 76.7% carvone and 9.8 to 22.3% limonene [9]. El Hassani et al. (2010), determined 70 to 73% for carvone in the dried plants [1]. Others *M. spicata* oils that revealed pulegone (26.71-29.56%) and piperitone (22.17-28.16%) have been identified [9]. Mint essential oils and their major compounds have very interesting biological properties. Antibacterial properties of *M. spicata* oils and ansecticidal activities of *M. x piperita* oils were showed [11,12].

In Senegal, mints are much consumed but there are few applications based on scientific studies nevertheless some of them have been briefly investigated for their chemical composition and antibacterial activity [6a,6b,13]. The objective of the present work is to study the chemical composition of essential oils of three varieties of Senegalese endemic mints: *Mentha citrata*, *Mentha x piperita* and *Mentha spicata* and to assess the effects of the drying in the shade on essential oils composition.

Yields were of 0.28 and 0.21% for *M. citrata*, 0.21 and 0.18% for *M. x piperita* and 0.10 and 0.19% for *M. spicata* in both fresh and dried plants respectively. Chromatographic study revealed 29 and 34 constituents in *M. citrata*, 38 and 40 in *M. x piperita* and 30 and 34 in *M. spicata* in the fresh and dried plants respectively. Analyses revealed a variation in the prominent components according to the drying (table 1). Monoterpenes contents were: 96.1 and 94.9% for *M. citrata*, 93.8 and 92.2% for *M. x piperita* and 95.6 and 95.4% for *M. spicata* in the freshand dried plants respectively.

Table 1: Chemical composition of essential oil

Compounds	Retention indices	*M. citrata*		*M. x piperita*		*M. spicata*	
		Fresh plants	Dried plants	Fresh plants	Dried plants	Fresh plants	Dried plants
α-Pinene	933	-	0.1	0.5	0.2	0.4	0.2
Sabinene	974	0.1	0.2	0.4	0.4	0.4	0.2
β-Pinene	978	0.1	0.4	0.8	0.6	0.6	0.4
Myrcene	990	**1.1**	**1.5**	0.3	0.3	0.8	0.5
Octan-3-ol	998	-	-	0.1	0.2	0.2	0.2
para-Cymene	1025	-	-	0.1	-	-	
Limonene	1030	0.2	0.3	**2.0**	**1.7**	**18.1**	**12.5**
1.8-Cineole	1034	2.0	3.1	**3.7**	**3.4**	0.4	0.3
(*Z*)-β-Ocimene	1037	**1.4**	**1.7**	-	0.1	-	-
(*E*)-β-Ocimene	1047	0.3	0.4	-	-	0.1	-
γ-Terpinene	1059	-	-	0.1	-	-	-
cis-Sabinene hydrate	1072	-	-	**1.3**	**1.3**	0.1	0.1
Terpinolene	1088	0.1	0.1	-	-	-	-
Linalool	1100	**45.8**	**42.0**	-	2.1	0.2	0.2
n-Nonanal	1105	-		-		0.1	0.1
1-Octen-3-yl acetate	1107	0.2	0.2	-	-	-	-
3-Octanol acetate	1119	0.1	0.1	-	-	0.2	0.1
allo-Ocimene	1124	-	-	-	-	0.1	0.1
Not identified	1153	-	-	0.2	0.1	-	-
Menthone	1159	-	1.0	**13.0**	**14.2**	-	T
Menthofurane	1166	-	0.1	**30.7**	**28.1**	-	-
Isomenthone	1169	-	0.6	1.0	1.4	-	-
Neomenthol	1172	-	-	1.6	1.5	-	-
δ-Terpineol	1173	0.1	0.1	-	-	-	-
Borneol	1175	-	-	-	-	0.3	0.4
Menthol	1180	-	0.1	**15.9**	**16.4**	-	-
Terpinen-4-ol	1184	-	-	0.3	-	-	-
Isomenthol	1191	-	-	0.1	0.2	-	-
α-Terpineol	1197	0.8	0.6	0.3	0.3	-	0.1
cis-Dihydrocarvone	1200	-	-	-	-	**1.9**	**2.0**
Not identified	1218	-	-	0.1	-	-	-
trans-Carveol	1221	-	-	-	-	**2.9**	**2.2**
Pulegone	1243	-	2.9	**17.6**	**13.8**	0.3	0.3
Carvone	1247	-	-	0.4	0.2	**67.8**	**74.7**
Linalyl acetate	1250	**42.7**	**38.5**	-	1.9	-	-
Piperitone	1259	-	-	0.1	0.2	-	-
Cis-carvoneoxide	1266	-	-	-	-	-	0.1
Neomenthyl acetate	1274	-	-	0.2	0.2	-	-
trans-Carvone oxide	1279	-	-	-	-	-	0.1
Menthyl acetate	1291	-	-	**2.6**	**3.1**	-	-
Isomenthyl acetate	1308	-	-	-	0.1	-	-
Dihydrocarvylacetate	1326	-	-	-	-	0.2	0.2
δ-Elemene	1336	-	-	-	0.1	0.1	0.1
Piperitenone	1344	-	-	-	0.1	-	-
Limonen-10-yl acetate	1352	0.3	0.4	0.4	0.1	-	-
cis-*trans*-Nepetalactone	1354	-	-	0.3	0.1	-	-
Nerol acetate	1358	0.3	0.2	-	-	-	-
cis-Carvyl acetate	1360	-		-		0.4	0.4
Geranyl acetate	1377	0.5	0.3	-	-	-	-
Not identified	1384	0.4	0.4	-	-	-	-
β-Bourbonene	1389	-	-	0.1	0.1	0.9	1.1
β-Elemene	1393	0.4	0.6	0.1	0.2	0.2	0.2
(*E*)-β-Caryophyllene	1426	0.9	1.6	**1.7**	**2.7**	**1.1**	**1.1**
Not identified	1448	-	-	1.5	0.3	-	-
ε-Muurolene	1451	0.1	0.2	-	-	0.1	0.1
(*E*)-β-Farnesene	1453	0.1	0.3	0.4	0.7	0.4	0.4
α-Humulene	1462	0.1	0.1	-	0.1	0.2	0.2
Germacrene D	1487	0.6	0.8	**1.4**	**2.6**	**1.1**	**1.0**
Bicyclogermacrene	1501	-	-	0.1	0.2	0.2	0.2
γ-Muurolene	1504	-	-	0.1	-	-	-
cis-Calamene	1526	0.1	-	-	-	0.1	T
Not identified	1542	-	-	-	-	-	-
Elemol	1553	0.7	0.6	-	-	-	-
Not identified	1559	-	-	0.1	-	-	-
Germacrene D-4-ol	1584	-	-	-	0.1	-	-
Caryophyllene oxide	1591	-	-	0.1	0.1	-	0.1
Viridiflorol	1603	0.3	0.3	0.3	0.5	-	-
Cubenol (1.10-di-*epi*)	1622	0.1	0.1	-	-	-	-
Not identified	1662	0.1	0.1	-	-	-	-
Monoterpenic hydrocarbons		**3.2**	**4.6**	**4.2**	**3.3**	**20.4**	**13.8**
Oxygenated monoterpenes		**92.9**	**90.3**	**89.6**	**88.9**	**75.2**	**81.6**
Sesquiterpenic hydrocarbons		**2.3**	**3.6**	**3.9**	**6.7**	**4.3**	**4.4**
Oxygenated sesquiterpenes		**1.1**	**1.0**	**0.4**	**0.7**	**0.0**	**0.1**
Not identified		**0.5**	**0.5**	**1.9**	**0.4**	**0.0**	**0.0**

t = trace (< 0.1%).

Essential oils of *M. citrata* contained mainly linalool and linalyl acetate. Linalool represented 45.8 and 42.0% and linalyl acetate constituted 42.7 and 38.5% in the fresh and dried plants respectively. Others representative components present in oils were 1.8-cineole with 2.0 and 3.1%, myrcene 1.1 and 1.5% and (*Z*)-β-ocimene 1.4 and 1.7%. Sesquiterpenes were dominated by (*E*)-β-caryophyllene that constituted 0.9 and 1.6.

Menthofuran was the most abundant compound of the essential oils of *M. x piperita*. It represented 30.7 and 28.1%, pulegone constituted 17.6 and 13.8% and 1.8-cineole 3.7 and 3.4% in the fresh and dried plants respectively. Menthol constituted 15.9 and 16.4% and menthone 13.0 and 14.2%. Others monoterpenes identified in the oils were menthyl acetate that represented 2.6 and 3.1%, isomenthone 1.0 and 1.4% and neomenthol 1.6 and 1.5%. The most representative sesquiterpenes were (*E*)-β-caryophyllene (1.7 and 2.7%) and germacrene D (1.4 and 2.6%).

The oils of *M. spicata* were characterized by carvone. It represented 67.8% in the fresh plants and 74.7% after drying. Limonene, the prominent monoterpenic hydrocarbon constituted 18.1% and 12.5% in the fresh and dried plants respectively. *cis*-Dihydrocarvone represented 1.9 and 2.0% and *trans*-carveol, 2.9 et 2.2% in the fresh and dried plants respectively. (*E*)-β-caryophyllene (1.1 and 1.1%) and germacrene D (1.1 and 1.0%) were the prominent sesquiterpenes present in the oils.

The yields of the oils were interesting according to the literature. In *Mentha x piperita* oils, Benayad (2013) obtained 1.72% after 20 days of drying in the shade [12], whereas Zheljazkova et al. (2012) obtained 0.188 to 0.979% [8]. In Senegal, a study revealed a yield of 0.075% in the fresh plants [6a]. All oils were dominated by the monoterpenes that decreased after drying, whereas sesquiterpenes increased. Monoterpenes represented more than 95% in *M. citrata* and *M. spicata*. These oils were richer in monoterpenes than those of *M. x piperita*. *M. x piperita* contained the major rate of sesquiterpenes, 7.7% after drying.

The major components of *M. citrata*: linalool and linalyl acetate decreased from 88.5% in the fresh plants to 80.5% after drying. As concern the same species Garlet et al. (2013) [4] and Padalia et al. (2013) [5] reported higher linalool percentages (53.5 to 45.0% and 59.7%) and lower linalyl acetate proportions (34.0 to 28.1% and 18.4%) than the senegalese oils, respectively. The compounds that represented 1 to 3% each of: 1.8-cineole, myrcene, (*Z*)-β-ocimene and (*E*)-β-caryophyllene, increased after drying.

In dried *M. x piperita* areal parts, menthofuran, pulegone and 1.8-cineole content are lower than in fresh material. According to the European legislation and because their potential deleterious properties, pulegone and menthofuran are regulated [14]. In foodstuffs which naturally contain these compounds, the maximum levels of pulegone and menthofuran are of 2000mg/kg and 3000mg/kg respectively in micro-freshening confectionery breath. In chewing-gums the maximum levels are 350mg/kg (pulegone) and 1000mg/kg (menthofuran). So the drying in the shade increased the quality of oils. The essential oils of *M. x piperita* from Senegal were rich in components from the menthol group: menthofuran, menthol, menthone, isomenthone, neomenthol and menthyl acetate that were constant, 64.8 and 64.7% in the fresh and dried plants respectively. These compounds were identified in other oils of *M. x piperita* [2,3,6a,7,8]. In Senegal, oils reported by Koyalta (1993) were higher in menthol (32.46-40.93%) and lower in menthofuran (3.13-4.40%) than these present oils [6a]. In addition, pulegone was not identified in oils of Koyalta (1993) [6a]. So the peculiarity of the present oils of *M. x piperita* from Senegal was its high contents in menthofuran and pulegone. Menthyl acetate, isomenthone, (*E*)-β-caryophyllene and germacrene D that represented between 1 to 3% each of, increased and neomenthol decreased.

Carvone increased after drying in the oils of *M. spicata*. Limonene decreased from fresh to dried plants. These compounds were identified in several oils. Generally, carvone varied from 40 to 70% in the essential oils of *M. spicata* and limonene from 10 to 20% [3,6b,9]. Koyalta (1990) previously identified carvone (74.5%) and limonene (11.6%) in the oils from Senegal [6b]. So the composition of the present Senegalese oils is very interesting. *trans*-Carveol decreased, whereas *cis*-Dihydrocarvone (*E*)-β-caryophyllene and germacrene D were constant. These components constituted 1 to 3% of the oil.

This study focus on three species of mints from Senegal shown that drying in the shade had effects on oils constituents. In *M. citrata* oils, the contents of the major compounds, linalool and linalyl acetate decreased after drying. The oils of *M. x piperita* were quite rich in menthol and menthone. But they also contained high levels of potential hepatotoxic components like pulegone and menthofuran that decreased with drying. This content can be reduced in that oils are not consumed as such, but added in the food products. Further studies could allow to assess the content of these both compounds in tea and hibiscus juice. Prominent components of *M. spicata* were carvone and limonene. The rate of carvone increased with drying whereas those of limonene decreased.

Experimental

Plant material: Plants were harvested in the micro-garden of the traffic circle of "Liberté 6 in Dakar" during the rainy season, 2013. The voucher specimens (LB_2, LB_3 and LB_4) were deposited in the herbarium of the "Institut Fondamental d'Afrique Noire de l'Université Cheikh Anta Diop de Dakar".

Essential oil extraction: Essential oils were extracted from the aerial parts of the plants. About 100g of both fresh and dried plants (let at 26-34°C in the shade for 7 days) were subjected to steam distillation using a Clevenger-type apparatus for 45min. The oils were stored in the refrigerator (4°C) in an amber vials to protect them against heat and light.

Gas Chromatography: Characterization of essential oils was carried out by gas chromatography. The following temperature conditions were used: initial temperature 40°C (5min), ramp 8°C/min and final temperature 280°C (5min). The detector and the injector temperature (splitless mode) was 290°C. The carrier gas was helium at a constant rate set at 1.5ml/min. The capillary column was an Optima-5-accent (Macherey-Nagel, Germany), 5% phenyl-95% methylsiloxane: 30m x 0.25mm, film thickness 0.25µm. 1 µl solutions of 10mg essential oil/20 ml n-hexane were injected on a Trace Ultra GC from Thermo Scientific (Ma, USA) fitted with a flame ionization detector (GC/FID). Air and hydrogen flows were 350ml/min and 35ml/min respectively.

The mass spectrometer was an Agilent 5973 Quadrupole (from Agilent Technologies, Belgium) coupled with a gas chromatograph Agilent 6890 (GC/MS). Mass spectra were recorded at 70 eV and the mass scanned range was from 35 to 350 amu.

The mass spectra were compared to those from Wiley 275 L, a computerized database and those given in the literature [15,16].

Pure compounds confirmed the identification of the major constituents.

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