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Research Article

CHEMICAL COMPOSITION OF ESSENTIAL OILS AND FLORAL WATERS OF *EUCALYPTUS CAMALDULENSIS* (Dehnh) FROM TWO LOCATIONS IN SENEGAL

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ABSTRACT. *Eucalyptus camaldulensis* Dehnh leaves from two locations in Senegal (Dakar and Saint Louis) were dried for 14 and 21 days prior to distillation of essential oils by steam distillation and extraction of floral water. Essential oil yields were 0.7 to 2.0%. Oils were analyzed by gas chromatography coupled to a flame ionization detector (GC-FID) and by gas chromatography coupled to a mass spectrometer (GC-MS) equipped with methyl-phenyl–polysiloxane 5% column. Floral waters chemical composition was analyzed by GC-FID and GC-MS equipped with a polar column (VF-Wax ms). Results showed qualitative and quantitative differences in composition between oils from Hann (Dakar) and Bango (Saint Louis). The main constituents of oils were hydrocarbon monoterpenes with 80.9-83.0% for Hann (Dakar) and 51.5-50.6% for Bango (Saint-Louis). Dakar oils contained *α*-phellandrene as the major compound, with 45.3% and 47.7% after 14 and 21 days of drying, respectively. Other compounds such as *p*-cymene, *α*-pinene and 1,8-cineole were identified. The Saint-Louis sample showed a very high content of *β*-phellandrene after 14 and 21 days of drying respectively, and *α*-pinene and *p*-cymene was also noted. Furthermore, the floral waters analysis revealed high concentrations of oxygenated compounds whose major part was present in oils. It was also noted qualitative and quantitative differences in floral waters composition. Dakar floral water contained 1,8-cineole (28.2%), terpinen-4-ol (20.3%) α-phellandrene-epoxide (15.9%) and *α*-terpineol (7%). The Saint-Louis floral water showed a high content of 1,8-cineole (19.2%), α- terpineol (15.4%), terpinen-4-ol (10.8%) and *trans*-pinocarveol (9.5%).

Keywords: *α-phellandrene, β-phellandrene, essential oils, Eucalyptus camaldulensis, floral water*

INTRODUCTION

Eucalyptus belongs to Myrtaceae family. Although originating from Australia, more than 700 species are grown worldwide [1]. *Eucalyptus camaldulensis* is the most widely

cultivated species in tropical and subtropical areas of the world [2]. Several species are present in Senegal, introduced in Senegal to prevent desertification and to satisfy the wood need of the population. *Eucalyptus* are well-adapted to Senegal [3]. Although the trees are widespread in the country, the use of essential oils of these species is still very low. *Eucalyptus* is planted mainly for its leaves, which have insecticidal effects and medicinal properties but also for their higher content in essential oils. These are exploited for their use in pharmacy and perfumery applications [4, 5, 6]. Cheng et al. [7] showed the effectiveness of *eucalyptus* essential oils against mosquito larvae. Several studies on the chemical composition of essential oils of *eucalyptus* reported 1.8-cineole as main compound: Francisco et al. [1] on *E. camaldulensis* of Maputo with 43.4% 1,8-cineole, *E. camaldulensis* of Morocco 50.69% and in Iran 69.46% and 54.37% [8, 9, 10]. Tsiri et al. [11] after working for one year to identify chemical composition of essential oils in leaves of *E. camaldulensis* from Greece collected and extracted monthly, noted qualitative and quantitative changes in the composition of oils. They found that 1,8-cineole was always the dominant component (25.3- 44.2%), followed by spathulenol and *β*-pinene with maximas of 19.8 and 18.6% respectively. A recent study undertaken by Lima et al. [12] on *E. camaldulensis* essential oils also showed 1,8-cineole as that main compound (46.74%) followed by alloaromadendrene (12.10%). Several factors such as the harvest area and the drying time can affect yields and the chemical composition of essential oils [13, 14].

Floral waters were obtained during steam distillation of leaves. During this process we obtained a share of water flavored hydrolat and also the essential oil that floats on the top of the floral water.

Some investigations on the volatile constituents of floral water show their chemical composition to be largely dominated by oxygenated compounds [15, 16].

This study was focused on the determination of the chemical composition of essential oils and floral water of *E. camaldulensis.* This is a part of an exploration of potential economic benefits of the forests of Senegal for applications in medical, food and perfumery applications.

MATERIALS AND METHODS

Plant material

The leaves of *E. camaldulensis* were collected in August 2013 in two different areas of Senegal: Hann-Dakar (14° 43' 40" North, 17° 26' 3" West) and Bango in Saint-Louis (16° 02' 00" North; 16° 30' 00" West). Identification of species was confirmed in the vegetable biology Department of Cheikh Anta Diop University (Dakar). Botanicals specimens were deposited in the herbarium of “Institut Fondamental d’Afrique Noire” (IFAN) of Cheikh Anta Diop University (Dakar) with HA1 (for Hann Dakar) and BS1 (for Bango Saint-Louis) codes. Freshly collected leaves were dried at room temperature for 14 and 21 days. For each sample, 150 g of leaves were submitted to steam distillation for 3 hours (with 1.5 L water) using a modified Clevenger-type apparatus. The oil yield was calculated relative to the dry matter.

Analysis of essential oils

The oils were analyzed by gas chromatography coupled with a flame ionization detector (GC-FID) and gas chromatography coupled with a mass spectrometer (GC-MS).

GC-FID: The gas chromatograph coupled with a flame ionization detector (HP 6890A GC) was equipped with a capillary column (optima-5-accent Macherey- Nagel, Germany), type 5% phenyl methyl polysiloxane. The column was 30m long, 0.25mm diameter and 0.25µm film thickness. Helium (He) was used as carrier gas at a flow rate of 30 mL/min. The oven temperature ranges from 40 to 280°C according to the following programming: 40 °C for 5 minutes and then a progression of 8° C/min until 280°C where it stabilizes for 5 minutes. The injector used in split mode was at 290°C at a flow rate of 30mL/min. The detector temperature was 290°C. The detector runs with compressed air and hydrogen with respective flows of 350 mL/min and 35 mL/min. The makeup gas (N_2) was used with a flow rate of 30 mL/min. The minimum peak area integration was fixed at 3000 units.

GC-MS: The GC (Agilent 6890) is equipped with a capillary column which is 30 m in length and a diameter of 0.25 mm and a film thickness of 0.25 µm. The oven temperature was programmed as follows: isotherm of 5 min at 40°C then a progression of 8°C/min up to 280°C where it is stabilized for 5 minutes. The injector, used in splitless mode, was at 240 °C. The carrier gas was Helium (He) with a flow rate of 30 mL/min. The GC is coupled with a mass spectrometer (MS Agilent 5973 FINNIGAN TRACE). Fragmentation was performed by electronic impact (70 eV). Mass spectrum (*m/z*) was set to 35 to 350.

The identification of the compounds was made using data from computer library (Wiley 275L) connected to the GC-MS and retention indices of components calculated using retention times of *n*-alkanes (that were injected after the oil at the same chromatographic conditions) compared to those of the literature [17, 18]. The results were confirmed by injection of standard synthetic compounds.

Floral water analysis

A liquid-liquid extraction was made to isolate components of floral waters of Dakar and Saint-Louis which was obtained during the extraction of the sample at 14 th day of drying. In a separatory bulb, 10 mL of floral water and 2 mL of *n*-hexane were mixed at 15 min. After decantation, the extract was dried over anhydrous sodium sulphate and analyzed by GC/FID and GC/MS equipped with polar columns (VF-Wax ms).

The gas chromatograph (Agilent type HP 6890 Series) coupled to a flame ionization detector (GC-FID) and gas chromatograph (Agilent 6890N) coupled to a mass spectrometer (5973 Agilent) were equipped with polar columns type VF-WAX ms (model Agilent P / N: cp9205). Column dimensions were: 30 m length; 0.25mm diameter and 0.25 µm film thickness. The carrier gas was helium with a flow rate of 1.8 mL/min. The oven temperature was programmed as follows: initially 50°C, it increased by 8°C/min until 250°C and an isothermal 15 minutes at 250 °C. The temperature of the flame ionization detector was 260°C, the hydrogen flow was 30 mL/min and that of air 400 mL/min. For each analysis, 0.5µL of the sample was injected in GC-FID and 1µL in GC-MS. A series of *n*-alkanes (C_7-C_{30}) was injected into each apparatus for the calculation of the retention indices. The energy of ionization in mass was 70 eV. The identification of the compounds was made using the spectral library (Wiley 275L) connected to the GC-MS and the retention indices found compared to those of the literature [19].

RESULTS AND DISCUSSION

Distillation

The yields of leaves essential oils based on the dried weight are presented in Table 1.

The highest essential oil yields were obtained from *E. camaldulensis* of Saint-Louis, with 1.6 and 2% after 14 and 21 days of drying, respectively (Table 1). The sample from Dakar provided 0.7 and 0.9%.

Table 1. *Essential oil yields of E. camaldulensis leaves from Dakar and Saint-Louis*

Drying time (Days)	Localities	
	Hann (Dakar) (%)	Bango (Saint-Louis) (%)
14	0.7	1.3
21	0.9	1.6

Gas Chromatography Analysis

The results of essential oils analysis are presented in Table 2.

A total of 26 compounds were identified in the volatile profile of the essential oils from Hann (Dakar), representing more than 97% of the total oil components which were detected (Table 2). The main constituent was α-phellandrene at 45.3% and 47.7% after 14 and 21 days of drying, respectively. These percentages of α-phellandrene and *p*-cymene (17.1; 16.1%) explain the high content of hydrocarbon monoterpenes with 80.9% and 83.0% at 14 and 21 days of drying. This chemical group was marked by the presence of α-thujene (2.3; 2.2%), α-pinene (3.3; 3.5%), limonene (1.9 and 1.9%) and γ-terpinene (4.8; 5.0%). Hydrocarbon sesquiterpenes such as aromadendrène (2.8, 2.3%), alloaromadendrene (0.7, 0.6%), α-gurjunene (0.7, 0.5%), and ledene (0.7; 0.6%) were present at 6.1% and 5.0% at 14 and 21 days of drying. Oxygenated sesquiterpenes were present with respective rates of 3.6 and 2.8% at at 14 and 21 days of drying. However, there was a low content of 1,8-cineole (4.8, 4.2%) who explains those of oxygenated monoterpenes (7.6 and 7.1%).

Thirty-eight (38) compounds were identified in oils from Bango (Saint-Louis) (Table 2), representing more than 95% of total oils after 14 and 21 days of drying. The hydrocarbon monoterpenes with 51.5% and 50.6% after 14 and 21 days of drying are the dominant oils, followed by oxygenated sesquiterpenes (23.7; 17.9%), oxygenated monoterpenes (8.3; 10.8%) and hydrocarbon sesquiterpenes (13.2; 15.8%). The main constituents in oils were β-phellandrene (18.8; 19.9%), α-pinene (18.4; 17.8%), *p*-cymene (9.4; 7.0%), α-terpineol (2.2; 2.1%), terpinen-4-ol (1.7; 1.9%), α-phellandrene (1.0; 1.6%) with a low rate of 1,8-cineole (1.3; 4.0%). These samples were characterized by the high presence of sesquiterpenes at the 14th and 21st day of drying with bicyclogermacrene (4.9 and 6.4%), β-carryophyllene (3.6; 3.9%), β-eudesmol (7.5; 6.6%), globulol (6.3; 4.5%), viridiflorol (5.6; 2.7%). Other compounds were present in small quantities.

Table 2. *Chemical composition of oils from Dakar and Saint-Louis*

Compounds and Retention Index		% of Compounds			
		14 (day)		21 (day)	
	Compounds	DK	SL	DK	SL
RI					
927	α-thujene	2.3	1.1	2.2	1.3
934	α-pinene	3.3	18.4	3.5	17.8
970	sabinene	-	0.1	-	0.1
978	β-pinene	0.2	0.9	0.2	0.8
990	myrcene	1.0	1.2	1.0	1.4
1006	α-phellandrene	45.3	1.0	47.7	1.6
1017	α-terpinene	1.1	0.1	1.2	0.2
1025	*p*-cymene	17.1	9.4	16.1	7.0
1030	limonene	1.9	-	1.9	-
1032	β-phellandrene	2.8	18.8	3.1	19.9
1034	1,8-cineole	4.8	1.3	4.2	4.0
1060	γ-terpinene	4.8	0.4	5.0	0.4
1086	α-terpinolene	1.1	0.1	1.1	0.1
1100	linalool	-	0.7	-	0.8
1145	*trans*-pinocarveol	-	0.2	-	0.3
1176	borneol	0.6	0.1	0.6	0.1
1184	terpinene-4-ol	1.7	1.7	1.8	1.9
1191	cryptone	-	0.6	-	0.8
1197	α-terpineol	0.5	2.2	0.5	2.1
1246	cuminal	-	0.2	-	0.1
1283	phellandral	-	0.9	-	0.4
1298	carvacrol	-	0.4	-	0.3
1336	bicycloelemene	-	2.2	-	3.0
1381	α-copaene	-	0.1	-	0.1
1393	β-elemene	-	0.4	-	0.4
1413	α-gurjunene	0.7	tr	0.5	0.1
1427	β-caryophyllene	0.9	3.6	0.8	3.9
1438	β-gurjunene	0.3	-	0.2	-
1445	aromadendrene	2.8	0.3	2.3	0.3
1467	alloaromadendrene	0.7	0.9	0.6	0.8
1487	germacreneD	-	0.1	-	0.2
1496	ledene	0.7	0.7	0.6	0.6
1502	bicyclogermacrene	-	4.9	-	6.4
1553	elemol	-	0.9	-	0.5
1579	spathunelol	0.2	0.3	0.1	0.3

Table 2. *(Continue)*

Compounds and Retention Index		% of Compounds			
		14 (day)		21 (day)	
RI	Compounds	DK	SL	DK	SL
1585	globulol	0.4	6.3	0.3	4.5
1593	viridiflorol	2.4	5.6	2.2	2.7
1604	sesquiterpene not identified	0.4	0.9	0.4	1.4
1633	γ-eudesmol	0.3	0.4	0.2	0.5
1640	hinesol	-	2.7	-	2.8
1664	β-eudesmol	0.3	7.5	-	6.6
	Hydrocarbon Monoterpenes	80.9	51.5	83.0	50.6
	Oxygenated Monoterpenes	7.6	8.3	7.1	10.8
	Hydrocarbon sesquiterpenes	6.1	13.2	5.0	15.8
	Oxygenated sesquiterpenes	3.6	23.7	2.8	17.9
	Total identified (%)	98.2	96.7	97.9	95.1

RI= Retention Index, DK= Dakar, SL= Saint-Louis Tr= trace < 0.1%

The chemical group percentages of essential oils from Dakar and Saint-Louis are displayed in Figures 1 & 2.

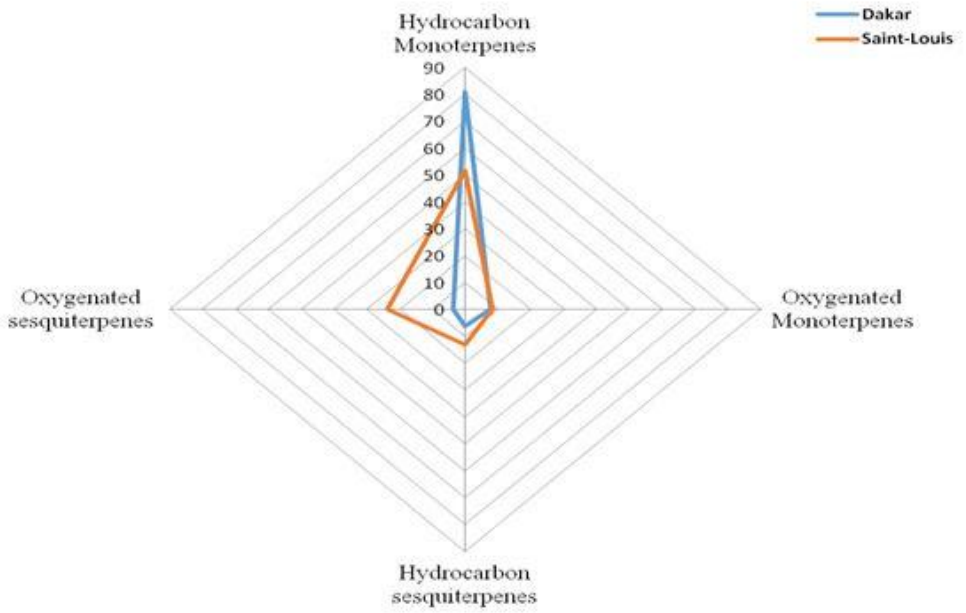

Fig.1. *Radar plot of chemical groups of essential oils from Dakar and Saint-Louis after 14 days of drying*

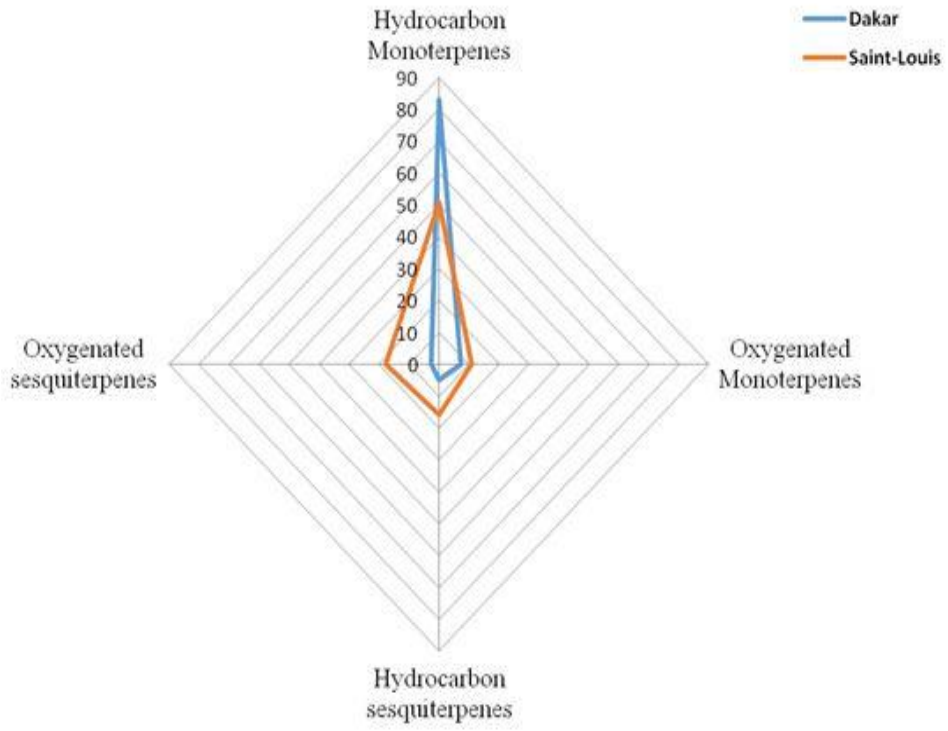

Fig. 2. *Radar plot of chemical groups of essential oils from Dakar and Saint-Louis after 21 days of drying*

In the floral water from Dakar *E. camaldulensis*, 25 compounds were identified representing 92.8% of the composition of the floral water. In the one of Saint-Louis, 26 compounds representing 88.7%. The results are shown in Table 3.

The analysis revealed 90.4% of oxygenated monoterpenes and 2.4% of oxygenated sesquiterpenes. The one of Saint-Louis content 74.5% of oxygenated monoterpenes and 14.2% of oxygenated sesquiterpenes. The major constituents of floral water Dakar were: 1.8-cineole (28.2%) while terpinen-4-ol (20.3%), *α*-phellandrene-epoxide (15.9%), and *α*-terpineol (7.0%) were also present at relatively high percentage. It was noted also the presence at a lower percentage of, *cis*-*p*-menthen-1-ol (2.7%), *p*-cymen-8-ol (2.3%), linalool (2.1%) and other compounds. The floral water of Saint-Louis show also 1,8-cineole (19.2%) with major constituents and *α*-terpineol (15.2%), terpinen-4-ol (10.8%) and trans-pinocarveol (9.5%). A high content of sesquiterpenes as spathulenol (5.3%), *β*-eudesmol (3.8%) was also noted in the floral water of Saint-Louis.

Table 3*. Chemical composition of floral waters of E. camaldulensis from Dakar and Saint-Louis*

RI	Compounds	Compounds (%) Dakar	Compounds (%) Saint-Louis
1210	1,8-cineole	28.2	19.2
1438	*cis*-linalool oxide	1.8	-
1541	linalool	2.1	0.8
1544	pyridinol	2	-
1557	*cis*-*p*-menthen-1-ol	2.7	1.1
1565	pinocarvone	0.5	2.7
1590	fenchol	-	2.6
1606	terpinen-4-ol	20.3	10.8
1624	myrtenal	-	3.5
1649	*trans*-pinocarveol	1.6	9.5
	ni	0.9	2.8
1676	carvotanacetone	1.5	-
1679	1,8-menthadien-4-ol	1.2	-
1688	α- terpineol	7	15.2
1692	borneol	0.6	1.8
1702	ni	1.5	1.2
1717	berbenone	0.2	0.6
1719	exo-2-hydroxycineoleacetate	-	0.7
1736	*trans*-piperitol	1.3	0.4
1783	ni	0.4	5.9
1797	α-phellandrene-epoxide	15.9	0.9
1825	*trans*-carveol	0.7	1.5
1838	*p*-cymen-8-ol	2.3	2.7
1878	*trans*-*p*-mentha-1(7)8-dien-2ol	0.5	0.5
1994	ni	-	1.3
2077	elemol	0.4	0.3
2088	ni	0.4	0.1
2095	globulol	0.2	0.9
2108	viridiflorol	0.6	0.1
2116	spathulenol	1	5.3
2136	carvacrol	-	0.5

***Table 3**. (Continue)*

RI	Compounds	Compounds (%) Dakar	Compounds (%) Saint-Louis
2158	*γ*-eudesmol	-	1.5
2198	ni	4	-
2211	*α*-eudesmol	-	1.8
2221	*β*-eudesmol	0.2	3.8
	oxygenated monoterpenes	90.4	74.5
	oxygenated sesquiterpenes	2.4	14.2
	Total identified (%)	92.8	90.8
	not identified	7.2	6.9

The essential oil yields are in concordance those obtained for the Eucalyptus genus [20, 21]. Likewise, yields obtained by Bachheti et al. [22] for *E. globulus*, *E. tereticornis* and *E. robusta* respectively after 3 hours of extraction, 1.1 %, 0.5% and 0.8% are close to ours.

The low yields of *E. camaldulensis* from Dakar (0.7; 0.9%) can be explained by the harvesting period- flowering had not yet begun. The increased yields from the 14th to 21st day (0.7 to 0.9% for Dakar and 1.6 to 2.0% for Saint-Louis) highlights the positive effect of drying on yields. According to Zrira et al. [23], the yield increased during the first two weeks of drying.

The chemical composition of Dakar and Saint-Louis oils revealed high percentage of hydrocarbon monoterpenes (*α*-phellandrene and *p*-cymene for Dakar and *β*-phellandrene, *α*-pinene and *p*-cymene for Saint-Louis). These chemical features were close to those reported from the same species in Taiwan. This species incorporates *α*-pinene (22.5%) and *α*-phellandrene (20.1%) as majority compounds and hydrocarbon monoterpenes as majority group [7]. The harvesting area had impacted the percentage of hydrocarbon monoterpenes in essential oils. The analysis revealed monoterpene percentages of 80.9 and 83.0% at 14 and 21 day of drying, respectively, for the Dakar sample, compared to 51.5 and 50.6% for those of Saint-Louis. These results were in contrast with those of Grbovic et al. [13] who reported no significant differences in hydrocarbon monoterpene percentages in *E. camaldulensis* essential oils from five areas of Montenegro. Major compounds of Saint-Louis's samples (*β*-phellandrene and *α*-pinene) were found in very low amounts in the Dakar sample. *p*-Cymene was present in Dakar sample oils (17.1% and 16.1%) compared to 9.4 and 7.0% in those of the Saint-Louis sample. Verdeguer et al. [24] found in *E. camaldulensis* essential oil from Spain 26.4% of hydrocarbon monoterpenes; 19.9% of oxygenated monoterpenes; 0.9% of hydrocarbon sesquiterpenes and 48.3% oxygenated sesquiterpenes. The authors reported spathulenol (41.5%) as a major component and percentages of *p*-cymene (21.9%) and 1,8-cineole (1.9%) close to those we report.

Oils from both of our samples contain low percentage of 1,8-cineole: 4.8 and 4.2% respectively after 14 and 21 days of drying from *E. camaldulensis* of Dakar against 1.3 and 4.0% from *E. camaldulensis* of Saint-Louis). This compound has often been reported

as a major essential oil by other researchers Francisco et al. [1] found on *E. camaldulensis* of Maputo 43.4% of 1,8-cineole, *E. camaldulensis* of Morocco gave 50.7% and those of Iran 54.4% [8, 10]. Medhi et al. [9] obtained with the same species growing in Tehran 69.5% 1.8-cineole. In Morocco, according to Zrira et al. [25] essential oils of six species of eucalyptus other than *E camaldulensis* are widely dominated by 1.8-cineole with percentages of 76.9% for *E. sideroxylon*; 61.4% for *E. astringents*; 69.9% for *E. salmonophloia*; 72.9% for *E. Salubris*; 58.5% for *E. brockwayi* and 46.9% for *E. torquata*. The percentage of 1.8-cineole does not present significant variations between the two studied areas. However, the low values of 1.8-cineole found in our study could be explained by the high presence of 1.8-cineole in the floral water (28.2%) in the floral water of Dakar and 19.2% in the floral water of Saint-Louis) which can be explained by the synergy of 1.8-cineole and oxygenated molecules highly present in floral waters. Though low in 1,8-cineole, 1,8-cineole percentages of our oils exceed those of an *E. camaldulensis*'s oil of Algeria [26, 4]. Sesquiterpenes concentrations were higher in the samples from Saint-Louis than in those from Dakar (Figures 1 and 2). We also noted the presence of many other compounds of oils of Saint-Louis that were not found in those of Dakar and vice versa. Figueiredo et al. [27] reported abiotic (geographic variation, climat pollution…) and biotic (genetic factors and evolution, diseases and pests…) factors can impact the essential oils compositions.

It was also interesting to note that 1,8-cineole, terpinen-4-ol and *α*-terpineol, found at lower percentages in the essential oils, were present in floral waters at high percentage. We noted also a presence of other oxygenated compounds in the floral waters, such as berbenone, *trans*-piperitol, *α*-phellandrene-epoxide, *trans*-carveol, *p*-cymen-8-ol which were not found in oils. It suggested that during extraction many hydrophilic oxygenated molecules partition into the floral waters. These results confirmed previous work on floral waters by several authors. They reported the richness of their floral waters in oxygenated and hydrophilic compounds [15, 16, 28]. Ndiaye et al. [29] had also found Senegalese *eucalyptus* floral waters rich in polar compounds. According to these authors, floral waters were mainly composed of 1,8-cineole who was the major compound of essential oils contrary to what was noted in this study. Some compounds for example myrtenal (3.5%), carvacrol (0.5%), *γ*-eudesmol (1.5%), *α*-eudesmol (1.8%) were present in the floral water of Saint-Louis and not in Dakar. The reverse was also noted with *cis*-linalool oxide (1.8%) pyridinol (2%) carvotanacetone (1.5%) 1,8-menthadien-4-ol (1.2%) in floral water of Dakar and not in Saint-Louis. This confirmed the qualitative difference found in oils from two locations in Senegal**.**

CONCLUSION

E. camaldulensis essential oils collected from Dakar and Saint-Louis are qualitatively and quantitatively different. It should be noted that the geographical origin of the species has a major impact on essential oil chemical composition. This might depend on abiotic and biotic factors or directly related to the specific growing conditions of the plant. According to the results obtained, *E. camaldulensis* of Dakar and Saint-Louis can be classified as eucalyptus chemotype with low percentage of 1,8-cineole and high percentage of hydrocarbon monoterpenes. The high percentage of 1,8-cineole found in aromatic waters of *E. camaldulensis* of Dakar and Saint-Louis could confer it in some cases a biological property comparable to those of essential oils rich in 1,8-cineole. This

study on chemical characterization of essential oil and floral water of *E. camaldulensis* from Senegal revealed important characteristics of these floral waters.

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