

Chemical Variability and Antibacterial Activity of Eucalyptus camaldulensis Essential Oils from Senegal

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Abstract

1,8-cineole, also known as eucalyptol, is the main constituent and the most important of the leaf oils of many species of the genus Eucalyptus. In present study, the essential oils isolated by hydrodistillation from the leaves of Eucalyptus camaldulensis from six different locations were analyzed by GC-FID and GC-MS. Essential oil yields ranged from 0.33% to 4.35%. In total, 34 constituents were identified, accounting for 94.0% to 99.8% of the total compositions. 1,8-cineole (46.4% - 84.4%) was the main constituent of all the essential oil samples, except for one sample collected in the Fatick region, which had p-cymene (46.4%) and 1,8-cineole (26.0%) as major compounds. The essential oil showed excellent activity against S. aureus, E. coli and E. faecalis $(IZ = 25.3 \pm 1.2 \text{ mm}; 18.7 \pm 0.6 \text{ mm}; 17.8 \pm 0.3 \text{ mm}, \text{respectively})$ and moderate activity against *P. aeruginosa* (IZ = 10.8 ± 0.8 mm). They may have potential applications in food and pharmaceutical products.

Keywords

Eucalyptus camaldulensis, Essential Oils, Antibacterial Activity, 1,8-Cineole and GC-SM

1. Introduction

Eucalyptus is one of the world's most widely planted genera [1]. E. camaldulensis is a native tree in Australia which has been broadly planted around the world. It belongs to the *Eucalyptus* genus of the Myrtaceae family which also includes around 700 species [2]. It is a highly adaptable tree with ability to tolerate extremes of drought and soil salinity. In Senegal, *E. camaldulensis* has been extensively planted to avert the phenomenon of soil salinization [3]. This species is used in the indigenous system of medicine to cure various human ailments such as diarrhea, chronic dysentery, malaria, infection of upper respiratory tract, and certain skin diseases [4].

Eucalyptus has been prized a rich source of essential oils. Essential oils of various species have been used in the pharmaceutical, cosmetics, food industries [5]. The European Pharmacopoeia standard for eucalyptus oil requires a minimum 1,8-cineole content of 70%. The chemical content in the essential oil depends on the species, geographic location, season, leafage, harvest time, and extraction method [6].

Among the different *E. camaldulensis* leaves studied in the previous literature, six chemotypes were distinguished: 1,8-cineole (16.0% - 70.4%) [7]-[16]; *p*-cymene (27.8% - 68.4%) [17] [18]; spathulenol (41.5%) [19]; *γ*-terpinene (57.4% - 72.5%) [20]; β -phellandrene (24.8%) [21] and ethanone (25.4%) [22].

Considering the huge biological potential of essential oils of this species, it is necessary to continue investigations in other agro-ecological zones to identify plants rich in 1,8-cineole for the plant valorization in pharmaceutical and agri-food products. Therefore, the aim of this study was to characterize the chemical composition and the antibacterial activity of *E. camaldulensis* oils collected in different areas in Senegal.

2. Experimental

2.1. Plant Material

Twenty-seven samples of *E. camaldulensis* leaf were collected in December 2018 from six localities of Senegal: Ziguinchor ($12^{\circ}33.126$ 'N, $16^{\circ}18.333$ 'W; five samples: 1 - 5), Mbao ($14^{\circ}45.31$ 'N, $17^{\circ}20.6$ 'W; five samples: 6 - 10). Ndiosmone ($14^{\circ}18.122$ 'N, $16^{\circ}11.255$ 'W; four samples: 11 - 14), Fatick ($14^{\circ}20.665$ 'N, $16^{\circ}21.957$ 'W; four samples: 15 - 18), Kaffrine ($14^{\circ}07$ '53"N, $15^{\circ}28$ '35"W; five samples: 19 - 23) and Saint-Louis ($16^{\circ}03.905$ 'N, $16^{\circ}26.607$ 'W; four samples: 24 - 27). Each leaf sample is harvested on the same tree.

2.2. Extraction of Essential Oils

Plant material was air-dried for 14 days at room temperature. Samples were hydrodistilled (5 h) using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [23]. The yields of essential oils (w/w, calculated on dry weight basis) were given in **Table 1**.

2.3. Chemical Compositions

The chromatographic analyses were carried out using a Perkin-Elmer Autosystem XL GC apparatus (Walthon, MA, USA) equipped with dual flame onization detection (FID) system and fused-silica capillary columns, namely, Rtx-1 (poly-dimethylsiloxane) and Rtx-wax (poly-ethyleneglycol) (60 m \times 0.22 mm i.d; film

Table 1. Chemical variability of the essential oils from *E. camaldulensis* leaves according to sample locations in Senegal.

	Compounds		Ir ac	Localities of sampling																											
N°_a}		Ir 🌶		Ir p ^d	Ziguinchor						:	Mba	5		Ndiosmone				Fatick					Kaffrine				S	aint-	-Louis	ouis
					1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
1 a-	Pinene	936	931	1015	5.1	16.7	3.3	13.0	10.2	13.8	15.2	9.8	22.0	12.5	12.4	18.9	29.5	27.7	1.2	7.8	17.9	11.8	13.5	2.0	7.5	2.0	1.5	1.0	8.9	1.2	1.5
2 a-	Fenchene	941	942	1047	-	-	-	-	-	-	-	-	-	-	0.1	0.1	-	0.1	-	-	0.1	0.1	-	-	0.1	-	-	-	-	-	-
3 Ca	mphene	950	944	1059	-	0.1	-	-	0.1	0.2	0.1	-	0.2	0.2	0.4	0.5	0.2	0.2		0.1	0.3	0.2	0.1	-	0.2	-	-	-	0.2	-	-
4 β-	Pinene	978	970	1108	0.2	5.7	0.2	0.3	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.3	0.2	0.1	0.1	0.1	0.1	0.2	-	0.3	-	-	-	0.1	-	-
5 M	yrcene	987	982	1154	0.6	0.5	0.7	0.5	0.3	-	0.2	0.1	0.1	0.1	-	-	-	-	0.6	0.2	-	-	0.4	-	-	0.2	-	-	-	0.1	0.1
6 <i>a</i> -	Phellandrene	1002	996	1164	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.8	1.5	-	-	-	-	-	1.2	-	-	-	0.3	-
7 p-	Cymene	1015	1013	1264	0.5	0.4	0.4	0.2	0.4	0.8	0.5	0.8	0.9	0.8	1.2	0.9	0.4	0.5	22.3	40.5	1.1	1.2	0.7	2.0	2.7	14.9	20.1	0.9	0.7	7.9	1.1
8 Li	monene	1025	1021	1200	13.2	15.5	7.5	5.7	9.9	5.0	5.6	12.9	10.3	11.4	2.3	3.0	10.6	4.6	3.6	8.6	5.5	4.9	6.6	7.6	6.8	4.7	10.0	2.1	4.3	3.1	4.1
9 1,8	8-Cineole	1024	1021	1209	70.9	53.5	76.6	66.6	73.9	74.3	69.4	55.3	60.0	70.7	73.3	72.1	53.7	57.0	46.4	26.0	68.2	71.9	71.7	84.4	76.0	69.4	57.6	83.3	71.7	73.68	30.5
10 γ-'	Terpinene	1051	1048	1239	0.9	0.6	0.6	0.5	0.5	0.3	0.4	0.4	0.2	0.4	-	-	0.4	0.2	1.6	0.6	0.1	0.1	0.6	0.4	0.5	0.6	5.4	0.1	0.1	1.3	0.3
11 p-	Cymenene	1075	1078	1432	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	0.1	0.1	0.1	-	-	-	-	-	-	-	-	-
12 Te	erpinolene	1082	1080	1278	0.7	0.3	2.5	0.2	0.2	-	0.1	0.1	0.2	0.1	-	-	0.1		0.4	0.1		0.2	0.2	-	-	-	-	-	-	-	-
13 Li	nalol	1086	1086	1533	0.1	0.1	0.1	0.2	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
14 <i>a</i> -	Fenchol	1099	1106	1561	-	-	-	-	-	-	-	-	-	-	0.3	0.3	0.2	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-
15 <i>a</i> -	Campholenal	1105	1106	1481	-	-	-	-	-	0.1	-	-	0.2	-	0.2	0.2	0.1	0.2	-	-	-	-	-	-	0.1	-	-	-	0.1	-	-
16 Tr	ans-pinocarvol	1126	1130	1650	-	0.1	-	-	0.2	0.6	0.3	0.2	1.0	0.3	4.6	1.8	0.3	1.0	0.1	1.2		3.3	0.2	0.1	0.7	0.1	-	-	3.8	-	-
17 Bo	orneol	1150	1158	1698	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.3	0.1	1.7	-	-	-	-	-	-	-	-	-	-
18 Te	erpinen-4-ol	1164	1163	1590	1.4	0.9	1.2	0.4	1.1	0.8	0.9	1.1	0.8	0.6	0.2		0.8	0.5	3.4	2.3	0.8	1.7	0.9	0.7	0.7	1.4	1.9	0.5	0.5	0.9	0.9
19 <i>a-</i> '	Terpineol	1176	1174	1684	3.0	2.9	2.2	2.7	2.5	2.4	1.9	1.9	1.5	1.7	1.2	1.2	2.4	3.5	1.5	0.6	2.0	1.3	2.3	1.3	1.5	1.2	0.9	0.9	1.3	1.2	1.9
20 Tr	ranscarveol	1200	1199	1824	-	-	-	-	-	-	-	-	-		-	-	-	-	-	0.2	-	-	-	-	-	-	-	0.7	0.6	0.5	0.4
	<i>is-p</i> -menth-1 (7), dien-2-ol	1217	1213	1871	0.1	0.1	-	-	-	-	0.1	0.2	0.2	0.3	1.1	0.6	0.1	0.2	-	-	-	-	0.1	0.1	0.8	0.4	0.4	-	-	-	-
22 Ca	arvone	1214	1224	1739	-	-	-	-	-	-	-	-	0.1	-	-	0.1		0.1	-	-	-	-	-	0.1	0.2	0.7	0.2	-	-	-	-
23 Ca	arvotanacetone	1220	1230	1656	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.8	0.8	0.2	0.1	-	-	-	-	-	-	-	0.2	-
24 Pij	peritone	1226	1234	1730	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.4	1.7	-	-	-	-	-	-	-	-	-	0.3	-
25 Ca	arvacrol	1278	1281	2219	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.9	1.3	-	-	-	-	-	0.4	-	-	-	0.5	-
26 Ar	omadandrene	1443	1447	1611	0.4	0.1	0.8	0.3		0.1	0.3	0.8	0.3	0.1	0.1		0.1	0.3	0.5	0.2	0.1	0.1	0.4	0.1	0.4	0.4	-	0.5	0.2	0.3	0.4
27 Al	loaromadendrene	1462	1457	1638	-	-	-	-	-	-	-	0.4	-	-	-	-	-	-	-	-	-	-	-	-	-	0.1	-	0.2	0.1	0.1	0.2
28 Le	dene	1491	1490	1695	0.2	0.4	0.4	2.4	-	-	1.5	-	0.3	-	-	-	-	1.7	-		-	-	-	-	-	-	-	-	-	-	-
29 Bi	cyclogermacrene	1494	1493	1712	-	-	-	-	-	-	-	7.4	-	-	-	-	-	-	0.2	0.8	-	-	-	-	-	-	-	-	-	-	-
30 Pa	lustrol	1569	1560	1920	-	-	-	-	-	-	-		-	-	-	-	-	-			-	-	-	-	-	-	-	-	-	-	-
31 Sp	athulenol	1572	1565	2119	-	-	-	-	-	-	-	2.8	-	-	-	-	-	-		0.6	-	-	-	-	-	-	-	-	-	-	-
32 Gl	obulol	1589	1575	2074	1.0	0.4	2.3	1.0	-	-	0.7	3.1	0.5	-	-	-	-	0.6	2.9	1.1	-	-	0.8	-	-	1.5	-	4.8	3.3	4.5	4.4
33 Vi	ridiflorol	1592	1591	2089	-	-	-	-	-	-	-	0.7	-	-	-	-	-	-	0.5		0.6	-	-	-	-	-	-	-	-	-	-
34 Gi	ıaiol	1593	1591	2090	-	-	-	-	-	-	-		-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.7	0.3	0.7	0.7
Hy	ydrocarbon monot	erpene	es		21.2	39.8	15.2	20.4	21.7	20.2	22.3	24.3	34.0	25.6	16.5	23.5	41.5	33.5	38.7	59.6	25.2	18.7	22.3	12.0	18.1	23.6	37.0	4.1	14.3	13.9	7.1
Oz	xygenated monote	rpenes			75.5	57.6	80.1	69.9	77.9	78.2	72.6	58.7	63.8	73.6	81.2	76.3	57.6	62.6	54.8	34.5	72.9	79.4	75.2	86.7	80.0	73.6	61.0	85.4	78.0	77.28	33.7
Hy	ydrocarbon sesqui	erpene	es		0.6	0.5	1.2	2.7	-	0.1	1.8	8.6	0.6	0.1	0.1	-	0.1	2.0	0.7	1.0	0.1	0.1	0.4	0.1	0.4	0.5	-	0.7	0.3	0.4	0.6
Oz	xygenated sesquite	rpenes			1.0	0.4	2.3	1.0	-	-	0.7	6.6	0.5	-	-	-	-	-	3.4	1.7	0.6	0.7	0.8	-	-	1.5	-	-	4.0	5.2	5.7
Тс	otal identified (%)				98.3	98.3	98.8	94.0	99.6	98.5	97.4	98.2	98.9	99.3	99.2	99.8	99.2	98.7	97.6	96.8	98.8	98.9	98.7	98.8	98.5	99.2	98.0	95.7	96.2	96.7 9	<i>€.5</i>
Yi	elds (w/w vs dry n	aterial)		2.51	1.45	1.91	2.86	2.40	1.35	2.25	1.60	1.67	1.37	0.93	1.70	1.75	1.93	0.74	0.33	2.11	1.30	1.37	2.34	1.67	1.76	1.90	2.25	2.75	1.63 2	2.19

^aOrder of elution is given on apolar column (Rtx-1). ^bRetention indices of literature on the apolar column (lRIa) [27]. ^cRetention indices on the apolar Rtx-1 column (RIa). ^detention indices on the polar Rtx-Wax column (RIp).

thickness 0.25 μ m). The oven temperature was programmed from 60°C to 230°C at 2°C/min and then held isothermally at 230°C for 35 min: hydrogen was employed as carrier gas (1 mL/min). The injector and detector temperatures were maintained at 280°C, and samples were injected (0.2 μ L of pure oil) in the split mode (1:50). Retention indices (RI) of compounds were determined relative to the retention times of a series of n-alkanes (C5 - C30) by linear interpolation using the Van den Dool and Kratz (1963) equation with the aid of software from Perkin-Elmer (Total Chrom navigator). The relative percentages of the oil constituents were calculated from the GC peak areas, without application of correction factors.

Samples were also analysed with a Perkin-Elmer Turbo mass detector (quadrupole) coupled to a Perkin-ElmerAutosystem XL, equipped with fused-silica capillary columns Rtx-1 and Rtx-Wax. The oven temperature was programmed from 60°C to 230°C at 2°C/min and then held isothermally at 230°C (35 min): hydrogen was employed as carrier gas (1 mL/min). The following chromatographic conditions were employed: injection volume, 0.2 μ L of pure oil; injector temperature, 280°C; split, 1:80; ion source temperature, 150°C; onization energy, 70 eV; MS (EI) acquired over the mass range, 35 - 350 Da; scan rate, 1 s. Identification of the components was based on: 1) comparison of their GC retention indices (RI) on non-polar and polar columns, determined from the retention times of a series of n-alkanes with linear interpolation, with those of authentic compounds or literature data; 2) on computer matching with commercial mass spectral libraries (23 - 25) and comparison of spectra with those of our personal library; and 3) comparison of RI and MS spectral data of authentic compounds or literature data.

2.4. Microbial Strains

The microorganisms used in the present investigation included reference strains from the American Type Culture Collection (ATCC): *E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853, *E. faecalis* ATCC 29212. All the strains were grown on Mueller-Hinton agar for the bacteria. These types of bacteria are the most frequently isolated in laboratories.

2.5. Determination of Antibacterial Activity

Antibacterial activity of the *E. camaldulensis* essential oil was evaluated using the agar disc diffusion method. Inocula were prepared by diluting overnight cultures in Mueller–Hinton broth (MHB; Oxoid) medium to approximately 106 CFU/mL. Filter paper discs (Whatman disc, 6 mm diameter) were impregnated with 20 μ L of the essential oil and placed onto the inoculated Petri dishes containing Mueller–Hinton 2 agar. In addition, reference disks without any oil and chloramphenicol (30 μ g/disc), were used for comparison. After incubation at 37°C ± 1°C for 18 - 24 h for bacteria, the diameters of inhibition zones were measured (mm) and recorded as the mean ± standard deviation. Each test was performed in trip-

licate separate. According to the width of the inhibition zone diameter expressed in mm, results were appreciated as follows: not sensitive (-) for diameter equal to or below 8.0 mm, moderately sensitive (+) for diameter between 8.0 and 14.0 mm, sensitive (++) for diameter between 14.0 and 20.0 mm and extremely sensitive (+++) for diameter equal to or longer than 20.0 mm.

3. Results and Discussion

3.1. Chemical Composition of Essential Oils

Yields of essential oils, calculated based on the mass of dry plant matter, were between 0.33% and 4.35% (Mean \pm SD: 1.78% \pm 0.59%). These results are in line with reports from the literature, indicating yields of 0.25% - 2.19% [7] [11] [14] [15] [16] [17] [19] [20].

The analysis of the leaf oils by GC/FID and GC/MS allowed the identification of 34 compounds representing 94.0% to 99.8% of the total chemical compositions (Table 1). 1,8-cineole (46.4% - 84.4%) was the main constituent of all the essential oil samples, except for sample No. 16 collected in the Fatick region, which had *p*-cymene (46.4%) and 1,8-cineole (26.0%) as major compounds. The *p*-cymene was also significantly present in samples No. 15 from Fatick and No. 22 and 23 from Kaffrine (22.3%, 14.9% and 20.1%, respectively). In addition to these two compounds, limonene (2.1% - 15.5%) and *a*-pinene (1.0% - 29.5%) were also found in the samples.

These results show an almost homogeneous chemotype in all harvest areas. However, the essential oils of the samples from Saint-Louis had a high 1,8-cineole content (80.5% - 83.3%) compared to the other samples and complied with the standards set by the European pharmacopoeia (1,8-cineole > 70%). Thus, these essential oils could be valued in the medical field.

To our knowledge, 1,8-cineole was the major component of the essential oil of *E. camaldulensis* in most studies worldwide [7]-[16]. This high *p*-cymene content was also described in samples from Italy (27.8% to 42.7%) and Turkey (68.4%).

3.2. Antibacterial Activity

The essential oil from sample N° 24 was used to study the antibacterial activity. Anti-bacterial screening of the essential oil was made by disc diffusion method against four bacteria (*E. coli* ATCC 25922, *S. aureus* ATCC 29213, *P. aeruginosa* ATCC 27853 and *E. faecalis* ATCC 29212). The results showed that the mean inhibition zones (IZ) of the essential oil were less than those of positive control, chloramphenicol (30 μ g/disc) (**Table 2**).

The essential of *E. camaldulensis* exhibited excellent activity against *S. aureus*, *E. coli* and *E. faecalis* (IZ = 25.3 ± 1.2 ; 18.7 ± 0.6 ; 17.8 ± 0.3 , respectively) and moderate activity against *P. aeruginosa* (IZ = 10.8 ± 0.8).

Previously, several studies have demonstrated the antimicrobial properties of 1,8-cineole-rich essential oils from eucalyptus species against a wide range of micro-organisms [24] [25] [26].

Mianoongonianao	Inhibition zone (mm)									
Microorganisms	Essential oil (20 μ L/disc) ^a	Chloramphenicol (30 µg/disc) ^b								
<i>E. coli</i> ATCC 25922	18.7 ± 0.6	30.2 ± 1.2								
S. aureus ATCC 29213	25.3 ± 1.2	29.6 ± 0.9								
E. faecalis ATCC 29212	17.8 ± 0.3	28.5 ± 1.6								
P. aeruginosa ATCC 27853	10.8 ± 0.8	14.3 ± 0.9								

Table 2. Antimicrobial activity of the essential oil from E. camaldulensis.

^aThe concentration of essential oil was 20 μ L/disc. ^bThe concentration of Chloramphenicol was 30 μ g/disk.

4. Conclusion

This study reported the chemical composition and the antibacterial activity of the leaf oils of *E. camaldulensis* from Senegal. 1,8-cineole was the main constituent of all the essential oil samples, except for one sample. The essential of *E. camaldulensis* exhibited excellent activity against *S. aureus*, *E. coli* and E. *faecalis* and moderate activity against *P. aeruginosa*. These results may have potential applications in pharmaceutical products.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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