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Essential oil of *Eucalyptus alba* L. Growing on the Salt Zone of Fatick (Senegal) as a Source of 1,8–Cineole and Their Antibacterial Activity

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ABSTRACT

Members of the genus *Eucalyptus* are potential sources of number of commercial essential oils and aromachemicals. In present study, the essential oils isolated by hydrodistillation from the leaves of *Eucalyptus alba* were analyzed by GC–FID and GC–MS. Essential oil yields ranged from 2.41 to 4.35%. In total 18 constituents were identified, accounting for 99.0 to 99.7% of the total compositions. 1,8-cineole was present in all samples as the major component (76.5–88.1%). In addition to this compound, others that were found including limonene (3.8–8.6%), *α*–terpineol (1.4–2.8%), globulol (1.3–6.3%) and *α*–pinene (1.5–1.8%). The essential oil showed a strong antibacterial activity against *S. aureus* ATCC 29213 which is translated by an MIC of 1.25 mg/mL and good activity against *E. coli* ATCC 25922 (6.25 mg/mL) and *E. faecalis* ATCC 29212 (6.25 mg/mL). To our knowledge, our study is the first report of *E. alba* essential oils characterized by a high 1,8–cineole content (>76.5%). They may have potential applications in food and pharmaceutical products.

Keywords: *Eucalyptus alba*, essential oils, antibacterial activity, 1,8–cineole and GC–SM

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INTRODUCTION

Essential oils are complex mixtures of potentially active substances used as flavoring agents and are constituted by several products available on the market. Currently, their importance is more prominent due to the increasing demand of food, cosmetics, and pharmaceutical industries. Recent scientific literature revealed the antimicrobial, antifungal, and antioxidant potential of volatile oils [1–7]. From a volatile oils application prospective, characterization is of great importance and is based on their chemical profiles.

1,8–cineole, also known as eucalyptol, has fresh, camphoraceous fragrance and pungent taste as characteristics and indeed is used extensively in food-flavor, pharmaceutical, and cosmetic industries. It is the main constituent and the most important of the leaf oils of many species of the genus *Eucalyptus*. The *Eucalyptus* corresponds to one of the principal genera of the Myrtaceae family, native to Australia and cultivated worldwide. *Eucalyptus* trees have perennial leaves that are odorous because of the presence of Essential Oils that are produced and stored in secretory cells. EOs obtained from *Eucalyptus* are usually rich in monoterpenes and in some cases sesquiterpenes. Many such EOs are used for pharmaceutical purposes and in perfumery. The eucalyptus oils used as pharmaceuticals are rich in 1,8–cineole [8–11].

E. alba is native to northern Australia, Papua New Guinea and Timor, and has been planted throughout tropical areas. Its current presence in tropical Africa is unclear, particularly because Eucalyptus alba hybridizes quickly with other *Eucalyptus* species [12]. It has been planted in Senegal, particularly in Fatick, to fight soil salinization. On top of that, the wood of this plant is used as pillowcases [13]. Unfortunately, this form of valorization fosters deforestation. Like other species of the genus *Eucalyptus*, these species could be valued for its therapeutic and fragrant properties. Some papers reported the

chemical composition of leaf essential oils [14–18] and biological activities [15,16]. The presence of 1,8–cineole has been reported at low levels, or even zero in the essential oils from the leaves of *E. alba*. Thus, the aim of this study was to characterize the chemical profile and antibacterial activity of the essential oils from leaves of *E. alba* collected in the saline region of Fatick in Senegal.

MATERIAL AND METHODS

Plant material

Four samples of *E. alba* fresh leaves were collected in 27 December 2018 from Fatick salt zone (Senegal) (14°20'24.99" N, 16°23'1.284'W). They have been planted to fight soil salinization phenomenon. Each leaf sample was harvested on the same tree. Plant material was identified by the technicians from the department of botanical of the Fundamental Institute of Black Africa (IFAN) of University Cheikh Anta Diop of Dakar (Senegal).

Extraction of essential oils

Plant material were air-dried for 14 days at room temperature. Samples were hydrodistilled (3 h) using a Clevenger-type apparatus according to the method recommended in the European Pharmacopoeia [19]. The yields of essential oils (w/w, calculated on dry weight basis) were given in the table 1.

GC and GC/MS Analysis

Analyses were carried out using a Perkin-Elmer Autosystem XL GC apparatus (Walthon, MA, USA) equipped with dual flame ionisation detection (FID) system and fused-silica capillarycolumns, namely, Rtx−1 (polydimethylsiloxane) and Rtx-wax (poly-ethyleneglycol) (60 m × 0.22 mm i.d; film thickness 0.25 μm). The oven temperature was programmed from 60 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). Injector and detector temperatures were maintained at 280°C, and samples were injected (0.2 μL 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 interpolationusing the Van den Dool and Kratz (1963) equation with the aid of software from Perkin–Elmer (Total Chrom navigator). Relative percentages of 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-Elmer Autosystem XL, equipped with fused-silica capillary columns Rtx-1 and Rtx-Wax. Oven temperature was programmed from 60 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 μL of pure oil; injector temperature, 280°C; split, 1:80; ion source temperature, 150°C; ionisation energy, 70 eV; MS (EI) acquired over the mass range, 35–350 Da; scan rate, 1 s.

Identification of the components was based on: (a) 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; (b) on computer matching with commercial mass spectral libraries [20–22] and comparison of spectra with those of our personal library; and (c) comparison of RI, MS and NMR spectral data of authentic compounds or literature data.

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, *E. faecalis* ATCC 29212 and *P. aeruginosa* ATCC 27853. All the strains were grown on Mueller–Hinton agar for the bacteria.

Determination of Antibacterial activity

Antibacterial activity of the essential oil (sample No. 2) was assayed using the agar disc diffusion method [23]. Inocula were prepared by diluting overnight cultures in Mueller–Hinton broth (MHB; Oxoid) medium to approximately 10^6 CFU/ml. Filter paper discs (Whatman disc, 6 mm diameter) were impregnated with 20 μl 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 μg/disc), were used for comparison. After incubation at 37 ± 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 triplicate 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.

Broth microdilution method – determination of the minimum inhibitory concentration (MIC)

For the determination of MIC, which represents the concentration that completely inhibit the growth of microorganisms, a microdilution broth susceptibility assay was used, as recommended by National Committee for Clinical Laboratory Standards [24]. All tests were performed in MHB supplemented with Tween 80 detergent to a final concentration of 0.5% (v/v). Dilutions series were prepared from 0.5 to 250.0 mg/mL of the oil or main components in a 96-well microtiter plate. 160 μL of MHB were added onto microplates and 20 μL of tested solution. Then, 20 μL of 1 × 10^6 CFU/mL of standard microorganism suspension were inoculated onto microplates. Plates were incubated at 37°C for 24 h. The same test was performed simultaneously for the growth control (MHB+ Tween 80) and sterility control (MHB + Tween 80 + test oil). The MIC is defined as the lowest concentration of the samples at which the bacterium does not demonstrate visible growth. The bacterium growth was indicated by turbidity.

RESULTS AND DISCUSSION

Chemical composition of essential oils

Essential oil yields, calculated with respect to the mass of dry plant material, are between 2.41 and 4.35%. These results differ from those in the literature which vary between 0.22–1.2% [14,16–18,25]. Thus, our results are promising for a subsequent valuation.

The analysis of the leaf essential oils by GC/FID and GC/MS allowed the identification of 18 compounds accounting for 99.0 to 99.7% of the total compositions (Table 1). 1,8–cineole was the main constituent present (76.5–88.1%) in all samples. In addition to this compound, others that were found included limonene (3.8–8.6%), *α*–terpineol (1.4–2.8%), globulol (1.3–6.3%) and *α*–pinene (1.5–1.8%).

To our knowledge, this high content of 1,8–cineole in the leaf essential oils of *E. alba* has never been described in the literature, while it is the most sought-after compound in *Eucalyptus oils* thanks to its therapeutic properties and its strong high added value. The chemical composition of leaf essential oil reported by Mondello et al. (1998) in Bangladesh was dominated by *β*–pinene (24.0%), *α*–pinene (12.9%), *β*–caryophyllene (7.8%) and limonene (5.2), while 1,8-cineole was present with a content of 1.4% [17]. A similar composition has been described in leaf essential oil from Burkina Faso. 1,8–cineole was absent in this oil which was predominantly

dominated by *β*–pinene (31.0%), α–pinene (20.1%), limonene (16.8%), *β*–caryophyllene (6.6%) and γ–terpinene (5.6%) [18]. This low 1,8–cineole (5.2%) content has also been reported by Cimanga et al. of the Democratic Republic of Congo. The essential oil consisted mainly of *β*–pinene (25.3%), *β*–terpineol (13.6%), *p*–cymene (7.4%) and *α*–terpineol (6.2%). %) and limonene (4.6%) [26]. The Oyedeji et al. (1999) study in Nigeria reported 1,8–cineole (13.3%) as the second major compound, the first compound being *α*–thujene (32.9%) followed by *p*–cymene (12.9%) and *β*–caryophyllene (7.8%) [25]. In Senegal (Kaolack), only one study reports the chemical composition of the essential oil of *E. alba*. It was predominantly dominated by 1,8–cineole (36.0-38.3%), *α*–pinene (19–26.4%), limonene (5.7–8.3), transpinocarveol (3.5–5%) and *β*–pinene (1.9–4.6%) [14]. This chemical variability may be due to climatic conditions, soil conditions and genetic mutations.

Table 1. Chemical composition of the leaf essential oils from *E. alba*.

N[a]	**Compounds**	***l*RIa[b]**	**RIa[c]**	**Rip[d]**	**1**	**2**	**3**	**4**
1	*α*-Pinene	936	931	1015	1.6	1.8	1.5	1.6
2	*β*-Pinene	978	970	1108	0.1	0.1	-	-
3	Myrcene	987	982	1154	-	0.3	-	-
4	*p*-Cymene	1015	1013	1264	0.7	0.7	0.7	1.1
5	**1,8-Cineole**	**1024**	**1021**	**1209**	**88.1**	**80.5**	**76.5**	**81.5**
6	Limonene	1025	1021	1200	3.8	5.4	4.1	8.6
7	*γ*-Terpinene	1051	1048	1239	0.2	0.4	0.2	0.3
8	Terpinolene	1082	1080	1278	-	0.1	-	0.1
9	Trans-pinocarvol	1126	1130	1650	0.2	-	0.1	-
10	Terpinen-4-ol	1164	1163	1590	0.5	1.4	0.7	1.6
11	*α*-Terpineol	1176	1174	1684	1.4	2.8	1.7	2.2
12	*Cis*-p-menth-1(7),8-dien-2-ol	1217	1213	1871	0.4	0.1	0.3	0.4
13	Carvone	1214	1224	1739	0.1	-	-	0.1
14	Aromadandrene	1443	1447	1611	0.7	1.1	2.6	0.4
15	Alloaromadendrene	1462	1457	1638	0.3	0.3	0.7	-
16	Epiglobulol	1552	1550	2013	-	0.6	0.7	-
17	Globulol	1589	1575	2074	1.3	2.9	6.3	1.8
18	Viridiflorol	1592	1591	2089	-	0.5	0.7	-
	Hydrocarbon monoterpenes				6.4	8.8	6.5	11.7
	Oxygenated monoterpenes				90.7	84.8	79.3	85.8
	Hydrocarbon sesquiterpenes				1.0	1.4	3.3	0.4
	Oxygenated sesquiterpenes				1.3	4.0	7.7	1.8
	Total identified (%)				99.4	99.0	96.8	99.7
	Yields (w/w vs dry material)				4.35	3.54	2.41	3.24

[a] Order of elution is given on apolar column (Rtx-1).

[b] Retention indices of literature on the apolar column (lRIa) [21]

[c] Retention indices on the apolar Rtx-1 column (RIa).

[d] Retention indices on the polar Rtx-Wax column (RIp).

Antibacterial activity

The essential oil from sample No. 2 was used to study the antibacterial activity. Antimicrobial screening of the essential oil was made by disk 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 µg/disc).

The essential of *E. alba* exhibited excellent activity against *S. aureus* ATCC 29213 (IZ = 26.8±1.4), good activity against *E. coli* ATCC 25922, *E. faecalis* ATCC 29212 (IZ = 19.4±1.1 and IZ = 16.2±0.8, respectively) and moderate activity against *P. aeruginosa* ATCC 27853 (IZ = 10.1±0.7).

The MIC of *E. alba* essential oil ranged from 1.25 to 25 mg/mL for the tested bacterial strains (Table 2). According to the results, the essential oil showed a strong antibacterial activity against *S. aureus* ATCC 29213 which is translated by an MIC of 1.25 mg/mL and good activity against *E. coli* ATCC 25922 (6.25 mg/mL) and *E. faecalis* ATCC 29212 (6.25 mg/mL). However, *P. aeruginosa* ATCC 27853 was less sensitive to the essential oil as their MIC was ≥ 25.00 mg/mL.

Table 2 : Antibacterial activity of the essential oil from *E. alba*

Microorganisms	Inhibition zone (mm)		MIC[c]
	Essential oil[a]	Chloramphenicol[b]	Essential oil (mg/mL)
E. coli ATCC 25922	19.4±1.1	30.2±1.2	6.25
E. faecalis ATCC 29212	16.2±0.8	28.5±1.6	6.25
S. aureus ATCC 29213	26.8±1.4	29.6±0.9	1,25
P. aeruginosa ATCC 27853	10.1±0.7	14.3±0.9	25.00

[a] The concentration of essential oil was 20 µL/disc. [b] The concentration of Chloramphenicol was 30 µg/disk. [c] Minimum inhibitory concentration.

CONCLUSION

This study reported the chemical composition and the antibacterial activity of the leaf oils of *E. alba* from Senegal. 1,8-cineole was present in all samples as the major component (76.5–88.1%). The essential oil showed a strong antibacterial activity against *S. aureus* ATCC 29213 and good activity against *E. coli* ATCC 25922 and *E. faecalis* ATCC 29212. To our knowledge, our study is the first report of *E. alba* essential oils characterized by a high 1,8–cineole content (>76.5%). They may have potential applications in food and pharmaceutical products.

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