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Chemical characterization of essential oil from the leaves of *Callistemon viminalis* (D.R.) and *Melaleuca leucadendron* (Linn.)

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ABSTRACT

Objective: To isolate and identify the compounds in the essential oils from the leaves of *Callistemon viminalis* (D.R.) and *Melaleuca leucadendron* (Linn.) collected in Dakar, Senegal.
Methods: The essential oils from the leaves of these two myrtaceaes were extracted by steam distillation and analyzed by gas chromatograph and gas chromatography–mass spectrometer.
Results: A total of 34 constituents were identified in the oil of *Callistemon viminalis* and the major compounds were 1.8-cineole (58.12%), limonene (9.72%), α-terpineol (9.56%), geranial (6.02%), δ-elemene (3.53%), myrcene (2.96%) and α-pinene (2.49%). For the essential oil of *Melaleuca leucadendron*, 43 constituents were identified, and 1.8-cineole (28.87%), epiglobulol (23.06%), α-pinene (12.22%), limonene (11.65%) and α-terpineol (7.06%) were major compounds.
Conclusions: Considering properties of the identified major compounds, essential oils of both studied myrtaceae could be used in the medicine field including the food, pharmaceutical and cosmetic industry.

1. Introduction

In the recent years, there has been an increased interest in the use of aromatic plants, which are used in different areas of life due to their various properties. According to the literature, essential oils have been known to possess antioxidant properties [1,2], expectorant, diuretic, antimicrobial and antifungal activities as well [3]. Besides their strict medicinal applications, essential oils are extensively used in many fields such as food industry, perfume products, cosmetics and chemical industry. Since antiquity, aromatic plants have been used for their medicinal properties. In Africa, plants have been traditionally used as insecticides for a long time [4,5], and their insecticidal and insect-repellent properties are proven by several researchers [4–9]. The essential oil of *Ocimum sanctum* (L). showed significant efficacy against *Dysdercus voelkeri*, a major pest of cotton [4]. According to Ogendo [6], the essential oils of *Ocimum gratissimum* (L.) revealed a remarkable insecticidal power against *Rhyzopertha dominica* (F.). Therefore, the chemical characterization of the essential oil is very important for the understanding of its properties.

However, in West Africa, the characteristics of the essential oils of used plants are hardly known. A sustained effort to search for a better understanding of the chemical composition and physico-chemical properties of essential oils from aromatic plants is needed to take better advantage of their opportunities. In this perspective, this work was carried out in order to characterize the essential oils of two myrtaceae [*Callistemon viminalis* D.R. (*C. viminalis*) and *Melaleuca leucadendron* (Linn.) (*M. leucadendron*)] grown in the Dakar region of Senegal.

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2. Materials and methods

2.1. Plant material

While the leaves and stems of *C. viminalis* were collected from the experimental field of the Faculty of Sciences and Technology of Cheikh Anta Diop University, Dakar and confirmed by Mr. Camara, a herbal botanist of the Vegetal Biology Department in October 2013, those of *M. leucadendron* were picked during the same period and confirmed by Mr. Gueye, a herbal botanist from the botanical garden at Hann Forest Park. The collected samples were dried during 6 days at ambient temperature and protected from sunlight.

2.2. Extraction of essential oils

Distillation by stripping with steam has been used to extract the essential oils from the dried leaves using a Clevenger-type apparatus for 2 h. Essential oils recovered were dried with anhydrous sodium sulfate and then stored in sealed tubes in the refrigerator until analysis.

2.3. Chemical analysis of essential oils

The analysis of the chemical composition of essential oils was performed by using a gas chromatograph with a flame ionization detector (GC-FID) and a gas chromatograph coupled with mass spectrometry (GC–MS).

GC-FID was equipped with a capillary column type 5% phenyl dimethylpolysiloxane (30 m × 0.25 mm inner diameter, film thickness 0.25 μm). The carrier gas used was helium (He) at a flow rate of 1.5 mL/min. Oven temperature ranged from 40 to 280 °C according to the following programming: initial temperature was 40 °C for 5 min and an increase of 8 °C/min to 280 °C where it stabilized for 5 min. The type injector split/splitless used splitless mode was at a temperature of 290 °C. The detector was set at 290 °C and operated by compressed air and hydrogen with respective flow rates of 350 mL/min and 35 mL/min. Gas make-up (dinitrogen N_2) was used with a flow rate of 30 mL/min.

The GC–MS was equipped with a capillary column and was used under conditions identical to those of GC-FID. The GC was coupled to mass spectrometry (MS FINNIGAN TRACE). Fragmentation was done by electron impact (70 eV) and the mass range was between 35 and 300 amu.

The components of the oil were identified by using the spectral library (Wiley 275 L) related to CG-MS with calculation of their retention indices which were afterwards compared with data published in the literature [10,11].

3. Results

3.1. Extracts physical properties and yields

The extraction of essential oils from the aerial parts of two myrtaceaes has enabled us to achieve interesting results such as 3.92% for *C. viminalis* and 1.28% for *M. leucadendron*. Therefore, the density of oils was determined: 0.88 for *C. viminalis* and 0.83 for *M. leucadendron*, while coloring was marked light yellow for *C. viminalis* essential oils and light green for *M. leucadendron*.

3.2. Chemical composition of essential oils

The identification of chemical constituents was carried out by the retention index determination from the retention times and by comparing the mass spectra to those of the apparatus database. The components identified in each essential oil sample were presented in their output orders in the following Table 1.

Table 1

Major chemical compounds in essential oils of *C. viminalis* and *M. leucadendron*.

Retention time	Calculated retention index	Representation of the families of chemical compounds	Identified compound	CV	ML
9.49	926	Hydrogenated monoterpenes	α-Thujene	–	0.08
9.68	926		α-Pinene	2.49	12.22
10.05	948		Caphene	0.06	0.05
10.68	973		Sabinene	–	0.53
10.79	978		β-Pinene	0.76	3.85
11.10	989		Myrcene	2.96	0.65
11.42	1002		Limonene	0.14	–
11.48	1005		α-Phellandrene	0.19	0.04
11.54	1008		δ-3-Carene	–	–
11.73	1017		α-Terpinene	–	0.26
11.91	1025		α-Terpinolene	0.06	–
11.91	1025		β-Cymene	–	0.32
12.03	1030		Limonene	9.72	11.65
12.05	1031		E-ocimene	–	–
				17.05	31.12
12.11	1034	Monoterpene oxides	1.8-Cineol	58.12	28.87
				58.12	28.87
12.39	1047	Monoterpene alcohols	β-Ocimene	–	0.09
12.65	1059		γ-Terpinene	0.52	0.80
13.23	1086		Terpinolene	0.15	0.54
13.43	1096		α-Terpineol		7.06
13.52	1099		Linalool	0.22	0.20
13.95	1121		Fenchol	–	0.09
14.53	1151		Isopulegol	–	0.15
14.75	1162		*cis*-Verbenol	0.09	

(*continued on next page*)

Table 1 (*continued*)

Retention time	Calculated retention index	Representation of the families of chemical compounds	Identified compound	CV	ML
14.95	1 172		Terpinen-4-ol	–	0.18
14.96	1 173		Borneol	0.35	–
15.11	1 180		Terpinen-4-ol	0.18	–
15.16	1 183		*p*-Cymene-8-ol	0.63	–
15.43	1 309		α-Terpineol	9.56	7.06
15.76	1 215		β-Citronellol	0.08	0.06
				11.11	8.36
15.95	1 225	Sesquiterpenes	Geranial	6.02	0.26
16.20	1 239		δ-Elemene	3.53	–
17.19	1 339		*cis*-Citral	–	0.07
17.69	1 325		Bicycloelemene	–	–
18.16	1 353		Eugenol	–	0.12
18.17	1 354		Geranyl acetate	0.76	–
18.56	1 377		Methyl acetate	0.16	–
18.76	1 389		β-Bourbonene	–	–
18.82	1 393		β-Elemene	–	–
18.93	1 399		Methyl eugenol	0.17	–
19.14	1 412		α-Copaene	–	0.05
19.35	1 426		β-Caryophyllene	0.63	–
19.36	1 426		α-Gurjunene	–	1.38
19.50	1 435		α-Bergamotene	–	0.06
19.62	1 443		Funebrene	–	–
19.65	1 445		(E)-β-farnesene	0.06	–
19.66	1 446		Aromadendrene	–	0.06
19.91	1 462		β-Eudesmene	–	0.23
19.92	1 462		α-Humulene	–	–
19.98	1 466		Alloaromadendrene	0.06	0.21
20.30	1 511		δ-Cadinene	0.13	0.05
20.31	1 487		Germacrene D	–	–
20.44	1 496		Bicyclogermacrene	–	–
20.45	1 521		β-Cadinene	–	0.53
20.46	1 507		Viridiflorene	0.08	0.07
20.53	1 502		β-Bisabolene	–	–
20.78	1 519		δ-Cadinene	–	0.10
20.84	1 522		Globulol	0.82	0.13
20.90	1 527		β-Sesquiphellandrene	–	–
21.13	1 542		α-Bisabolene	–	–
21.21	1 547		Calacorene	0.17	–
				–	2.74
21.67	1 577	Sesquiterpenols	Palustrol	–	0.25
21.85	1 590		Spathulenol	0.10	–
22.02	1 603		Caryophyllene epoxide	0.12	0.82
22.05	1 605		Epiglobulol	–	23.06
22.18	1 614		Ledol	–	1.86
22.25	1 618		Rosifoliol	0.16	–
22.34	1 626		Eudesm-7 (11)-en-4-ol	–	–
22.53	1 639		γ-Eudesmol	0.12	0.21
22.59	1 643		α-Cadinol	–	0.37
22.66	1 648		β-Eudesmol	–	0.42
22.88	1 664		Bulnesol	–	0.87
22.92	1 666		Viridiflorol	–	–
23.29	1 693		Bergamotol, Z-α-trans	–	–
				–	27.50
Total				99.37	99.47

CV: *C. viminalis*; ML: *M. leucadendron*; –: Mean trace.

4. Discussion

The comparative analysis of extraction results showed that the leaves of *C. viminalis* were much richer in essential oils than *M. leucadendron* ones. The extraction percentage (3.92%) obtained for *C. viminalis* was also very high compared to those in the literature that is between 0.85% and 0.90% [12,13]. However, the essential oil obtained from *M. leucadendron* is comparable to that found (1.20%) for the leaves of the Egyptian species picked in February 2001 [14]. The physical properties of the two myrtaceae showed differences in densities, as well as in the level of coloration, light-green for the *M. leucadendron* essential oil while that of *C. viminalis* was light yellow.

In the essential oil of *C. viminalis*, 34 compounds which represent 99.37% of the whole were identified. The major compounds were: 1.8-cineole (58.12%), limonene (9.72%), α-terpineol (9.56%), geranial (6.02%), δ-elemene (3.53%), myrcene (2.96%) and α-pinene (2.49%). The concentrations of the other constituents were less than 1%. These results are near to those obtained for the essential oil of *C. viminalis* studied in

Cameroon and Madagascar [12–15]. The major compounds are the same with a slight difference in their contents of α-pinene. The Madagascar *C. viminalis* essential oil has very high levels of α-pinene (28.70%), compared to that of Dakar (2.49%) and Cameroon (0.38%). Actually, something very interesting was noticed about the chemical composition of these essential oils; the species of Dakar showed the presence of geranial and δ-elemene with significant average grades 6.02% and 3.53%, respectively, while these two components haven't been identified in oil of *C. viminalis* from Cameroon and Madagascar. However, the essential oils of the Cameroonian and Madagascar species showed the presence of δ-3-carene (8.6%) and *p*-cymene (8.46%), respectively.

The apportion of the *C. viminalis* essential oil chemical compound families studied shows a predominance of monoterpene oxides represented by 1.8-cineole (58.12%), followed hydrogenated monoterpenes (17.05%) and monoterpene alcohols (11.11%) (Table 1).

For the essential oil of *M. leucadendron*, 43 compounds representing 99.47% of the whole were identified. It is mainly composed of 1.8-cineole (28.87%), epiglobulol (23.06%), α-pinene (12.22%), limonene (11.65%), α-terpineol (7.06%), β-pinene (3.85%), ledol (globulol) (1.86%) and α-gurjunene (1.38%). This biochemical profile obtained for *Melaleuca* oil is comparable with the Cairene species with the absence of the epiglobulol [14]. However, a difference between the major constituents of the essential oils was quantitatively observed. The essential oil of *M. leucadendron* collected in Egypt is much richer in 1.8-cineole (64.30% against 28.87%), and also α-terpineol (11.02% against 7.06%). Conversely, the essential oil of the species from Dakar showed higher levels of α-pinene, limonene and β-pinene with respective averages of 12.22%, 11.65% and 3.85% against 4.24%, 6.70% and 1.67% for the chemotype of Cairo. Epiglobulol (23.06%) and α-gurjunene (1.38%) found in *M. leucadendron* essential oil of Dakar were not reported with the chemotype of Cairo.

Unlike the essential *C. viminalis* oil, that of *M. leucadendron* showed a predominance of hydrogenated monoterpenes (31.12%), followed by monoterpene oxides (28.87%) and sesquiterpene alcohols or sesquiterpenols (27.50%) (Table 1). Other chemical families such as monoterpene alcohols (8.36%) and sesquiterpenes (2.74%), are also present in the essential oil of *Melaleuca*.

Both myrtaceae have shown chemical compositions with interesting majority compounds, such as 1.8-cineole, limonene, α-terpineol and α-pinene with insecticidal properties have already been demonstrated. Indeed, the insecticidal properties of 1.8-cineole have been demonstrated against several insects [16]. Similarly, a study on its biological activity against stored foodstuffs weevils demonstrated the repellent effect of this constituent against *Sitophilus granarius* and *Sitophilus zeamais* [16]. In addition, several studies were conducted on herbs for insecticidal effects against the maize weevil. And in most of these plants, it is noted the presence of eucalyptol (1.8-cineol) or α-pinene or limonene in the chemical composition of the essential oils from the aerial parts of the plants [17,18]. In fact, the insecticidal properties of α-pinene against several insects, have also been demonstrated, and in other cases against *Tribolium confusum* [19]. Similar effects were also noted with α-terpineol [16–20]. Moreover, the kind *Callistemon* in general is traditionally used as an insecticide and also to treat bronchitis [21]. The insecticidal properties of isolated chromenes of the hexane extract of the stems and leaves of *C. viminalis* have been demonstrated against *Musca domestica*, *Aphis fabae* and *Thrips tabaci* [22]. In addition, antibacterial and anthelmintic properties of the leaves essential oil have been demonstrated [23]. On the other hand, *M. leucadendron* more commonly known as niaouli, with its aromatic oil has medicinal properties according to several researchers [24,25]. It is better known as the tea tree or honey myrtle. *M. leucadendron* is used as an insecticide and insect repellent, a powerful general antiseptic (pulmonary, intestinal and urinary), a stimulating and invigorating product [25]. The essential oil of *Melaleuca* genus is used to treat respiratory tract infections and relieve pain, including toothache [26].

Facing these remarkable qualities noted for both myrtaceae, we can hypothesize that the biological tests planned will allow us to achieve good results that we can later exploit in many areas of life, including medicine, to protect stocks for insect's attack, *etc.*

Essential oils are composed of chemical molecules that determine their properties, fields of action, toxicities and use precautions. It is therefore essential to characterize the essential oils for their better development. The chromatographic analysis of the studied essential oil (*M. leucadendron* and *C. viminalis*) reveals a different chromatographic profile between both the raw essential oils. Quantitative variations among identical compounds detected have also been observed. Based on the properties of these chemical families of compounds, essential oils from these plants could have a strong insecticidal activity. Considering the properties of the identified major compounds, essential oils of both studied myrtaceae have enormous potential in various fields including food, pharmaceutical and cosmetic industry.

Conflict of interest statement

We declare that we have no conflict of interest.

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