Asian Journal of Applied Chemistry Research

5(2): 46-52, 2020; Article no.AJACR.56791
ISSN: 2582-0273

Chemical Composition and Antibacterial Activity of Essential Oil of *Melaleuca quinquenervia* (Cav.) S.T. Blake (Myrtaceae)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJACR/2020/v5i230132
Editor(s):
(1) Dr. Angélica Machi Lazarin, State University of Maringá, Brazil.
Reviewers:
(1) Khairul Anuar Mat Amin, Universiti Malaysia Terengganu, Malaysia.
(2) Alice Sato, Universidade Federal do Estado do Rio de Janeiro, Brazil.
(3) Aline Pereira Pedrosa, Federal University of Rio de Janeiro Rio de Janeiro, Brazil.
Complete Peer review History: http://www.sdiarticle4.com/review-history/56791

Original Research Article

Received 20 February 2020
Accepted 27 April 2020
Published 28 April 2020

ABSTRACT

For the first time, this study aimed to determine the chemical composition and the antibacterial activity of *Melaleuca quinquenervia* leaf essential oils from Senegal. Ten samples of leaves from *M. quinquenervia* were collected on two Senegal localities. The essential oils were obtained by hydrodistillation and analyzed by GC/FID and GC/MS. The oil yields ofdried leaves ranged 1.65 to 3.74%. Oilsamples were dominated by 1,8–cineole (24.6–49.3%), viridiflorol (14.9–35.7%), α–terpineol (6.3–12.7%), α–pinene (5.0–11.5%) and limonene (3.7–7.3%). The antibacterial

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activity was evaluated using a paper disc diffusion method. The essential oil exhibited excellent activity against *S. aureus*, moderate activity against *E. coli* and *E. faecalis* and no activity against *P. aeruginosa*.

Keywords: Melaleuca quinquenervia; essential oils; antibacterial activity and GC–SM.

1. INTRODUCTION

The genus *Melaleuca* belongs to the large Australian plant family Myrtaceae. In Australia there are about 200 *Melaleuca* species, which are mainly trees oftemperate to subtropical zones. Among this genus, some species areharvested for their essential oils, including *Melaleuca quinquenervia*, commonly called "niaouli". The *M. quinquenervia* leaves are considered to be a source of 1,8–cineole-rich essentialoil, called Niaouli oil, which is used in pharmaceutical preparations forthe relief from cough and colds, rheumatism, and neuralgia as well as inaromatherapy [1–4].

In recent years, some studies have investigated about chemical composition and bioactivities of *M. quinquenervia* essential oils. These oils have showngreat variability in composition according different geographic locations. Monoterpenoids (1,8–cineole, *p*–cymene and α–terpineol), and sesquiterpenoids ((*E*)–nerolidol, viridiflorol, β–caryophyllene and longifolene) were reported as the mainconstituents of most essential oil samples from *M. quinquenervia*. Most of the previous reports on essential oil compositions from this species concerning plants growing in Australia [3], New Caledonia [2,3], Madagascar [5], Benin[6], Egypt [7], Mauritius [8], Cuba [9,10], USA [11], Brazil [12], India [13] and Pakistan [14].

In Senegal, *M. quinquenervia* has been extensively planted to avoidthe phenomenon of soil salinization [15,16] and no study on the chemical composition of the oil has been carried out. Thus, the aim of this study was to characterize the chemical composition and the antibacterial activity of the essential oils from leaves of *M. quinquenervia* harvested in the areas of Tanma Lakeand Mbao Forest, two localities in Senegal.

2. MATERIALS AND METHODS

2.1Plant Material

Ten samples of *M. quinquenervia* fresh leaves were collected from two localities from Senegal: Tanma Lake (14°55'39.068" N, 17°4'15.099" O, five samples: 1-5) and Mbao Forest (14°45'31.039" N, 17°20'6.928" O, five samples: 6-10). Each leaf of one sample was harvested on the same tree. The plant material was identified by the technicians from the department of Botanical of the Fundamental Institute of Black Africa (IFAN) of Cheikh Anta Diop University of Dakar (Senegal).

2.2 Essential Oil Isolation

Plant material were 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 [17]. The yields of essential oils (w/w, calculated on dry weight basis) were given in the Table 1.

2.3 GC and GC/MS Analysis

The chromatographic analyses were carried out using a Perkin-Elmer Autosystem XL GC apparatus (Walthon, MA, USA) equipped with dual flame ionization 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). The 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) [18] 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-Elmer Autosystem XL, equipped with fused-silica capillary columns Rtx-1 and Rtx-Wax. The 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; ionization 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 [19–21] and comparison of spectra with those of our personal library; and (c) 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): two Gram-negative (*Escherichiacoli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and two Gram-positive (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212). All the strains were grown on Mueller–Hinton agar for the bacteria.

2.5 Determination of Antibacterial Activity

Antibacterial activity of the essential oil from sample 1 was evaluatedusing the agar disc diffusion method [22]. 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 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 – 24h 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.

3. RESULTS AND DISCUSSION

3.1 Chemical Composition of Essential Oils

The essential oil yields calculated in relation to the mass of dry vegetable matter were between 1.65–2.72% for samples collected in Tanma Lake and between 2.22–3.74% for those harvested at Mbao Forest. This difference in essentialoil yields may be due to the nature of the soil. During sampling, we found that the Mbao Forest soil was sandy and wet, while the Tanma Lake soil was hard and dry. In addition, Tanma Lake is a salt zone.

The analysis of the leaf essential oils by GC/FID and GC/MS allowed the identification of 32 compounds accounting for 97.8 to 99.5% of the total compositions (Table 1). All essential oils consisted mainly of 1,8–cineole 10 (24.6–49.3%), viridiflorol25 (14.9–35.7%), α–terpineol 17(6.3–12.7%), α–pinene2 (5.0–11.5%) and limonene 9(3.4–7.3%). These results show an almost homogenous chemotype on the two harvest zones.

This chemotype has been described in the literature. A study in southeastern Australia reported 1,8–cineole (10.0–75.0%), viridiflorol (13.0–66.0%), α–terpineol (0.5–14.0%) and β–caryophyllene (0.5–28.0%) as predominant compounds [3]. A study conducted in Florida showed the chemotype 1,8–cineole (28.6%), viridiflorol (26.3%), α–terpineol (8.7%), limonene (6.7%) and β–caryophyllene (6.5%) [11]. In Cuba, the essential oil consisted mainly of 1,8–cineole (28.8%), viridiflorol (25.3%), limonene (13.6%) and α–pinene (8.7%) [9].

In previously study on the essential oil composition of *Melaleuca leucadendron* from west Senegal (Dakar), it was reported to contain 1,8–cineole (28.87%), epiglobulol (23.6%), α–pinene (12.22%), limonene (11.65%) and α-terpineol (7.06%) as the major constituents [23]. However, the essential oil of *M. leucadendra* is characterized by a particularly high abundance of Methyl eugenol [12,24]. Until the taxonomic revision of the *Melaleuca leucadendra* complex by blake (who referred to it as *M leucadendron*), the name has been used in a very broad sense to describe any one of many closely related broad-leaved *Melaleuca* [25].

Table 1. Chemical composition of the leaf essential oils from *M. quinquenervia*

					LAC TANMA					MBAO FOREST				
N[a]	**Compounds**	**IRIa**[b]	**RI*a***[c]	**RI*p***[d]	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
1	α-Thujene	932	922	1023	-	-	-	-	-	0.1	0,1	0.1	0.1	-
2	α-Pinene	936	931	1015	6.6	5.0	8.0	11.5	7.5	7.1	6.5	7.2	5.7	**6.6**
3	Camphene	950	944	1059	0.1	-	-	0.2	0.2	-	-	0.1	-	0.1
4	β-Pinene	978	970	1108	2.3	1.7	1.1	2.5	1.9	2.0	0.3	2.6	2.3	1.8
5	Myrcene	987	982	1154	0.2	0.1	0.1	0.1	-	0.4	0.8	0.6	0.7	0.3
6	α-Phellandrene	1002	996	1164	-	-	-	-	-	-	0.2	0.1	0.1	-
7	α-Terpinene	1013	1010	1174	0.1	-	-	-	-	0.2	0.4	0.3	0.1	0.1
8	p-Cymene	1015	1013	1264	0.7	0.5	0.8	1.2	0.8	0.1	0.7	0.2	0.3	0.5
9	Limonene	1025	1021	1200	6.7	6.0	3.4	6.5	7.3	5.8	5.9	7.3	7.0	7.0
10	1,8-Cineole	1024	1021	1209	30.6	34.3	41.6	31.9	28.7	24.6	36.1	28.3	49.3	40.3
11	Trans-β-Ocimene	1041	1040	1243	-	-	-	-	-	0.1	0.1	0.2	0.1	0.1
12	γ-Terpinene	1051	1048	1239	0.5	0.3	-	0.2	0.5	0.6	0.7	0.8	0.6	0.6
13	Terpinolene	1082	1080	1278	0.2	-	-	-	0.1	0.3	0.2	0.4	0.2	0.2
14	Linalool	1086	1086	1533	0.1	0.1	-	0.1	0.1	0.2	0.6	0.5	0.1	0.3
15	Pinocarvone	1137	1137	1558	-	-	0.3	0.4	0.1	-	-	-	-	-
16	Terpinen-4-ol	1164	1163	1590	0.7	0.4	0.2	0.4	0.7	0.7	0.9	0.8	0.6	0.8
17	α-Terpineol	1176	1174	1684	8.3	9.0	6.3	6.6	8.3	6.9	9.3	7.9	12.7	10.3
18	β-Caryophyllene	1421	1435	1591	0.2	0.2	-	0.1	0.2	0.2	0.5	0.3	0.1	0.1
19	α-Humulene	1455	1450	1660	0.1	-	-	-	0.1	-	0.3	0.1	-	-
20	β-Selinene	1486	1482	1712	0.1	0.1	-	0.1	0.1	-	-	-	-	-
21	Ledene	1491	1490	1695	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1	-	0.1
22	Palustrol	1569	1560	1920	0.3	0.4	0.3	0.4	0.5	0.2	-	0.3	0.1	0.2
23	Caryophyllene oxyde	1570	1568	1959	1.7	2.6	2.1	2.5	2.5	0.6	0.1	0.7	0.6	0.7
24	Globulol	1589	1575	2074	0.4	0.5	0.4	0.4	0.5	0.4	0.1	0.3	0.2	0.3
25	Viridiflorol	1592	1591	2089	31.3	33.8	29.9	27.8	33.0	35.7	28.7	33.0	14.9	24.5
26	Guaiol	1593	1591	2090	0.4	0.7	0.4	0.7	0.7	0.2	0.2	0.2	0.1	0.2
27	Ledol	1600	1596	2029	2.0	2.2	2.0	1.8	2.2	2.0	1.8	2.9	0.9	1.5
28	γ-Eudesmol	1618	1617	2197	0.4	0.5	0.4	0.5	0.6	0.2	0.4	0.1	0.1	0.2
29	τ-Cadinol	1633	1632	2169	0.8	0.8	0.4	0.5	0.8	1.0	-	0.4	0.2	0.5
30	Hinesol	1632	1637	2201	1.5	0.2	1.7	0.6	-	3.2	1.1	1.3	1.1	0.8
31	Pogostol	1647	1645	2225	1.5	-	-	1.3	1.6	3.5	1.4	1.3	1.0	0.9

32	Bulnesol	1665	1651	2204	0.4	-	-	-	-	1.4	1.7	0.5	0.3	0.2
	Hydrocarbon monoterpenes				17,4	13.6	13.4	22.2	18.3	16.7	15.9	19.9	17.2	17.3
	Oxygenated monoterpenes				39.7	43.8	48.4	39.4	37.9	32.4	46.9	37.5	62.7	51.7
	Hydrocarbon sesquiterpenes				0,5	0.4	0.1	0.3	0.6	0.3	0.9	0.5	0.1	0.2
	Oxygenated sesquiterpenes				40.7	41.7	37.6	36.5	42.4	48.4	35.5	41.0	19.5	30.0
	Total identified (%)				98.3	99.5	99.5	98.4	99.2	97.8	99.2	98.9	99.5	99.2
	Yields (w/w vs dry material)				2.62	1.65	2.15	2.23	2.72	3.74	2.22	2.96	3.74	2.86

[a] *Order of elution is given on apolar column (Rtx-1).*
[b] *Retention indices of literature on the apolar column (IRIa) [26].*
[c] *Retention indices on the apolar Rtx-1 column (RIa).*
[d] *Retention indices on the polar Rtx-Wax column (RIp).*

Table 2. Antimicrobial activity of the essential oil from *M. quinquenervia*

Microorganisms	Inhibition zone (mm)	
	Essential oil (20 µL/disc)	Chloramphenicol (30 µg/disc)
E. coli ATCC 25922	11.4±0.8	30.2±1.2
S. aureus ATCC 29213	16.1±0.6	29.6±0.9
E. faecalis ATCC 29212	10.7±0.5	28.5±1.6
P. aeruginosa ATCC 27853	7.2±0.3	14.3±0.9

3.2 Antibacterial Activity

The essential oil from sample 1 was used to assessthe antibacterial activity. Antibacterial screening of the essential oil was carried outby the 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 µg/disc) (Table 2).

The essential oil of *M. quinquenervia* exhibited excellent activity against *S. aureus* (IZ =16.1±0.6), moderate activity *against E. coli* and *E. faecalis* (IZ = 11.4±0.8 and IZ = 10.7±0.5, respectively) and no activity against *P. aeruginosa*.

4. CONCLUSION

This study reported for the first time the chemical composition and the antibacterial activity of essential oils of *M. quinquenervia* from Senegal. The oil samples were dominated by 1,8−cineole, viridiflorol, α-terpineol, α-pinene and limonene. The essential oil has shownanexcellent activity against *S. aureus*and moderate activity against *E. coli* and *E. faecalis*. These results may have potential applications in pharmaceutical products.

ACKNOWLEDGEMENTS

We thank the Embassy of France in Senegal and the Territorial Authority of Corsica for their financial support.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sdiarticle4.com/review-history/56791