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Chemical composition of *Cinnamosma madagascariensis* (Cannelaceae) essential oil and its larvicidal potential against the filariasis vector *Culex quinquefasciatus* Say

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ABSTRACT

Madagascar flora is diverse and unique. *Cinnamosma madagascariensis* is an endemic species widely present in the forests of Madagascar. This plant has important traditional uses ranging from management of dementia, epilepsy, headache to malaria. Few data have been reported about the chemical composition of the essential oil, and no studies have been published on its bioactivity against mosquitoes. Here, we focus on the chemical composition of essential oils extracted from *C. madagascariensis* stem bark and leaves, and their larvicidal potential against the filariasis vector *Culex quinquefasciatus*. GC-MS analysis revealed differences between the chemical volatile profiles of leaves and bark oils. In the former, linalool (30.1%), limonene (12.0%), myrcene (8.9%) and α-pinene (8.4%) were the major constituents, while in the latter β-pinene (33.3%), α-pinene (19.3%) and limonene (12.0%) were the most representative compounds. Acute toxicity experiments conducted on larvae of the filariasis vector *C. quinquefasciatus* led to LC_{50} of 61.6 μL L^{-1} and 80.1 μL L^{-1} for the bark and the leaf essential oils, respectively. Overall, the chance to use compounds from the *C. madagascariensis* bark and leaf essential oils against filariasis vectors seems promising, since they are effective at moderate doses and could be an advantageous alternative to build newer and safer mosquito control tools. To the best of our knowledge, this is the first report about the chemical composition of *C. madagascariensis* essential oils.

1. Introduction

Madagascar has been singled out by the international scientific and conservation community as one of the richest countries in the world in terms of biodiversity, endemism and range of habitats. Its flora is diverse and unique. Of approximately 10,000 native higher plant species, about 8000 species are thought to be endemic to the island (Du Puy and Moat, 1998). *Cinnamosma madagascariensis* is one of the three species included in the genus *Cinnamosma* (Cannelaceae family) that encompasses trees endemic to forests of Madagascar (Perrier de la Bathie, 1954). *C. madagascariensis* is known under different Malagasy vernacular names: '*mandravasarotra*' (mandrava = destroy, *sarotra* = hard, difficulties), '*fanalamangidy*' (fanala = action of removing, *mangidy* = bitter, negative things) and '*hazontromba*' (tree for spirit possession). In fact, the plant has several traditional uses. Leaves are used in fumigation during the ritual ceremony of spirit possession called '*tromba*' or to expel bad spirits when a person is supposed to be possessed by evil spirits or for the management of brain disorders such as dementia (*kasoa*), epilepsy and headaches (Beaujard, 1988; Rakotobe et al., 1993). Other uses of *C. madagascariensis* concern treatment of dental decay, malaria and complications after childbirth (Razafindraibe et al., 2013). Interestingly, *Cinnamosma* species are also used against gastrointestinal, respiratory and parasitic infections (Randrianarivelo et al., 2009).

Lymphatic filariasis, commonly known as elephantiasis, is a neglected tropical disease. Its successful prevention and control is a challenge of noteworthy importance in modern parasitology (Benelli, 2015a). More than 1.4 billion people in 73 countries are living in areas where lymphatic filariasis is transmitted and are at risk of being infected. Globally, an estimated 25 million humans suffer with genital disease and over 15 million people are afflicted with lymphedema (WHO, 2014). Eliminating lymphatic filariasis can prevent unnecessary

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Table 1
Chemical composition of the essential oils from leaves and stem bark of *Cinnamosma madagascariensis*.

N.	Component[a]	RI Exp.[b]	RI Lit.[c]	Percentage (%) [d]		ID[e,f]
				Leaves	Stem bark	
1	α-Thujene	920	924	0.1	1.0	RI, MS
2	α-Pinene	925	938	8.4	19.3	Std
3	Camphene	938	946	0.2	0.3	Std
4	Verbenene	944	961	0.1		RI, MS
5	Sabinene	965	969	0.8	0.1	RI, MS
6	β-pinene	967	974	7.1	33.3	Std
7	Myrcene	988	988	8.9	0.6	Std
8	*p*-Mentha-1(7),8-diene	1001	1003	0.4		RI, MS
9	α-Phellandrene	1002	1002		0.2	Std
10	α-Terpinene	1013	1014	0.1	0.6	RI, MS
11	*p*-Cymene	1021	1020	0.6	3.0	Std
12	Limonene	1024	1024	12.0	12.0	Std
13	1,8-Cineole	1026	1026	1.5	0.4	Std
14	(E)-β-ocimene	1046	1044	1.0		RI, MS
15	γ-Terpinene	1054	1054	0.2	0.8	Std
16	*cis*-Linalool oxide	1069	1067	0.2		RI, MS
17	Terpinolene	1084	1086	0.1	0.6	Std
18	*trans*-Linalool oxide	1085	1084	0.2		RI, MS
19	Linalool	1102	1095	30.1	6.6	Std
20	Hotrienol	1104		0.6		RI, MS
21	α-Campholenal	1122	1122	0.1		RI, MS
22	*trans*-Pinocarveol	1131	1135	0.5		Std
23	Pinocarvone	1156	1160	0.3		RI, MS
24	*p*-Mentha-1,5-dien-8-ol	1164	1166	0.1		RI, MS
25	Terpinen-4-ol	1171	1174	0.7	0.8	Std
26	Cryptone	1184	1183	0.1		RI, MS
27	α-Terpineol	1186	1186	0.5	0.2	Std
28	Myrtenal	1189	1195	0.3		Std
29	Myrtenol	1191	1194	0.3		Std
30	Verbenone	1204	1204	0.1		Std
31	Thymol, methyl ether	1229	1232		0.1	RI, MS
32	Carvacrol, methyl ether	1242	1241		0.1	RI, MS
33	Lynalyl acetate	1256	1254	0.1		RI, MS
34	Isobornyl acetate	1279	1287	0.1	0.2	Std
35	Myrtenyl acetate	1319	1324		tr	RI, MS
36	α-Cubebene	1340	1345	0.2	0.2	RI, MS
37	α-Terpinyl acetate	1344	1346	0.1	tr	RI, MS
38	α-ylangene	1351	1373		0.3	RI, MS
39	α-Copaene	1364	1374	1.1	2.7	RI, MS
40	β-Elemene	1382	1389	0.3	0.4	RI, MS
41	(E)-Caryophyllene	1404	1417	3.1	3.3	Std
42	α-*trans*-Bergamotene	1426	1432	0.1	0.5	RI, MS
43	β-Copaene	1429	1430	0.1		RI, MS
44	α-Humulene	1442	1452	2.4	3.5	Std
45	*allo*-Aromadendrene	1445	1439	0.1	0.1	RI, MS
46	4,5-di-epi-aristolochene	1455	1471		0.2	RI, MS
47	*trans*-Cadina-1(6),4-diene	1461	1475	0.1		RI, MS
48	γ-Muurolene	1464	1478		0.5	RI,MS
49	Germacrene D	1468	1484	1.2	0.4	RI, MS
50	β-Selinene	1480	1489	0.2	0.2	RI, MS
51	*ar*-Curcumene	1474	1479	0.2	0.1	RI,MS
52	*trans*-Muurola-4(14),5-diene	1476	1493	0.3	0.2	RI, MS
53	α-Selinene	1480	1498		0.4	RI, MS
54	epi-Cubebol	1481	1493	0.2		RI, MS
55	α-Zingiberene	1487	1493	0.4		RI, MS
56	α-Muurolene	1488	1500		0.4	RI, MS
57	Viridiflorene	1492	1496		0.3	RI, MS
58	α-Bulnesene	1502	1509	0.1	0.3	RI, MS
59	*trans*-Calamanene	1518	1521	0.7	0.4	RI, MS
60	δ-Cadinene	1519	1522	1.4	1.6	RI, MS
61	*trans*-Cadina-1,4-diene	1529	1533	0.1	0.1	RI, MS
62	Liguloxide	1533	1534		0.2	RI, MS
63	α-Calacorene	1538	1544	0.5	0.4	RI, MS
64	Hedycaryol	1542	1546	0.5		RI, MS
65	β-Calacorene	1562	1564	0.1		RI, MS
66	Caryophyllene oxide	1579	1582	4.2	0.8	Std
67	Viridiflorol	1587	1592	0.3	0.1	Std
68	Humulene epoxide II	1603	1608	1.4	0.5	RI, MS
69	1,10-di-epi-Cubenol	1615	1618		0.3	RI, MS
70	Muurola-4,10(14)-dien-1-β-ol	1626	1630	1.3		RI, MS
71	epi-α-Cadinol	1635	1638		0.1	RI, MS
72	Caryophylla-4(12),8(13)-dien-5--ol[g]	1637	1639	0.3		RI, MS
73	β-Eudesmol	1645	1651	1.0	0.4	RI, MS

Table 1 (*continued*)

N.	Component[a]	RI Exp.[b]	RI Lit.[c]	Percentage (%) [d]		ID[e,f]
				Leaves	Stem bark	
74	α-Eudesmol	1648	1652	0.3	0.2	RI, MS
75	α-Cadinol	1649	1652	0.1		RI, MS
76	Cadalene	1672	1675	0.1		RI, MS
	Total identified (%)			97.9	99.2	
	Grouped compounds					
	Monoterpene hydrocarbons			40.0	71.9	
	Oxygenated monoterpenes			35.7	8.4	
	Sesquiterpene hydrocarbons			12.6	16.3	
	Oxygenated sesquiterpenes			9.7	2.6	

[a] Compounds are listed in order of their elution from a HP-5MS column.
[b] Linear retention index on HP-5MS column, experimentally determined using homologous series of C8-C30 alkanes.
[c] Linear retention index taken from Adams (2007).
[d] Percentage values are means of three determinations, with a RSD% in all cases below 10%.
[e] Identification methods: std., based on comparison with authentic compounds; MS, based on comparison with WILEY, ADAMS, FFNSC2 and NIST 08 MS databases; RI, based on comparison of LRI with those reported in ADAMS, FFNSC 2 and NIST 08.
[f] tr, % below 0.1%.
[g] Correct isomer not identified.

suffering and contribute to the reduction of poverty. Lymphatic filariasis is caused by Filariodidea nematodes, namely *Wuchereria bancrofti*, which is responsible for 90% of cases, *Brugia malayi* and *B. timori*. Microfilariae are transmitted to humans by different mosquitoes.

Culex species, with special reference to *Culex quinquefasciatus* Say, are the most common vectors across urban and semi-urban areas of Asia (Chadee et al., 2002). To deal with this important plague, WHO has launched its "Global Programme to Eliminate Lymphatic Filariasis" in 2000. In 2012, the WHO neglected tropical diseases roadmap reconfirmed the target date for achieving elimination by 2020. In this framework, besides preventive chemotherapy and morbidity management, vector control in select settings contributed to the elimination of lymphatic filariasis, as well as to the elimination of arboviruses, in the absence of large-scale preventive chemotherapy (WHO, 2014; Benelli and Mehlhorn, 2016; Benelli et al., 2016).

More generally, the recent outbreaks of mosquito-borne diseases highlighted the pivotal importance of mosquito vector control in tropical and subtropical areas worldwide, as well as emerging alerts in other parts (Benelli, 2016a, 2016b, 2016c; Nicoletti et al., 2016). However, mosquito control is facing a number of important and timely challenges, mainly due to the rapid development of pesticide resistance (Naqqash et al., 2016) and the limited success of biocontrol programs on Culicidae (Benelli and Mehlhorn, 2016; Benelli, 2016d). In this framework, screening botanicals for their mosquitocidal and repellent activity may offer effective and eco-friendly tools in the fight against mosquitoes (see Benelli, 2015b; Pavela, 2015a; Pavela and Benelli, 2016 for reviews).

Despite the several uses as a traditional remedy, *C. madagascariensis* has been poorly explored for secondary metabolites and their biological activity (Vecchietti et al., 1979; Harinantenaina et al., 2008). Therefore, continuing our studies on Malagasy aromatic plants with reported traditional uses (Nicoletti et al., 2012; Maggi et al., 2013; Rakotosaona et al., 2015; Randrianarivo et al., 2016), we herein focus on the chemical composition of essential oils extracted from *C. madagascariensis* stem bark and leaves, and their larvicidal potential against the filariasis vector *C. quinquefasciatus*.

2. Materials and methods

2.1. Plant material

Fresh leaves and stem barks of *C. madagascariensis* Danguy were collected in November 2014 at Ambohitantely-Ankazobe, central highlands of Madagascar. Botanical identification was made at the Department of Botany of the Parc Botanique et Zoologique de Tsimbazaza, Antananarivo. A voucher specimen has been kept at the herbarium of the Institut Malgache de Recherches Appliquées under the accession code MAD-1059.

2.2. Distillation of essential oils

Ten kilograms of leaves and 5 kg of stem barks were separately extracted by steam distillation using a portable alembic for 4 h. A total of 27.5 mL of essential oils were obtained from the leaves and 6.7 mL from the stem barks.

2.3. GC-MS analysis

Chemical analysis of *C. madagascariensis* essential oils was achieved on an Agilent 6890 N gas chromatograph coupled to a 5973 N mass spectrometer, using an HP-5 MS (5% phenylmethylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 μm film thickness; J&W Scientific, Folsom) capillary column. The temperature programme was the following: 5 min at 60 °C, subsequently 4 °C/min up to 220 °C, then 11 °C/min up to 280 °C, held for 15 min. Injector and detector temperatures were set to 280 °C. The carrier gas was He, with a flow rate of 1 mL/min. The split ratio employed was 1:50. Mass spectra were acquired (*m*/*z* 29–400) in electron-impact (EI) mode with an ionization voltage of 70 eV. Before injection, the essential oils were diluted 1:100 in *n*-hexane, and then 2 μL of the solution were injected into GC-MS system. The main oil constituents were identified by co-injection with authentic standards available in the laboratory. Moreover, the identification was carried out by interactive combination of chromatographic linear retention indices and MS data that were consistent with those reported in commercial libraries (Adams, 2007; NIST08, 2008; FFNSC2, 2012). For each sample, the analysis was repeated three times and the relative percentage average values were reported. Quantification of volatile components was achieved by peak-area internal normalisation without using correction factors.

2.4. Mosquito rearing

C. quinquefasciatus was reared in the laboratory as described by Pavela (2015b). The larvae were fed on dog biscuits and yeast powder in the ratio 3:1. Adults were provided with a 10% sucrose solution (w:v) and a 1-week-old chick for blood feeding. Early fourth instar larvae were used in the study. All the tested insects were treated and maintained at a temperature of 25 ± 1 °C, 50%–70% R.H. and 16:8 photoperiod (L:D). All experiments were performed under the same conditions.

Table 2
Acute toxicity of *Cinnamosma madagascariensis* bark and leaf essential oil against fourth instar larvae of the filariasis vector *Culex quinquefasciatus* Say.

Cinnamosma madagascariensis	LC_{50} (μL L^{-1})	LCI-UCI	LC_{90} (μL L^{-1})	LCI-UCI	χ^2
Bark essential oil	61.6	53.7–69.9	158.9	125.3–189.7	0.021 n.s.
Leaf essential oil	80.1	75.3–84.1	112.3	104.1–127.2	4.379 n.s.

LC_{50} = lethal concentration that kills 50% of the exposed organisms.
LC_{90} = lethal concentration that kills 90% of the exposed organisms.
LCI = 95% lower confidence interval.
UCI = 95% upper confidence interval.
χ^2 = chi square.
n.s. = not significant ($\alpha = 0.05$).

2.5. Larvicidal potential

Mosquito larvicidal trials were carried out according to WHO (1996) standard procedures, with slight modifications (Pavela, 2015b). The *C. madagascariensis* essential oils were diluted in dimethyl sulfoxide in order to prepare a serial dilution of the test dosage. For experimental treatment, 1 ml of serial dilution was added to 224 ml of distilled water in a 500-ml glass bowl and shaken gently to produce a homogeneous test solution. The *C. quinquefasciatus* larvae were transferred in water into a bowl of the prepared test solution, with final surface area of 125 cm^2 (25 larvae/beaker). Four duplicate trials were carried out for every sample concentration, and for each trial, a negative control was included using distilled water containing the same amount of dimethyl sulfoxide as the test sample. A different series of concentrations (resulting from the previous screening) was used for each essential oil in order to obtain mortality ranging between 10% and 90%. At least five concentrations were selected for the calculation of lethal doses. Mortality was determined after 24 h of exposure, during which no food was offered to the larvae.

2.6. Data analysis

In acute toxicity experiments, LC_{50}, LC_{90}, regression equation, 95% confidence limits (CI95) and chi square values were calculated using probit analysis (Finney, 1971).

3. Results and discussion

The chemical compositions of essential oils from leaves and bark of *C. madagascariensis* are reported in Table 1. A total of 76 volatile components were identified (63 in leaf essential oil, 51 in bark essential oil), corresponding to 97.9%–99.2% of the total compositions, respectively. Noteworthy, differences were observed between the chemical volatile profiles of leaves and bark. In the former, the major chemical classes were given by monoterpene hydrocarbons (40.0%) and oxygenated monoterpenes (35.7%), with linalool (30.1%), limonene (12.0%), myrcene (8.9%) and α-pinene (8.4%) as the predominant compounds. In the latter, monoterpene hydrocarbons constituted the major fraction of the oil (71.9%), with β-pinene (33.3%), α-pinene (19.3%) and limonene (12.0%) as the most representative compounds. Interestingly, linalool, which was the major volatile component of leaves, was herein occurring at lower percentages (6.6%) in the bark. Another noteworthy chemical class was given by sesquiterpene hydrocarbons (12.6% in leaves, 16.3% in bark), with (*E*)-caryophyllene (3.1%–3.3%) and α-humulene (2.4%–3.5%) as the most representative compounds. To the best of our knowledge, this study represents the first investigation on *C. madagascariensis* essential oil. Previously, the essential oil from *C. fragrans* was investigated and two main chemotypes (i.e. linalool-type and 1,8-cineole-type) were reported (Randrianarivelo et al., 2010). On this basis, the essential oil of *C. madagascariensis* leaves can be classified into the linalool chemotype, previously reported for this genus (see Randrianarivelo et al., 2010).

Our acute toxicity experiments showed differential larvicidal activity exerted by the *C. madagascariensis* bark and leaf essential oils (Table 2). Indeed, LC_{50} calculated against fourth instar larvae of *C. quinquefasciatus* were 61.6 μL L^{-1} and 80.1 μL L^{-1} for the bark and the leaf essential oils, respectively. The overall activity can be justified by the presence of some monoterpene constituents with documented insecticide power, such as linalool (Lopez et al., 2012), limonene (Hebeish et al., 2008), α- and β-pinene (Michaelakis et al., 2009). In particular, the toxicity of the bark essential oil may be related to its higher content of α-pinene (19.3% vs 8.4%) and β-pinene (33.3% vs 7.1%). These compounds were already reported as highly effective against *C. quinquefasciatus* (Pavela, 2015b; Govindarajan et al., 2016), with special reference to the latter because of the presence of exocyclic double bonds in the molecule structure (Perumalsamy et al., 2009).

In recent years, the indigenous flora of European, African and Asian countries has been extensively screened for insecticidal, repellent and antiplasmodial bioactivities of plant extracts and essential oils (Benelli, 2015a, 2015b; Pavela, 2015a, 2015b; Govindarajan and Benelli, 2016a, 2016b, 2016c; Pavela and Benelli, 2016). However, searching for the mosquitocidal activity of *Cinnamosma* essential oils, we faced a severe shortage of literature. More generally, concerning the larvicidal action of plant essential oils against mosquito vectors of economic importance, Pavela (2015a, 2015b) recently showed that essential oils with $LC_{50} \leq 100$ ppm were obtained from 122 plants. In this scenario, our results are of interest, even though it should also be noted that some of the surveyed species showed a higher larvicidal potential compared to *C. madagascariensis* bark and leaf essential oils (i.e. seven essential oils showed $LC_{50} < 10$ ppm).

4. Conclusions

Overall, the chance to use compounds from the *C. madagascariensis* bark and leaf essential oil against filariasis vectors seems promising, since they are effective at moderate doses and could be an advantageous alternative to build newer and safer mosquito control tools. Further research on the bioactivity of the single constituents of this essential oil on Culicidae vectors (see also Govindarajan and Benelli, 2016d), as well as additional efforts to shed light on the possible mechanism(s) of action against mosquito young instars, are urgently required.

Conflict of Interest

The authors declare no competing interests.

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