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ORIGINAL PAPER

Traditional herbal remedies and dietary spices from Cameroon as novel sources of larvicides against filariasis mosquitoes?

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Abstract In Cameroon, many dietary spices are used by traditional healers to cure several diseases such as cancer and microbial infections. *Aframomum daniellii*, *Dichrostachys cinerea* and *Echinops giganteus* are Cameroonian spices widely used as flavourings and as food additives. Moreover, they are traditionally herbal remedies employed to treat several diseases, as well as to control populations of insect pests. In this research, we analysed the chemical composition of *A. daniellii*, *D. cinerea* and *E. giganteus* essential oils and we evaluated their larvicidal potential against larvae of the filariasis and West Nile virus vector *Culex quinquefasciatus*. The essential oils were obtained from different plant parts by hydrodistillation and their composition was analysed by GC-MS. The three spices exhibited different volatile chemical profiles, being characterized by 1,8-cineole, sabinene and β-pinene (*A. daniellii*), geraniol and terpinen-4-ol (*D. cinerea*), and silphiperfol-6-ene and presilphiperfolan-8-ol (*E. giganteus*). Results showed that the highest larvicidal toxicity on *Cx. quinquefasciatus* was exerted by *D. cinerea* essential oil ($LC_{50} = 39.1$ μL L^{-1}), followed by *A. daniellii* (pericarp essential oil: $LC_{50} = 65.5$ μL L^{-1}; leaves: $LC_{50} =$ 65.5μL L^{-1}; seeds: $LC_{50} = 106.5$μL L^{-1}) and *E. giganteus* ($LC_{50} = 227.4$ μL L^{-1}). Overall, the chance to use the *D. cinerea* essential oil against *Cx. quinquefasciatus* young instars seems promising, since it is effective at moderate doses and could be an advantageous alternative to build newer mosquito control tools.

Keywords *Aframomum daniellii* · *Dichrostachys cinerea* · *Echinops giganteus* · Essential oil · *Culex quinquefasciatus* · Rift Valley fever · West Nile virus

Introduction

Spices and herbs have been used in African countries since ancient times not only to give taste and flavour to foods but also as food preservatives and to prevent and cure diseases (Dzoyem et al. 2014). This aspect has recently attracted the attention of many scientists and encouraged them to conduct screening for biological activities. Hopefully, this will lead to new information on plant applications and new perspective on the potential use of plant-derived products (Mouhssen 2004). In Cameroon, there are many dietary spices that are used by traditional healers to cure several diseases such as cancer and microbial infections (Tekwu et al. 2012; Kuete et al. 2011). Among them, *Aframomum daniellii* (Hook. f.) K. Schum, *Dichrostachys cinerea* (L.) Wight & Arn. and *Echinops giganteus* A. Rich. are commonly known to people, especially in the western region of Cameroon, for their use as flavour ingredients of the

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traditional soup called *Nah poh* (Tchiégang and Mbougueng 2005). However, important uses as herbal medicines are recorded as well.

A. daniellii is a perennial herb, 3–4 m tall, belonging to the Zingiberaceae family and known as 'African cardamom'. Its olive-brown and shiny seeds are used to flavour foodstuffs while its essential oil in perfumery and dye manufacturing (Menut et al. 1991). In the traditional medicine, the seeds are used as laxative and antihelminthic, and the root as purgative (Bouquet 1969). Previously, Odukoya et al. (1999) demonstrated the anti-inflammatory activity of essential oil of *A. daniellii* seeds. Fasoyiro (2007) reported the preservative properties of the petroleum ether and ethanol fractions of *A. danielli* in stored grains of soybean, cowpea and maize against fungal infestation.

D. cinerea [syn. *Dichrostachys glomerata* (Forssk.) Chiov.], belonging to the Mimosaceae family, is a deciduous tree growing in Cameroon and other tropical countries. In Kenya, South Africa and Tanzania, the decoctions of the leaves and roots are used against venereal disease, eye injury, skin rash and pimple, snake bite, wound and as astringent, detoxifying, antalgic and aphrodisiac. The root is used for chest complaints and the twigs for gonorrhoea and syphilis. The smoke of the leaf and the root are used for pulmonary tuberculosis (Deniz 2009).

E. giganteus, belonging to the Asteraceae family, is a branched herb, 60–150 cm tall, with roots that are widely used in the traditional medicine of Cameroon and Nigeria for the treatment of various diseases and illnesses such as heart and gastric troubles, to calm stomach ache, to give carminative help and reduce the effects of alcohol, and reduces asthma attacks (Menut et al. 1997; Tene et al. 2004). In previous studies, the methanolic extract of this plant has been shown to have antibacterial (Fankam et al. 2011), antifungal (Dzoyem et al. 2014) and antioxidant effects (Abdou et al. 2010). The cytotoxicity of crude methanol extract of roots has also been demonstrated (Kuete et al. 2011; Kuete et al. 2013). Overall, these three plant species are traditionally herbal remedies employed to treat several diseases and parasites, as well as to control populations of arthropod pests (Adebayo et al. 2016; Ahua et al. 2007; Karunamoorthi and Hailu 2014).

The recent outbreaks of mosquito-borne diseases (e.g. Zika virus in Brazil and French Polynesia) highlighted the pivotal importance of mosquito vector control in tropical and subtropical areas worldwide (Benelli and Mehlhorn 2016), as well as emerging alerts in other parts (Benelli 2016a, b, c; Nicoletti et al. 2016). *Culex quinquefasciatus* Say (Diptera: Culicidae) is a vector of many pathogens and parasites of humans, and both domestic and wild animals. In the USA, the viruses transmitted by this species include West Nile, St. Louis encephalitis and Western equine encephalitis virus. Outside the USA, *Cx. quinquefasciatus* is a common vector across urban and semi-urban areas of Asia responsible for transmitting filarial nematodes (tropical Africa and Southeast Asia) (Benelli 2015a) and the Rift Valley fever virus (Africa) (Foster and Walker 2002).

Notably, 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 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 *Brugia timori*. Microfilariae are transmitted to humans by different mosquitoes (Chadee et al. 2002). Within the WHO 'Global Programme to eliminate Lymphatic Filariasis', besides preventive chemotherapy and morbidity management, vector control in select settings has been emphasized to boost the elimination of lymphatic filariasis (WHO 2014; Benelli et al. 2016; Pavela et al. 2016).

Mosquito control is facing a number of relevant challenges nowadays, which are mainly due to the rapid development of pesticide resistance (Naqqash et al. 2016) and the spread of invasive mosquito vectors in novel areas worldwide (Benelli and Mehlhorn 2016). Thus, screening botanicals for their mosquitocidal and repellent activity may offer effective and eco-friendly tools in the fight against mosquitoes (Amer and Mehlhorn 2006a, b, c, d; Benelli 2015b; Pavela 2015a; Pavela and Benelli 2016). Therefore, as part of our continuous search for bioactivity of volatile components from Cameroonian spices (Fogang et al. 2012; Fogang et al. 2014; Woguem et al. 2014), in this research we analysed the chemical composition of *A. daniellii*, *D. cinerea* and *E. giganteus* essential oils and we evaluated their larvicidal potential against larvae of the filariasis and West Nile vector *Cx. quinquefasciatus*.

Materials and methods

Plant materials

Fresh fruits of *A. danielli* and *D. cinerea*, and the roots of *E. giganteus* were collected respectively from the villages Bamougoum and Bafoussam's market (Cameroon, Western Region). The peel fruit of *A. danielli* and pericarp of *D. cinerea* were then removed and the seed used; the roots of *E. giganteus* were cleaned with water, washed and sliced into small pieces. These plant parts were dried at room temperature for 1 week (panels a, b, and c of Fig. 1, respectively). The identification of the three plants was made by a taxonomist at the Cameroon National Herbarium (Yaoundé), where voucher specimens were deposited under the following reference numbers: 43130/HNC/Cam for *A. danielli*, 42920/HNC for *D. cinerea* and 23647/SRF-Cam for *E. giganteus*.

Fig. 1 **a** Dry seeds of *A. danielli*. **b** Dry fruits of *D. cinerea*. **c** Dry roots of *E. giganteus*

Extraction of the essential oils

Dried seeds, pericarp and leaves of *A. danielli*, seeds of *D. cinerea*, and roots of *E. giganteus* were ground and hydrodistilled in a Clevenger-type apparatus for 6 h. The collected oils of *A. danielli* and *E. giganteus* were dried over anhydrous sodium sulphate. On the other hand, the essential oil of *D. cinerea* was completely soluble in H_2O after hydrodistillation; thus, the aromatic H_2O collected was extracted with hexane. The hexane fraction was retained and the solvent was then removed with a rotary evaporator at 35 °C. The oil yields were expressed in percentage on a dry weight basis (*w*/*w*); the extracted oils were conserved in dark vials and stored in a refrigerator at 4 °C until used.

GC-MS analysis

Gas chromatographic analysis of essential oils of *A. daniellii*, *D. cinerea* and *E. giganteus* was performed on an Agilent 6890N gas chromatograph equipped with a 5973N mass spectrometer. For separation of volatiles, a HP-5 MS (5 % phenyl methylpolysiloxane, 30 m, 0.25 mm i.d., 0.1 μm film thickness; J & W Scientific, Folsom) capillary column was used with the following temperature programme: 5 min at 60 °C, then 4 °C/min to 220 °C, then 11 °C/min to 280 °C, held for 15 min, for a total run of 65 min. Other conditions are as follows: injector and detector temperatures, 280 °C; carrier gas, He; flow rate, 1 mL/min; split ratio, 1:50; acquisition range, 29–400 *m*/*z* in electron-impact (EI) mode; ionization voltage, 70 eV. The essential oils were diluted 1:100 in *n*-hexane, then 2 μL was injected into GC-MS. For identification of the essential oil components, co-injection with commercial standards was used whenever possible, together with correspondence of retention indices (RIs) and mass spectra (MS) with respect to those reported in commercial libraries (Adams 2007; NIST 08 2008; FFNSC2 2012) and literature (Menut et al. 1997; El-Sayed 2016). Semi-quantification of essential oil components was made by peak area normalization considering the same GC response of the detector towards all volatile constituents.

Mosquito rearing

Cx. 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 chicken 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.

Larvicidal toxicity

Mosquito larvicidal trials were carried out according to WHO (1996) standard procedures, with slight modifications (Pavela 2015b). The *A. daniellii*, *D. cinerea* and *E. giganteus* 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 *Cx. 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.

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).

Results and discussion

Chemical composition of the essential oils

The volatile components identified in the essential oils of *A. daniellii*, *D. cinerea* and *E. giganteus* growing in Cameroon are reported in Table 1. Overall, different oil chemical profiles were exhibited confirming the different membership of the investigated species to different botanical families (i.e. Zingiberaceae, Mimosaceae and Asteraceae, respectively). A total of 94 volatile components were identified in the essential oils obtained from different parts of *A. daniellii*, accounting for 95.5–99.3 % of the total compositions (Table 1). Overall, these oils were mostly characterized by monoterpene hydrocarbons (67.2 % in seeds, 52.6 % in pericarp and 59.8 % in leaves), with minor contributions of oxygenates monoterpenes (13.0 % in seeds, 28.0 % in pericarp and 11.0 % in leaves), sesquiterpene hydrocarbons (12.1 % in seeds, 5.3 % in pericarp and 20.0 % in leaves) and oxygenated sesquiterpenes (3.2 % in seeds, 13.2 % in pericarp and 8.4 % in leaves). Nonetheless, the chemical profiles of the three plant parts were quite different. The most abundant volatile components of seeds were 1,8-cineole (48.8 %), β-pinene (11.2 %) and α-terpineol (10.8 %). In the essential oil obtained from fruit pericarp, β-pinene (17.6 %), sabinene (11.7 %) and linalool (10.2 %) were the major constituents. Finally, in the essential oil from leaves, sabinene (43.9 %) and (*E*)-caryophyllene (16.6 %) were the most abundant compounds. The chemical profile of *A. daniellii* reported in this study was totally consistent with that previously reported for the seeds collected in Nigeria (Adegoke et al. 1998), where 1,8-cineole (59.8 %) and β-pinene (13.2 %) were the major essential oil constituents. On the other hand, this is the first investigation of the essential oils obtained from pericarp and leaves, and no data are available for comparative purposes.

This study reports the first phytochemical analysis of the volatile components obtained from seeds of *D. cinerea*. Forty-nine volatile components were identified representing 76.0 % of the total volatile profile (Table 1). The chemical composition was dominated by oxygenated monoterpenes (50.6 %), with minor amounts of oxygenated sesquiterpenes (12.1 %). Geraniol (18.2 %), terpinen-4-ol (7.5 %), linalool (4.0 %) and umbellulone (3.8 %) were the most representative compounds. Among components of different chemical nature (12.7 %) occurring in the volatile oil, the pyrazine ligustrazin (5.1 %), the phenylpropanoid elemicin (3.0 %) and the fatty acid decanoic acid (2.8 %) were the most abundant.

Thirty-five volatile components were detected in the root essential oil from *E. giganteus*. This oil was entirely composed of sesquiterpenoids (94.3 %), with hydrocarbons being more abundant than oxygen-containing compounds (54.7 vs 39.6 %, respectively) (Table 1). The most abundant compounds were tricyclic sesquiterpenoids such as silphiperfol-6-ene (23.0 %) and presilphiperfolan-8-ol (22.7 %), followed by presilphiperfol-7-ene (7.8 %), cameroonan-7-α-ol (7.8 %) and (*E*)-caryophyllene (6.3 %). These results were consistent with those obtained by Menut et al. (1997), although some discrepancies were found. They consisted in a lower amount of presilphiperfolan-8-ol (6.5 %) and the absence of cameroonan-7-α-ol that is the major contributor to the strong woody and patchouli-like smell of the root oil (Taber and Nelson, 2011). Conversely, we did not find presilphiperfol-7(8)-ene that was characterized as a new compound by these authors. Tricyclic sesquiterpenes are secondary metabolites with a restricted distribution in higher plants. Thus, their presence in *E. giganteus* has chemotaxonomic importance. As regards their biosynthesis, some authors proposed that the silphiperfolenes may origin by multiple rearrangement from the caryophyllene cation via a presilphiperfolane cation (Bohlmann et al. 1981; Bohlmann and Jakupovic 1980). These molecules have not been investigated for biological activities so far.

Larvicidal toxicity

Recently, the indigenous flora of African and Asian countries has been extensively screened for insecticidal and repellent activity of endemic plant species. Moreover, botanicals may be also useful sources of antiplasmodial molecules, as recently elucidated by the recent Nobel Prize to Y. Tu, due to the discovery of artemisinin for malaria treatment (Benelli and Mehlhorn 2016; Benelli 2016d). Particularly, in Africa, ethnobotanical research projects carried out in the regions of today's Ethiopia, South Africa, Nigeria, Kenya and Tanzania indicate that the native inhabitants of the African study regions traditionally use 64 plant species as mosquito repellents, belonging to 30 families (Pavela and Benelli 2016), including *Echinops kebericho* (Karunamoorthi and Hailu 2014). More generally, aromatic plants (i.e. *Citrus* spp., *Eucalyptus* spp., *Lantana camara*, *Ocimum* spp. and *Lippia javanica*) were the most commonly used in all the study regions (Pavela and Benelli 2016). In the majority of cases, the essential oils are the active substances contained in these plants, which provide significant insecticidal, antiovipositional and repellent effects (Benelli 2015a, b; Pavela and Benelli 2016).

Our results showed that the highest acute larvicidal activity on *Cx. quinquefasciatus* was exerted by *D. cinerea* essential oil ($LC_{50} = 39.1$ μL L^{-1}), followed by *A. daniellii* (pericarp: $LC_{50} = 65.5$ μL L^{-1}; leaves: $LC_{50} = 65.5$ μL L^{-1}; seeds: $LC_{50} = 106.5$ μL L^{-1}) and *E. giganteus* ($LC_{50} = 227.4$ μL L^{-1}) (Table 2). Notably, when searching for the antiparasitic and antivectorial properties of *D. cinerea*, *A. daniellii* and *E. giganteus* essential oils, we faced a severe shortage of literature (Adebayo et al. 2016; Ahua et al. 2007; Karunamoorthi and Hailu 2014). In particular, limited knowledge is available about the larvicidal and repellent activity of

Table 1 Chemical composition of the essential oils from *A. daniellii*, *D. cinerea* and *E. giganteus*

No.	Component[a]	RI calc.[b]	RI lit.[c]	*Aframomum daniellii* (%)[d]			*Dichrostachys cinerea* (%)[d]	*Echinops giganteus* (%)[d]	ID[e]
				Seeds	Pericarp	Leaves			
1	α-Thujene	916	924	tr	0.5	1.0			RI, MS
2	α-Pinene	921	932	2.9	4.4	2.4		tr[f]	Std
3	Camphene	933	946	tr	0.6	tr			RI, MS
4	Sabinene	959	969	0.5	11.7	43.9			RI, MS
5	β-Pinene	963	974	11.2	17.6	5.8	0.1		Std
6	Dehydro-1,8-cineole	979	988	0.3					RI, MS
7	Myrcene	982	988	0.3	1.1	1.5	0.3	tr	Std
8	δ-2-Carene	992	1001		0.1				RI, MS
9	α-Phellandrene	996	1004	0.1	5.5	tr			Std
10	δ-3-Carene	1003	1008		0.2			tr	Std
11	α-Terpinene	1009	1014	0.1	0.5	0.9			RI, MS
12	p-Cymene	1016	1020	0.7	2.6	1.0	0.1		Std
13	Limonene	1020	1024	1.5	3.8	0.7	tr	tr	Std
14	1,8-Cineole	1021	1026	48.8	5.9	0.5	2.3		Std
15	Ethylhexanol	1031	1030				0.2		RI, MS
16	(*Z*)-β-Ocimene	1032	1032		0.3				RI, MS
17	(*E*)-β-Ocimene	1041	1044	0.1	1.5	0.3			RI, MS
18	γ-Terpinene	1050	1054	1.0	1.1	1.9			Std
19	*cis*-Sabinene hydrate	1057	1065		0.7	1.1			RI, MS
20	*cis*-Linalool oxide	1071	1067				0.1		RI, MS
21	Terpinolene	1079	1086	0.1	0.9	0.4		tr	Std
22	Ligustrazin	1081	1083				5.1		RI, MS
23	*trans*-Sabinene hydrate	1089	1098		0.7	0.9			RI, MS
24	Linalool	1096	1095		10.2	1.8	4.0		Std
25	2-Methyl butyl-2-methyl butyrate	1102	1100	tr					RI, MS
26	*cis*-*p*-Menth-2-en-1-ol	1113	1118		0.1	0.2	0.3		RI, MS
27	α-Campholenal	1123	1122				0.1		RI, MS
28	*trans*-Pinocarveol	1128	1135	tr	tr		0.7		Std
29	*trans*-*p*-Menth-2-en-1-ol	1131	1136			0.1	0.3		RI, MS
30	Geijerene	1130	1138		0.1				RI, MS
31	Camphor	1132	1141		1.0				Std
32	*cis*-Verbenol	1142	1137				0.2		RI, MS
33	*trans*-Pinocamphone	1151	1158				0.4		RI, MS
34	Pinocarvone	1152	1160		0.1		0.1		RI, MS
35	Borneol	1156	1165	tr	0.4		0.7		Std
36	δ-Terpineol	1158	1162	0.4	0.0				RI, MS
37	*p*-Mentha-1,5-dien-8-ol	1158	1166	0.4					RI, MS
38	*cis*-Pinocamphone	1162	1172		0.0		0.8		RI, MS
39	Umbelullone	1166	1167				3.8		RI, MS
40	Terpinen-4-ol	1167	1174	0.3	2.3	3.7	7.5		Std
41	*cis*-Pinocarveol	1175	1182		0.2				RI, MS
42	*p*-Cymen-8-ol	1178	1179		0.0		0.3		RI, MS
43	α-Terpineol	1181	1186	10.8	1.4	0.2	3.3		Std
44	Myrtenal	1184	1195		0.7	0.1	0.2		Std
45	Myrtenol	1186	1194	0.1	2.1	0.4	1.1		Std
46	*cis*-Piperitol	1199	1195		0.0	tr			RI, MS
47	*trans*-Piperitol	1205	1207				0.1		RI, MS

Table 1 (continued)

No.	Component[a]	RI calc.[b]	RI lit.[c]	*Aframomum daniellii* (%)[d]			*Dichrostachys cinerea* (%)[d]	*Echinops giganteus* (%)[d]	ID[e]
				Seeds	Pericarp	Leaves			
48	*trans*-Carveol	1217	1215				0.2		RI, MS
49	Thymol, methyl ether	1224	1232	tr					RI, MS
50	Nerol	1229	1227				0.2		Std
51	Citronellol	1231	1223				0.3		Std
52	Carvacrol, methyl ether	1237	1241	0.4					RI, MS
53	Neral	1241	1235				0.2		Std
54	Piperitone	1250	1249				0.3		RI, MS
55	Geraniol	1251	1249		0.3		18.2		Std
56	*trans*-Ascaridol glycol	1262	1266			tr			RI, MS
57	Isobornyl acetate	1276	1283		0.6	tr			Std
58	Thymol	1291	1289	tr			0.9		Std
59	*trans*-Sabinyl acetate	1291	1289			tr			RI, MS
60	Methyl myrtenate	1292	1293				2.0		RI, MS
61	Carvacrol	1301	1298	0.1	tr		0.7		Std
62	*cis*-Pinocarvyl acetate	1303	1311		0.2	0.1			RI, MS
63	Myrtenyl acetate	1316	1324		1.0	1.9			RI, MS
64	Silphiperfol-5-ene	1318	1326					2.1	RI, MS
65	δ-Elemene	1326	1335		0.1				RI, MS
66	Presilphiperfol-7-ene	1328	1334					7.8	RI, MS
67	Silphinene	1333	1340					1.7	RI, MS
68	7-Epi-silphiperfol-5-ene	1336	1349					3.5	RI, MS
69	α-Terpinyl acetate	1341	1346	0.2	0.1		0.3		RI, MS
70	α-Copaene	1362	1374	0.3	0.1	0.2			Std
71	β-Bourbonene	1369	1387			tr			RI, MS
72	Modheph-2-ene	1362	1382					3.0	RI, MS
73	Silphiperfol-6-ene	1373	1377					23.0	RI, MS
74	β-Cubebene	1377	1387	tr		tr			RI, MS
75	α-Isocomene	1379	1387					2.4	RI, MS
76	β-Elemene	1380	1389	1.1	0.2	tr			RI, MS
77	Decanoic acid	1380	1386				2.8		RI, MS
78	Cyperene	1381	1398	0.1	0.4				RI, MS
79	*iso*-Longifolene	1383	1389					tr	RI, MS
80	β-Isocomene	1400	1407					2.1	RI, MS
81	α-Gurjunene	1400	1409					tr	Std
82	(*E*)-Caryophyllene	1402	1417	0.7	1.7	16.6		6.3	Std
83	α-trans-Bergamotene	1425	1432	0.8		0.1			RI, MS
84	α-Guaiene	1425	1437	0.8					RI, MS
85	α-Humulene	1436	1452	0.3	0.3	1.5		2.0	Std
86	Geranyl acetone	1449	1453				1.2		RI, MS
87	(*E*)-β-Farnesene	1450	1454	0.1		tr		tr	RI, MS
88	γ-Gurjunene	1456	1475	0.7					RI, MS
89	Germacrene D	1465	1484		1.5	0.3		0.3	RI, MS
90	Selina-4,11-diene	1467	1474	1.0	0.1	0.1			RI, MS
91	β-Selinene	1469	1489	1.0	0.1				RI, MS
92	γ-Muurolene	1477	1478	0.6					RI, MS
93	*ar*-Curcumene	1472	1479		0.0			0.1	RI, MS
94	Bicyclogermacrene	1480	1500		0.2	0.1			RI, MS

Table 1 (continued)

No.	Component[a]	RI calc.[b]	RI lit.[c]	*Aframomum daniellii* (%)[d]			*Dichrostachys cinerea* (%)[d]	*Echinops giganteus* (%)[d]	ID[e]
				Seeds	Pericarp	Leaves			
95	α-Zingiberene	1485	1493		0.1				RI, MS
96	*epi*-Cubebol	1489	1493					0.1	RI, MS
97	α-Bulnesene	1491	1509	1.2					RI, MS
98	(*Z*)-α-Bisabolene	1493	1506			0.1			RI, MS
99	Silphiperfolan-6-α-ol	1496	1507					1.0	RI, MS
100	β-Bisabolene	1498	1505	3.2	0.5	0.9			RI, MS
101	Cameroonan-7-α-ol	1500	1510					7.1	RI, MS
102	Sesquicineole	1501	1515		0.3				RI, MS
103	*trans*-Calamenene	1508	1521			tr			RI, MS
104	*delta*-Cadinene	1510	1522	tr	0.1	tr		0.3	RI, MS
105	Silphiperfolan-7-β-ol	1510	1519					2.5	RI, MS
106	Silphiperfolan-6-β-ol	1535	1546					1.7	RI, MS
107	Hedycaryol	1536	1546		2.1	1.5			RI, MS
108	Elemicin	1556	1555				3.0		RI, MS
109	(*E*)-Nerolidol	1556	1561	tr	0.1	0.7			Std
110	Spathulenol	1560	1577		0.1				RI, MS
111	Isoaromadendrene epoxide	1560	1572				1.8		RI, MS
112	Prenopsan-8-ol	1564	1575					3.2	RI, MS
113	Caryophyllene oxide	1564	1582		0.6	2.2	1.1		Std
114	Presilphiperfolan-8-ol	1578	1585					22.7	MS
115	Guaiol	1583	1600	tr	0.7	0.5			RI, MS
116	Humulene epoxide II	1590	1608		0.1	0.1			RI, MS
117	10-*epi*-γ-Eudesmol	1600	1622	0.7	0.3	0.3			RI, MS
118	Eremoligenol	1611	1629	0.2	0.1	0.4			RI, MS
119	γ-Eudesmol	1615	1630		0.6	0.4			RI, MS
120	1,10-di-*epi*-Cubenol	1619	1618					0.1	RI, MS
121	β-Eudesmol	1631	1649	1.3	2.2	1.5			RI, MS
122	α-Acorenol	1628	1632				1.0		RI, MS
123	Caryophylla-4(12),8(13)-dien-5-ol[g]	1630	1639			0.2		tr	RI, MS
124	*epi*-α-Muurolol	1635	1640				0.7	0.4	RI, MS
125	α-Muurolol	1640	1644					0.1	RI, MS
126	α-Eudesmol	1636	1652	0.2	1.3	0.5			RI, MS
127	*neo*-Interemedeol	1636	1658	0.2	1.3				RI, MS
128	Intermedeol	1639	1666	0.4	1.8	0.1			RI, MS
129	α-Cadinol	1647	1652				1.4	0.4	RI, MS
130	Ageratochromene	1655	1658				0.8		RI, MS
131	*epi*-β-Bisabolol	1657	1670		0.1				RI, MS
132	*epi*-α-Bisabolol	1671	1683		0.2				RI, MS
133	α-Bisabolol	1673	1685		1.4	0.4			Std
134	3-Oxo-β-ionone	1678	1685				0.9		RI, MS
135	Cyperotundone	1688	1695				0.9		RI, MS
136	(2*E*-6*Z*)-Farnesol	1709	1698				2.4		RI, MS
137	Curcuphenol	1716	1717					0.4	RI, MS
138	(2*E*-6*Z*)Farnesal	1718	1713				1.0		RI, MS
139	(2*E*-6*E*)-Farnesal	1737	1740				1.7		RI, MS
140	*n*-Tricosane	2300	2300		0.1				Std

Table 1 (continued)

No.	Component[a]	RI calc.[b]	RI lit.[c]	*Aframomum daniellii* (%)[d]			*Dichrostachys cinerea* (%)[d]	*Echinops giganteus* (%)[d]	ID[e]
				Seeds	Pericarp	Leaves			
	Oil yield (%)			2.5			0.4	1.8	
	Total identified (%)			95.5	99.2	99.3	76.0	94.3	
	Grouped compounds (%)								
	Monoterpene hydrocarbons			67.2	52.6	59.8	0.6	tr	
	Oxygenated monoterpenes			13.0	28.0	11.0	50.6		
	Sesquiterpene hydrocarbons			12.1	5.3	20.0		54.7	
	Oxygenated sesquiterpenes			3.2	13.2	8.4	12.1	39.6	
	Others			tr	0.1		12.7		

[a] Components are reported in order of their elution from a HP-5MS capillary column

[b] Retention index experimentally determined using a mixture of C_8-C_{30} of *n*-alkanes

[c] Retention index taken from Adams (2007) and/or NIST 08 (2008) for apolar capillary column

[d] Relative percentage values are means of three determinations with a RSD% below 10 % for the most abundant components

[e] Identification methods: std, based on comparison with authentic compounds; MS, based on comparison with WILEY, ADAMS, FFNSC2 and NIST 08 MS databases

[f] Traces, % <0.1

[g] Correct isomer not identified

these plants against culicid vectors (Adebayo et al. 2016; see Karunamoorthi and Hailu 2014 also about close-related *Echinops* species traditionally used as mosquito repellents). To the best of our knowledge, only the essential oil from the fruits of *A. daniellii* has been tested on fourth instar larvae of *Aedes aegypti*, *Anopheles gambiae* and *Culex pipiens fatigans*, where 0.02–0.04 % of the oil produced 90–100 % mortality on the larvae of all the three mosquitoes (Adebayo et al. 2016). More generally, concerning the larvicidal action of plant essential oils against mosquito vectors of economic importance, Pavela (2015a) recently showed that essential oils with LC_{50} ≤100 ppm were obtained from 122 plants. In this scenario, our results are of interest, since the *D. cinerea* oil achieved a $LC_{50} = 39.1$ μL L^{-1}, which far encompasses the larvicidal potential of the majority of essential oils currently tested as mosquito larvicides (e.g. Conti et al. 2012, 2014; Benelli et al. 2015; Pavela 2015a).

The notable effects of *D. cinerea* essential oil could be given by some of its major constituents such as geraniol (18.2 %) and terpinen-4-ol (7.5 %). In fact, geraniol is a monoterpene alcohol that possesses documented larvicidal and repellent effects on mosquitoes (Chen and Viljoen 2010) and is incorporated in eco-friendly formulations to be used as alternative tools for mosquito vector control (Chuaycharoensuk et al. 2012). In addition, geraniol is also used as tick repellent and acaricidal agent (Khallaayoune et al. 2009; Jeon et al. 2009). Terpinen-4-ol exhibited significant larvicidal effects against several mosquitoes including *Cx. quinquefasciatus* (Govindarajan et al. 2016a). As regards *A. daniellii*, the higher larvicidal activity of pericarp and leaves may be justified by the presence of sabinene (11.7 % in pericarp oil, 43.9 % in leaves oil) and linalool (10.2 % in pericarp oil) that were previously proven to be effective against *Anopheles stephensi*, *Ae. aegypti* and *Cx. quinquefasciatus* (Govindarajan 2010).

Overall, the chance to use the *D. cinerea* essential oil against filariasis and West Nile virus mosquito vectors seems promising, since it is effective at moderate doses and could be an advantageous alternative to build newer mosquito control tools. Further

Table 2 Acute toxicity of *A. daniellii*, *D. cinerea* and *E. giganteus* essential oils against the fourth instar larvae of *Cx. quinquefasciatus*

Essential oil	LC_{50} (μL L^{-1})	LCI-UCI	LC_{90} (μL L^{-1})	LCI-UCI	χ^2
Aframomum daniellii seeds	106.5	97.2–118.1	233.7	188.3–311.3	2.001 n.s.
Aframomum daniellii pericarp	65.5	59.4–71.9	142.2	119.9–152.3	1.835 n.s.
Aframomum daniellii leaves	73.6	68.8–78.4	117.9	104.1–150.3	0.689 n.s.
Dichrostachys cinerea	39.1	25.3–42.7	96.1	84.5–99.7	0.259 n.s.
Echinops giganteus	227.4	198.5–232.1	564.7	548.9–612.3	0.102 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 ($\alpha = 0.05$)

research on the impact of *D. cinerea* essential oil and its major constituents on non-target aquatic organisms (Govindarajan and Benelli 2016; Govindarajan et al. 2016a, b) is urgently required.

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