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Insecticidal Activity and Chemical Composition of Volatile Oils from *Hyptis martiusii* Benth

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The essential oils from leaves and inflorescences of *Hyptis martiusii* Benth were analyzed by GC-MS. Twenty-six compounds representing 93.2% of the essential oil of leaves were characterized; Δ-3-carene (22.5%), 1,8-cineole (24.27%), β-caryophyllene (6.15%), and bicyclogermacrene (6.32%) were found as the major components. In the essential oil of inflorescences 27 compounds representing 87.7% of the oil were identified. The major components were Δ-3-carene (13.5%), α-pinene (5.78%), β-caryophyllene (6.59%), viridiflorene (8.25%), and germacrene B (5.21%). The essential oil of leaves and 1,8-cineole showed pronounced insecticidal effect against *Aedes aegypti* larvae and *Bemisia argentifolii*, the vectors of dengue fever and white fly fruit plague, respectively.

KEYWORDS: ***Hyptis martiusii*; insecticide; essential oil; 1,8-cineole; *Aedes aegypti*; *Bemisia argentifolii***

INTRODUCTION

The genus *Hyptis* (Labiatae) is very large (400 species), and many of its reported species are known for their medicinal use as indigenous drugs (*1*−*3*). Widespread throughout tropical America, they are used as antifungal, antibacterial, and anticonvulsant (*4*, *5*) agents, in various gastrointestinal ailments, and as cures for fungal diseases and malaria (*6*). In addition, many species of *Hyptis* are used against stored product pests and other pest insects, and they are commonly used against mosquitoes (*7*). The leaves of those species are also largely used as potent insect repellants by native populations of many parts of the world (*8*−*10*).

Despite their reported use for medicinal purposes, a literature survey revealed that in Brazil the phytochemical analysis of plants belonging to the *Hyptis* genus has been limited to a few species. As part of investigative efforts to find biologically active essential oils from northeastern Brazil flora, combined with the previously reported use of leaves of many species of *Hyptis* as natural insecticide, we report herein the insecticidal activity and chemical composition of the essential oils from leaves and inflorescences of *H. martiusii* Benth.

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H. martiusii Benth is a small shrub that grows in abundance in northeastern Brazil, where it is popularly known as "cidreira-do-mato" (Port. Lit.: Wild *Lippia*). No phytochemical investigation on this species has so far been reported according to a literature survey.

The essential oil from leaves of *H. martiusii* was tested against *Bemisia argentifolii* (white fly), a common pest of edible fruits of commercial value such as melon and watermelon, and *Aedes aegypti* larvae, the vector for the transmission of dengue and yellow fevers, responsible for serious public health hazards in Brazil, Africa, and Asia. The insecticidal activity of the major component, 1,8-cineole, isolated from the essential oil of leaves is also reported.

MATERIALS AND METHODS

Plant Material. The entire plant was collected in August 1999, at the flowering stage, from plant populations growing wild in Chapada do Araripe (Araripe's plateau), Moreilândia County, Pernambuco State, northeastern Brazil. A voucher specimen (no. 25046) has been identified by Dr. Afrânio G. Fernandes and deposited at the Herbário Prisco Bezerra (EAC), Departamento de Biologia, Universidade Federal do Ceará, Brazil.

Analytical Conditions. Analysis of the volatile constituents was performed by GC-MS on a Hewlett-Packard 5971 GC-MS instrument

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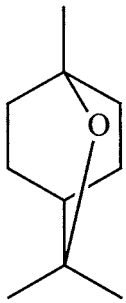

Figure 1. Structure of 1,8-cineole.

equipped with a dimethylpolysiloxane DB-5 fused silica capillary column (30 m × 0.25 mm; 0.25 μm film thickness); helium was used as carrier gas at a flow rate of 1 mL/min, and the temperature was programmed from 35 to 180 °C at a 4 °C/min rate and from 180 to 280 °C at a 20 °C/min rate. The injector and detector were maintained at 250 and 200 °C, respectively. The mass spectra were taken over the *m*/*z* 28−400 range with an ionizing voltage of 70 eV. Identification of individual components of the essential oils was performed by computer library MS search based on their Kovat's retention indices as a preselection routine (*11*), as well as by visual inspection of published spectral data (*12*).

Isolation of the Volatile Constituents. The entire *H. martiusii* plant was separated into fresh leaves and inflorescences and separately submitted to hydrodistillation for 3 h in a Clevenger-type apparatus. The essential oils obtained from the leaves and inflorescences were dried over anhydrous sodium sulfate to yield clear yellowish oils in 0.4 and 0.3% yields, respectively.

^{13}C NMR. The oxygenated monoterpene 1,8-cineole (**Figure 1**) was obtained from the leaves' oil after separation by column chromatography and analyzed by ^{13}C NMR spectroscopy. The analysis was performed with $CDCl_3$ solution using a Brucker Avance DPX 300 (300 MHz for ^{1}H, 75 MHz for ^{13}C).

Bioassay Tests. *Origin of the B. argentifolii Population.* The insect parents were obtained from an experimental crop of melons maintained by the Experimental Station of Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), a governmental institution devoted to agricultural research, in Paraipaba County, State of Ceará (Brazil). The colony was kept captive in special "cages" with melon plants at the Laboratory of Entomology of EMBRAPA Agroindustria Tropical, in Fortaleza City.

Cultivation of Host Plants. Melon seeds were sowed in conical plastic vessels (10 cm height, 10 and 13 cm of diameter, bottom and top, respectively) over a 7 cm layer of a substrate obtained directly from an arboretum used by EMBRAPA. Each vessel received tree seeds of the melon variety Hale's Best Jumbo and was isolated from the external environment by a piece of tulle held by a wire frame inside the vessel. They were kept under these conditions for 15 days when at least four to five fully developed leaves emerged.

Massal Rearing of B. argentifolii. Vessels with seedlings were introduced into plastic cages (35 × 48 × 32 cm). The upper part and lateral wall were covered with pieces of tulle. Several vessels with plants of different ages were kept in the same cage in order to have the insect colony in different developing stages. Senescent plants were discarded and replaced by young ones.

Insecticidal Activity Assays against B. argentifolii. Seven-day-old leaves without insects were harvested. Testing substances were applied on the abaxial face of each leaf by a hand sprayer and individually adjusted by its peciole to small vials with distilled water. Each vial, containing one leaf, was then stored in an individual transparent acrylic cup (9.5 cm height, 5.0 and 7.0 cm diameter, bottom and top, respectively), partially covered with a transparent polyethylene film. Ten adult insects were introduced in each cup, the polyethylene cover was closed, and the cups were stored in a BOD chamber at 24 °C and photoperiod of 14 h. Results were recorded 72 h later by counting the layed eggs and the number of survivors. The experiments had a complete randomized block design with eight replicates for treatment.

Table 1. Effect of the Essential Oil from Leaves of *H. martiusii* against *B. argentifolii*

concn (mg/L)	av (%) of dead insects after 72 h	av (%) of layed eggs after 72 h
2000	93.7	0.1
1000	63.7	23.9
500	56.2	20.9
250	61.2	21.7
witness	25.0	32.2

Table 2. Effects of 1,8-Cineole against *B. argentifolii*

treatment	av (%) of dead insects after 72 h	av (%) of layed eggs after 72 h
1,8-cineole (1000 mg/L)	91.2	9.2
witness	22.5	26.4

Table 3. Effect of the Essential Oil from Leaves of *H. martiusii* and 1,8-Cineole against *A. aegypti* Larvae

	av (%) of dead larvae after 24 h	
concn (mg/L)	*H. martiusii* oil	1,8-cineole
500	100	100
250	99	100
100	22	100
50	0	53
25	0	10

An individual leaf was considered to be the experimental plot. The results are presented in **Tables 1** and **2**.

Origin of the A. aegypti Larvae. The Entomology Laboratory of Fundação Nacional de Saúde (FNS) provided the *A. aegypti* eggs used in the bioassays. The eggs were immersed in chlorine-free tap water, and larvae emerged after a few minutes. Only the larvae at the third stage were selected for this study (*13*).

Larvicidal Bioassay against A. aegypti. Testing substances were placed in a beaker and dissolved in DMSO (0.3 mL) and water (19.7 mL) at concentrations ranging from 1 to 500 ppm, followed by addition of 50 larvae at the third stage. A mortality count was conducted 24 h after treatment. A control solution using DMSO and water did not present larvicidal activity. Tests were done in triplicate, and the results are presented in **Table 3**.

RESULTS AND DISCUSSION

Chemical Analysis. The results obtained from qualitative analysis of the essential oils are shown in **Table 4**. As a total, 26 constituents representing 93.2% of the essential oil from leaves and 27 constituents representing 87.7% of the essential oil from inflorescences were identified.

The hydrocarbon and oxygenated compounds represented about 60.5% and 32.7% of the total essential oil of leaves, respectively. The main components were 1,8-cineole (24.3%) and Δ-carene (22.5%), with minor amounts of the sesquiterpenes bicyclogermacrene (6.3%) and β-caryophyllene (6.1%). The essential oil composition of the leaves from *H. martiusii* resembles the volatile oils reported for the congeners *H. suaveolens* and *H. goyazensis* (*9*), both containing 1,8-cineole, 37.0 and 36.8%, respectively, as the main component.

From **Table 4** it can be seen that the hydrocarbon percentage is high for both oils (73.0 and 60.5% for inflorescence and leaves, respectively), but oxygenated monoterpenes (27.0 versus 2.3%) predominate in the leaf oil, whereas oxygenated sesquiterpenes (12.5 versus 5.7%) predominate in the inflorescence oil.

Table 4. Percentage Composition of Essential Oils from Leaves (I) and Inflorescences (II) of *H. martiusii* Benth

component	RI[a]	I (%)	II (%)
α-pinene	934	3.91	5.78
β-pinene	975	1.93	3.18
β-myrcene	991	1.39	1.03
α-phellandrene	1003	1.03	0.88
Δ-3-carene	1011	22.51	13.50
o-cymene	1020	0.59	
p-cymene	1023	2.34	
limonene	1029		4.56
1,8-cineole	1031	24.27	2.28
γ-terpinene	1059	1.40	1.32
terpinolene	1088	1.42	1.03
camphor	1145	2.16	
4-terpineol	1180	0.53	
α-cubebene	1351	0.54	1.08
α-copaene	1376	0.91	1.40
α-gurjunene	1409		1.10
β-caryophyllene	1417	6.15	6.59
γ-elemene	1430		1.62
aromadendrene	1436	2.79	2.14
α-humulene	1449	1.72	1.77
allo-aromadendrene	1456		1.02
γ-muurulene	1472	1.51	
β-selinene	1478		0.81
bicyclogermacrene	1486	6.32	
viridiflorene	1487		8.25
γ-cadinene	1502	0.78	1.71
δ-cadinene	1510	1.92	4.01
selina-3,7(11)-diene	1527	1.33	4.89
germacrene B	1540		5.21
ledol	1549	0.53	2.30
spathulenol	1554	0.73	
caryophyllene oxide	1561	3.68	4.60
globulol	1568	0.83	0.97
viridiflorol	1580		4.64
total		**93.16**	**87.70**

[a] Retention index.

Insecticidal Activities. The experiments showed that the volatile constituents of leaves induced 100% mortality in *A. aegypti* larvae after 1 day at a dosage of 250 mg/L (**Table 1**). However, *B. argentifolii* mortality was reached only after 3 days at 2000 mg/L (**Table 2**).

The oxygenated monoterpene 1,8-cineole was the major constituent of the tested oil. It was obtained in the pure form by column chromatography of the leaf oil and characterized by comparison of its spectral data (GC-EIMS and ^{13}C NMR) to the values reported in the literature (*12*, *14*). 1,8-Cineole was tested against both insects under identical conditions to compare its activity with that of the investigated oil (**Table 3**). In this experiment we found a high mortality rate of *A. aegypti* larvae after 1 day under dosages as low as 100 mg/L, whereas *B. argentifolii* showed high mortality only at 1000 mg/L after 3 days.

From these results it can be concluded that the larvae of *A. aegypti* are susceptible to the composition of the essential oil and suggested that the oil activities can be attributed, to a considerable degree, to the presence of 1,8-cineole as the main component; however, synergistic action of the other components cannot be disregarded.

There is evidence in the literature for the 1,8-cineole activity against ticks (*15*), *Lavandula spica* (*16*), *Rhyzopertha dominica*, and *Tribolium castaneum* (*17*). It plays an important role in plant resistance to insects and has low toxicity to mammals, which, in accordance with the present conclusions, can suggest its use as an ecologically safe alternative insecticide.

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