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Chemical Composition and Larvicidal Activity of the Essential Oils from *Eupatorium betonicaeforme* (D.C.) Baker (Asteraceae)

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The volatile composition of the essential oils from leaves and roots of *Eupatorium betonicaeforme* (D.C.) Baker was analyzed by GC−MS. A total of 12 compounds were identified. β-Caryophyllene (12.4−41.7%), α-humulene (11.7−14.6%), γ-muurolene (10.4−19.0%), bicyclogermacrene (15.0−17.5%), 2,2-dimethyl-6-vinylchroman-4-one (10.3−25.5%), and 2-senecioyl-4-vinylphenol (8.5−41.0%) were the most prominent constituents. The former two compounds were isolated and characterized by spectroscopic data. The essential oils and the isolated compounds were tested against *Aedes aegypti* larvae survival. The results obtained show that the essential oil from roots and 2,2-dimethyl-6-vinylchroman-4-one (10.3−25.5%) could be considered as natural larvicidal agents.

KEYWORDS: ***Eupatorium betonicaeforme*; essential oil; *Aedes aegypti*; larvicidal activity; 2,2-dimethyl-6-vinylchroman-4-one**

INTRODUCTION

The growing demand for natural products has intensified in the past decades. It is well-known that plant-derived natural products are extensively used as biologically active compounds, particularly in the area of infectious diseases, which represent a serious problem to health, being one of the main causes of morbidity and mortality worldwide (*1*, *2*). Mosquitoes are responsible for more diseases than any other group of arthropods, being the major vectors for the transmission of malaria, filariasis, dengue fever, yellow fever, and several viral diseases (*2*, *3*). The mosquito *Aedes aegypti* is responsible for yellow fever in Central and South America and West Africa. It is also the vector of dengue hemorrhagic fever, which is endemic to Southeast Asia, the Pacific Islands, Africa, and the Americas (*4*). Recently, in Brazil, the incidence of dengue fever has increased significantly. For 2000, 180.137 cases of dengue were registered, approximately 80% of the total in the Americas (*5*). The continuous use of synthetic insecticides and insect growth regulators for the eradication of *A. aegypti* has been effective but nevertheless has led to the outbreak of insect species showing pesticide resistance. It has also provoked undesirable effects, including toxicity to nontarget organisms, and fostered environmental and human health concerns (*3*, *6*, *7*). The use of natural products can be considered an important alternative strategy for the control of *A. aegypti* larvae, since they constitute a rich source of bioactive compounds that are biodegradable to nontoxic products and potentially suitable for use in integrated management programs. Thus, essential oils may be an alternative to currently used insecticidal agents. In fact, the chemical composition of the essential oils, as well as their antimicrobial, antifungal, antitermite, larvicidal, and insecticidal properties (*8*−*11*), has been enough investigated.

As part of pharmacological−phytochemical integrated studies of medicinal plants from northeastern Brazil flora, we are investigating the chemical composition of plants from the genus *Eupatorium* (Asteraceae). Several plants of this genus are widely used in folk medicine in different parts of the world due their medicinal properties, such as antimicrobial, astringent, disinfectant, hepatoprotective, antiherpetic, antirheumatic, and analgesic, as well as in the treatment of fever, headache, stomach ulcer, diarrhea, and malaria (*12*−*15*). Concomitantly, essential oils, pure compounds, and extracts of *Eupatorium* species have shown several biological activities, including antibacterial, ant-repelling, antifungal, anti-inflammatory, and antioxidant (*16*−*19*). Thus, the genus seems to be a promising resource of new biologically active drugs.

The purpose of this work is to present the chemical composition of the essential oils from leaves and roots of *E. betonicaeforme* (D.C.) Baker, as well as their larvicidal properties against *A. aegypti* larvae.

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Table 1. Volatile Constituents Identified for *E. betonicaeforme*

compound[a]	KI[b]	relative contents (%) oil A[c]	oil B[d]	oil A[c]	oil B[d]
α-gurjunene	1378	0.7			
β-caryophyllene	1398	12.4	36.1	36.3	41.7
α-humulene	1430	5.0	13.3	14.6	11.7
β-farnesene	1435	0.7			
γ-muurolene	1456	3.8	20.3	10.4	19.0
β-selinene	1460	0.4	1.0	1.0	1.2
bicyclogermacrene	1500	3.7	15.0	15.0	17.5
δ-cadinene	1495	0.4	1.2	1.0	1.0
spathulenol	1545	0.7	2.3		2.3
unknown	1547	0.7			
unknown	1549	0.8		2.2	
unknown[e]	1563	1.7		0.8	
caryophyllene oxide	1547		1.6		3.4
unknown	1550		2.0		0.9
unknown	1557		0.7		
2,2-dimethyl-6-vinylchroman-4-one[f]	1582	25.5		10.3	
unknown	1595		1.1		
unknown	1599		1.0		
unknown	1611	0.4	1.9		
unknown	1618	0.5			
unknown	1633	1.3			
unknown	1655		0.9		
2-senecioyl-4-vinylphenol[f]	1658	41.0		8.5	
unknown	1851		1.5		
total identified		93.6	90.7	97.1	97.8

[a] Listed in order of elution from a DB-5 capillary column. [b] KI = Kovats retention index in reference to C_8–C_{26} *n*-alkanes on a DB-5 column. [c] Oils from roots. [d] Oils from aerial parts. [e] Probably an isomer of 2,2-dimethyl-6-vinylchroman-4-one. [f] Compounds identified by MS and ^{1}H and ^{13}C NMR spectral data. [g] Probably a dihydro derivative of 2-senecioyl-4-vinylphenol.

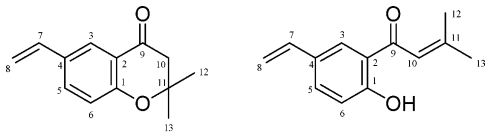
MATERIALS AND METHODS

Plant Material. *E. betonicaeforme* (D.C.) Baker (Asteraceae, tribe Eupatorieae), is a perennial subshrub, 60−90 cm high, with opposite peciolate leaves and several inflorescences of light purple color, of extensive and expressive but irregular dispersion in Brazil, Uruguay, and Argentina (*20*). Several specimens of a single population of *E. betonicaeforme* from the same site, in the flowering stage, were collected in May 2002 and June 2003, in Acarape County, State of Ceará, Northeast of Brazil. Voucher specimens (No. 32430), have been deposited at the Herbário Prisco Bezerra of the Departamento de Biologia, Universidade Federal do Ceará, Brazil.

Extraction of the Essential Oils. The fresh plant parts, i.e., ground roots (390 and 1.256 g, samples A and A′, respectively) and aerial parts (1.290 and 1.180 g, samples B and B′, respectively), were subjected to hydrodistillation for 2 h in a Clevenger-type apparatus. The obtained essential oils were dried over anhydrous sodium sulfate and, after filtration, stored under refrigeration until analyzed and tested. The samples A, B, A′, and B′ were isolated in yields of 0.014%, 0.005% 0.021%, and 0.007% (w/w on fresh weight basis), respectively.

Analysis of the Essential Oils. The volatile constituents from roots and aerial parts of *E. betonicaeforme* (**Table 1**) were analyzed by GC−MS on a Hewlett-Packard Model 5971 GC/MS using a (5%-phenyl)-methylpolysiloxane DB-5 capillary column (30 m × 0.25 mm) with film thickness 0.1 μm, carrier gas helium, and flow rate 1 mL/min with split mode. The injector temperature and detector temperature were 250 °C and 200 °C, respectively. The column temperature was programmed at 4 °C/min from 35 to 180 °C and then at 10 °C/min from 180 to 250 °C. Mass spectra were recorded from 30 to 450 *m/z*. Individual components were identified by matching their 70 eV mass spectra with those of the spectrometer database using the Wiley L-built library and two other computer library MS searches using retention indices as a preselection routine (*21, 22*) as well as by visual comparison of the fragmentation pattern with those reported in the literature (*23, 24*).

Chromatography of the Root Essential Oil. An aliquot of the essential oil from roots (240 mg, sample A) was subjected to chromatography over silica gel (24 g, 150−230 mesh) and eluted sequentially with petroleum ether, *n*-hexane/$CHCl_3$ gradients, and finally with EtOAc. Eighteen 10-mL fractions were obtained that were combined on the basis of TLC profiles. Compound **1** (25 mg) was isolated from the fraction D/38−43, eluted with petroleum ether, while compound **2** (34 mg) was isolated from a more polar fraction, H/71−74, eluted with *n*-hexane−$CHCl_3$ (1:1).



1 **2**

Figure 1. Structures of compounds **1** and **2** isolated from the oil of roots of *E. betonicaeforme*.

Characterization of the Isolated Compounds. The structures of compounds **1** and **2** (**Figure 1**) were established from one- and two-dimensional NMR experiments as well as by direct comparison of the ^{1}H NMR, IR, and MS data with literature values (*25*). To the best of our knowledge, the ^{13}C NMR data of both compounds are being reported for the first time.

2,2-Dimethyl-6-vinylchroman-4-one (**1**) was obtained as yellow crystals: mp 43−44 °C; ^{13}C NMR (125 MHz, $CDCl_3$) 159.9 (C-1), 120.3 (C-2), 124.6 (C-3), 130.9 (C-4), 133.9 (C-5), 118.9 (C-6), 135.7 (C-7), 113.5 (C-8), 192.8 (C-9), 49.2 (C-10), 79.7 (C-11), 27.0 (C-12/C-13).

2-Senecioyl-4-vinylphenol (**2**) was obtained as a colorless oil: ^{13}C NMR (125 MHz, $CDCl_3$) 163.2 (C-1), 120.5 (C-2), 128.2 (C-3), 128.6 (C-4), 133.1 (C-5), 118.8 (C-6), 135.8 (C-7), 112.4 (C-8), 196.3 (C-9), 120.0 (C-10), 158.6 (C-11), 28.5 (C-12), 21.6 (C-13).

Larvicidal Bioassay. Portions of essential oils and the pure isolated compounds **1** and **2** (5−500 μg/mL) were placed in a beaker (50 mL) and dissolved in H_2O:DMSO (98.5:1.5). Fifty instar III larvae of *A. aegypti* were delivered to each beaker. After 24 h, at room temperature, the number of dead larvae was counted and the lethal percentage calculated. A control using DMSO and water was carried out in parallel. For each sample, three independent experiments were run. The bioassays were performed at Laboratório de Entomologia, Núcleo de Endemias da Secretaria de Saúde do Estado do Ceará, Brazil.

RESULTS AND DISCUSSION

Table 1 shows the identified constituents and their percentage composition, as well as their Kovats indices (KI) values listed in order of elution from the DB-5 capillary column. A total of 12 compounds were identified ranging 90.7 to 97.8% of the whole oil samples. As can be noticed from **Table 1**, the first harvesting yielded oils (A and B) almost 2-fold richer in constituents than the second harvesting (A′ & B′), even though the major constituents common to both leaf oils (B and B′) have shown a steady percentage: β-caryophyllene (36.0−41.7%), γ-muurolene (20.3−19.0%), bicyclogermacrene (15.0−17.5%), and α-humulene (13.3−11.7%). On the other hand, the roots oils (A and A′) showed a large increasing in the amount of several major hydrocarbons, for instance β-caryophyllene (12.4−36.1%), α-humulene (5.0−14.6%), γ-muurolene (3.8−10.4%), and bicyclogermacrene (3.7−15.0%). The contrary is observed for the oxygenated compounds; for example, the concentration of 2,2-dimethyl-6-vinylchroman-4-one (25.5−10.3%) has decreased more than twice, while there is almost 5 times less 2-senecioyl-4-vinylphenol (41.0−8.5%). From our data there is not a solid argument to surely explain these figures, particularly because there is no control of the edafoclimatic conditions, but one can maybe speculate on the influence, over the soil, of the irregular raining conditions during the only two seasons (raining and drought) of Northeastern Brazil. Thus, the roots would undergo a greater influence than the aerial parts. In the preliminary analysis of the oils from roots (**Figures 2**

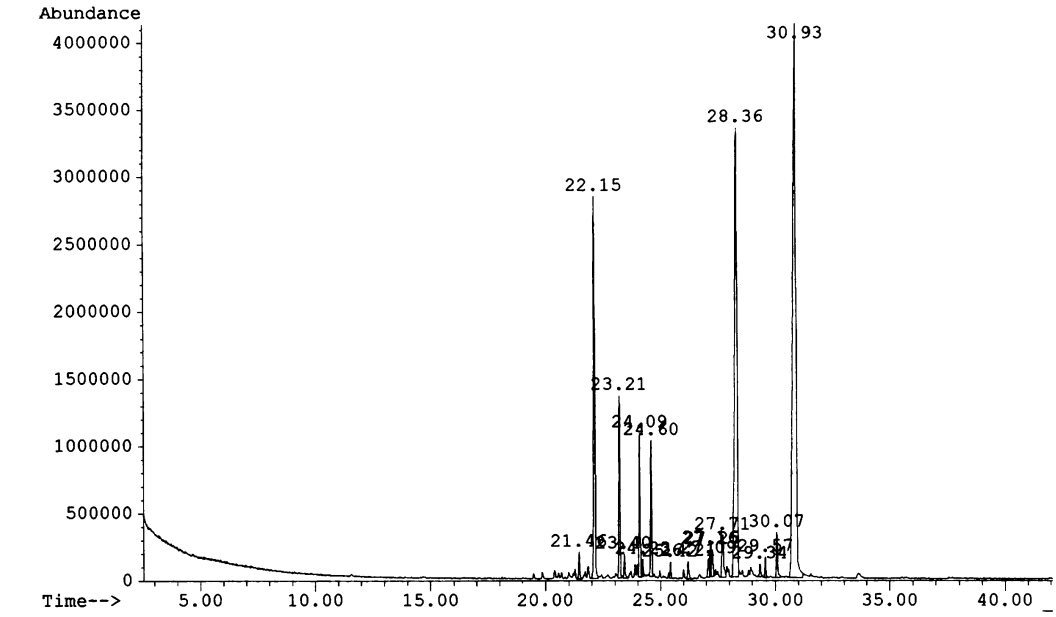


Figure 2. GC chromatogram of the essential oil from roots of *E. betonicaeforme*.

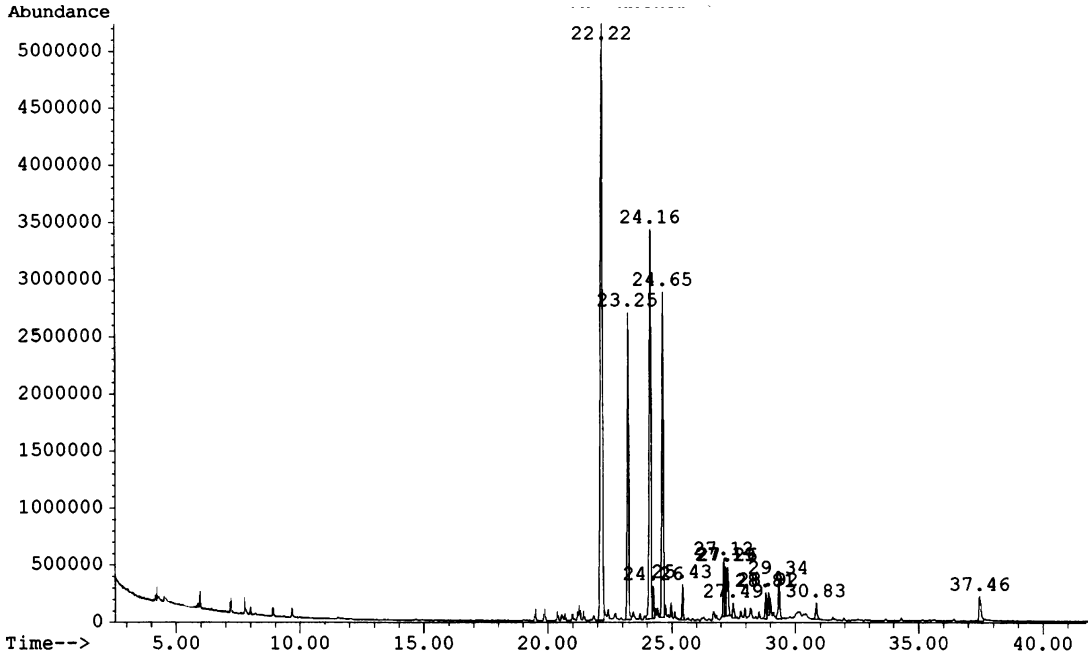


Figure 3. GC chromatogram of the essential oil from leaves of *E. betonicaeforme*.

and **3**), the peaks corresponding to 2,2-dimethyl-6-vinylchroman-4-one (t_R 28.36 min) and 2-senecioyl-4-vinylphenol (t_R 30.93 min) were not identified from their Kovats retention indices and mass spectra. For this reason, an aliquot of the essential oil from roots (oil A) was subjected to silica gel chromatography, and both compounds, in the pure forms, were isolated and characterized by NMR spectral data and comparison with literature values (*25*).

The bioassay results (**Table 2**) showed that the essential oil from leaves (oil B′) was less active, whereas the essential oil from roots (oil A′) demonstrated a significant larvicidal effect. The two major compounds of the essential oil from roots (oil A) were isolated, and their larvicidal potential was also evaluated. Compound **1** (2,2-dimethyl-6-vinylchroman-4-one) was far more active then compound **2** (2-senecioyl-4-vinylphenol). Thus, compound **1** is probably the major active principle responsible for the larvicidal action, causing 84% larval mortality at 12.5 μg/mL; however, the synergistical action of other constituents cannot be disregarded.

Table 2. Larvicidal Activity of the Volatile Constituents *E. betonicaeforme*

	larval mortality (%)			
concn (μg/mL)	oil A	oil B	1	2
500	100	100	nt[a]	nt
250	100	100	nt	nt
100	100	26	100	100
50	100	nt	100	38
25	64	nt	nt	nt
12.5	7	nt	84	36
5	nt	nt	37	nt

[a] nt = not tested.

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