Composition and antimicrobial activity of the essential oil from aerial parts of *Baccharis trinervis* (Lam.) Pers.

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Dedicated to Professor Otto Richard Gottlieb on the occasion of his 85th birthday
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Abstract
The essential oil from the aerial parts of *Baccharis trinervis* (Lam.) Pers., obtained by hydrodistillation, was analyzed by GC-MS, as well as by ^{1}H and ^{13}C NMR. α-Thujene, α-pinene, sabinene, β-pinene, β-phellandrene, (*E*)-lachnophyllum acid methylester and (*Z*)-lachnophyllum acid methyl ester were found to be the major components among the identified constituents. Evaluation of the *in vitro* antimicrobial activity of the essential oil against four bacteria and one fungus is also reported.

Keywords: *Baccharis trinervis*, essential oil, methyl (*Z*)-dec-2-en-4,6-diynoate, methyl (*E*)-dec-2-en-4,6-diynoate, polyacetylenes, antimicrobial activity

Introduction

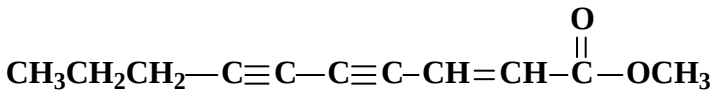
The genus *Baccharis* (Asteraceae, tribe Asterae, subtribe Baccharidinae) comprises about 500 species, most of them found in Brazil and the Andes Mountains range.[1] Species of this genus have been used traditionally for its febrifuge, antirheumatic, antispasmodic and diuretic effects, as well as for headaches, diabetes, hepatobiliary disorders and skin ulceration.[2,3] Regarding to biological activities, antifungal, antiviral, antileukemic, analgesic, antioxidant and anti-inflammatory properties have been reported for plants of this genus.[2,4-7] About 100 species have

been investigated chemically and several classes of secondary metabolites such as sesquiterpenes, diterpenes, chromenes, flavones and trichothecenes have been isolated.[1,8-11] *B. trinervis* is a perennial shrub, used in folk medicine as antiseptic, digestive and to treat snake-bites and it is widely distributed from Mexico to Argentina. Previous phytochemical studies with this species showed the presence of polyacetylenes, flavones and a large number of *neo*-clerodane type diterpenes.[12-14] On the other hand, no reports related to the chemical analysis of the volatile constituents has been found in the literature. Thus, the aim of this work is the identification of the volatile components of its aerial parts by gas chromatography - mass spectrometry (GC–MS). In addition, two of the major components were isolated and characterized by NMR spectroscopy. The antimicrobial activities, against one Gram-positive, three Gram-negative bacteria and one pathogenic fungus have also been evaluated.

Results and Discussion

Aerial parts of *B. trinervis* have been collected at the same site at two different dates: September, 2000 and October 2001 yielding, after hydrodistillation, oil I and oil II, respectively. The results of the analysis are presented in Table 1, where the components are listed in order of elution on a DB-5 column. A total of 18 volatile constituents were identified in oil I, while 26 components were identified in oil II, representing 98.7% and 95.8% of oils, respectively. As can be seen from Table 1, the compositions of both oils were similar for the most representative compounds. The major volatile constituents ($\geq$6.6%) were α-thujene, α-pinene, sabinene, β-pinene, β-phelladrene, methyl (*Z*)-dec-2-en-4,6-diynoate [(*Z*)-lachnophyllum acid methyl ester] and methyl (*E*)-dec-2-en-4,6-diynoate [(*E*)-lachnophyllum acid methyl ester]. The letters are also known as dihydromatricaria esters and have been isolated from another *Baccharis* species.[12] Camphene, *trans*-sabinene hydrate, *cis*-sabinene hydrate, *cis*-*p*-menth-2-en-1-ol, *trans*-*p*-menth-2-en-1-ol, γ-terpinene, cryptone, α-terpineol, neryl acetate, α-copaene, α-humulene and γ-cadinene were only identified in oil II. Nevertheless, these compounds correspond just to 7.7% of the total oil composition. In the preliminary analysis of the oil the peaks corresponding to methyl (*Z*)-dec-2-en-4,6-diynoate and methyl (*E*)-dec-2-en-4,6-diynoate could not be identified from their retention indices and mass spectra. For this reason, a sample of the essential oil was subjected to Si gel chromatography and a fraction enriched with both unknown compounds was obtained. ^{1}H and ^{13}C NMR data, particularly by COSY, HMQC and HMBC analysis, of this fraction allowed the identification of both components as the matricaria ester-type polyacetylenes, methyl (*Z*)-dec-2-en-4,6-diynoate **1** and methyl (*E*)-dec-2-en-4,6-diynoate **2**. Polyacetylenes are some of the most important class of compounds present in the essential oils of several Asteraceae species. These type of compounds (C_{17}-esters) were previously isolated from aerial parts of *B. trinervis* and are regarded as biogenetically important constituents.[12]

The antimicrobial activities of the essential oil from the aerial parts of *B. trinervis* are summarized in Table 2. The volatile constituents of this plant proved to be active against all the tested microorganisms.



1 methyl (*Z*)-dec-2-en-4,6-diynoate
2 methyl (*E*)-dec-2-en-4,6-diynoate
Figure 1

Table 1. Volatile constituents of the aerial parts of *Baccharis trinervis*

Compound[a]	RI[b]	Oil I	Oil II
α-Thujene	918	10.5	6.6
α-Pinene	924	8.7	5.7
Camphene	932	-	0.3
Sabinene	962	10.9	14.2
β-Pinene	964	6.7	1.4
β-Myrcene	979	2.3	2.8
β-Phellandrene	1017	19.8	18.4
(*Z*)-β-Ocimene	1026	4.9	2.3
(*E*)-β-Ocimene	1036	4.6	1.9
γ-Terpinene	1051	0.6	-
cis-Sabinene hydrate	1055	-	0.3
trans-Sabinene hydrate	1080	-	0.2
cis-*p*-Menth-2-en-1-ol	1103	-	0.4
trans-*p*-Menth-2-en-1-ol	1123	-	0.2
Terpinen-4-ol	1168	1.3	2.9
Cryptone	1173	-	1.5
α-Terpineol	1177	-	0.4
cis-Chrysanthenyl acetate	1254	1.6	1.8
α-Terpinyl acetate	1340	1.7	1.8
Neryl acetate	1353	-	0.3
α-Copaene	1357	-	0.2
β-Elemene	1379	0.8	1.9
trans-Caryophyllene	1404	1.9	3.0
α-Humulene	1431	-	0.6
γ-Muurolene	1464	0.6	-
Bicyclogermacrene	1476	0.5	0.5
Methyl (*Z*)-dec-2-en-4,6-diynoate[c]	1493	6.6	14.6
Methyl (*E*)-dec-2-en-4,6-diynoate[c]	1502	14.7	10.7
Total identified		98.7	94.9

[a] Order of elution on DB-5 capillary column. [b] RI = Retention index in reference to $C_8 - C_{26}$ *n*-alkanes on DB-5 column. [c] Compounds identified by MS, ^1H and ^{13}C NMR spectral data.

Table 2. Antimicrobial activity of the essential oil of *Bacharis trinervis*

	MIC (mg/mL)						
Organism	Control	22.8	11.4	5.7	2.85	1.43	0.71
S. aureus	29[a]	22	14	13	12	11	10
E. coli	22[b]	18	15	11	10	9	8
S. cholerae-suis	22[b]	23	15	11	10	9	8
P. aeruginosa	13[c]	15	13	12	11	10	8
C. albicans	23[d]	24	23	14	12	9	7

Diameter of inhibitory zones (mm) at indicated concentrations (mg/mL). Positive control substances (concentration): [a] chloramphenicol (30 μg), [b] amikacin (30 μg), [c] ampicillin (10 μg) and [d] intraconazole (160 μg).

Experimental Section

General Procedures. The mass spectra were carried out on a Hewlett Packard 5971 mass spectrometer. The NMR spectra were recorded on a Bruker Avance DRX-500 instrument at 500 MHz (^{1}H) and 125 MHz (^{13}C), using the residual $CHCl_3$ (δ_H 7.24 / δ_C 77.23) as internal standard. Silica gel 60 (Merck, 70-230 mesh) was used for column chromatography and precoated Silica gel plates (Merck, kieselgel 60 F_{254}, 0.20 mm) were used for analytical chromatography.

Plant material. Aerial parts of *B. trinervis* were collected in September 2000 (oil I) and October 2001 (oil II) from their natural habitat in the Meruóca mountain, State of Ceará (Northeast of Brazil). A voucher specimen (# 30.120) is deposited at the Herbarium Prisco Bezerra (EAC) and authenticated by Dr. E. P. Nunes of the Departamento de Biologia, Universidade Federal do Ceará.

Isolation of the essential oils. Fresh aerial parts (500 g of each harvesting) were subjected to hydrodistillation for 2 h in a modified Clevenger apparatus. Anhydrous Na_2SO_4 was poured into the oil layer, after separation, filtered through a small cotton patch and stored in a refrigerator before analysis. The oil yields were 0.20% (oil I) and 0.23% (oil II).

Oil fractionation. An aliquot of 306 mg of oil II, was subjected to silica gel 60 (particle size 0.063 – 0.200 mm) column chromatography eluting with a gradient of petroleum ether/CH_2Cl_2 (100:0 to 80:20). Thirteen fractions (10 mL) were collected, monitored by TLC, and combined to yield three major fractions, Fr. A (75.5 mg), Fr. B (30.0 mg) and Fr. C (78.2 mg). The fraction C, constituted by the two major compounds was analyzed by ^{1}H and ^{13}C NMR spectroscopy.

Oils analysis. The oils from the aerial parts of *B. trinervis* were analyzed by GC/MS (Hewlett-Packard 5971) employing the following conditions: dimethylpolysiloxane DB-5 fused silica capillary column (30 m x 0.25 mm, film thickness 0.1 μm); carrier gas: helium (1 mL/min);

injector temperature: 250ºC; detector temperature 200ºC; column temperature: 35°-180ºC at 4ºC/min, then 180°-250ºC at 10ºC/min; mass spectra: electronic impact 70 eV. Individual components were identified by two computer library MS searches using retention indices as a preselection routine,[15,16] as well as by visual comparison of the fragmentation pattern with those reported in the literature.[17] Silica gel column chromatography of the crude oil allowed the isolation of both *Z* and *E* trichothecenes which structures were identified by NMR analysis and comparison with ^{13}C NMR data from literature, showing a good matching with Bohlmann finding.[18]

(Z)-Lachnophyllum acid methyl ester. ^{13}C NMR (125 MHz, $CDCl_3$): 165.2 (C-1), 131.1 (C-3), 122.9 (C-2), 90.5 (C-7), 86.9 (C-5), 71.2 (C-4), 65.6 (C-6), 51.9 (-OCH_3), 22.1 (C-8), 22.0 (C-9), 13.8 (C-10). ^{1}H RMN (500 MHz, $CDCl_3$): 6.20 (d, J = 11.3 Hz, H-3), 6.16 (d, J = 11.3 Hz, H-2), 3.76 (s, OCH_3), 2.34 (t, J = 7.1 Hz, 2H-8), 1.58 (m, 2H-9), 1.00 (t, J = 7.4 Hz, 3H-10). EI-MS (70 eV) *m/z* (rel. int.): 176 [M^+] (100).

(E)-Lachnophyllum acid methyl ester. ^{13}C NMR (125 MHz, $CDCl_3$): 166.1 (C-1), 132.4 (C-3), 124.9 (C-2), 89.4 (C-7), 83.7 (C-5), 71.7 (C-4), 65.3 (C-6), 52.3 (-OCH_3), 22.1 (C-8), 22.0 (C-9), 13.8 (C-10). ^{1}H RMN (500 MHz, $CDCl_3$): 6.75 (d, J = 16.1 Hz, H-3), 6.29 (d, J = 16.1 Hz, H-2), 3.75 (s, OCH_3), 2.34 (t, J = 7.1 Hz, 2H-8), 1.58 (m, 2H-9), 0,99 (t, J = 7.4 Hz, 3H-10): EI-MS (70 eV) *m/z* (rel. int.): 176 [M^+] (100).

Biological assays. The agar well diffusion method was employed for the determination of the antimicrobial activities of the essential oil II against a panel of the following microorganisms: *Staphylococcus aureus* (ATCC 6538p), *Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 10536), *Salmomonella chorelae-suis* (ATCC 10708), and *Candida albicans* (ATCC 10231). The culture medium used for bacteria was Mueller-Hinton agar, while Sabouraud agar was used for growing the fungus. Plates for the assay were prepared by dispersing 50 mL of sterile agar medium in sterile Petri plates. The sterile agar plates were streaked with a dilution of the test organism (1 mL of broth culture in 9 mL of sterile water). Wells (5 mm in diameter) were created in the agar and 25 μL of solutions ranging from 22.8 to 0.71 mg/mL were delivered into them. After incubation for 20 h at 35 °C, all plates were examined for any zones of growing inhibition, and the diameters of these zones measured in millimeters. Standard antibiotics (chloramphenicol, amicacin and ampicillin) were used in order to control the sensitivity of the tested bacteria, while intraconazole was used in order to control the tested fungus. $CHCl_3$ as the solvent control was also analyzed. The minimal inhibitory concentrations (MICs) were determined by, applying to the agar plates 25 μl of $CHCl_3$ solutions of the samples, starting with a maximum concentration of 22.8 mg/mL, and then reducing it by successive two-fold dilutions of that stock solution. Each experiment was performed in triplicate.

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