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Chemical characterization and antimicrobial evaluation of the essential oils from *Baccharis uncinella* D.C. and *Baccharis semiserrata* D.C. (Asteraceae)

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Due to the biological properties associated to the *Baccharis* genus and the limited information on its antimicrobial properties, the present work investigates the chemical composition and antibacterial, antifungal and antiprotozoal activities of the essential oils of two species, *B. uncinella* from Campo Alegre and *B. semiserrata* from Atalanta, both places located in Santa Catarina State, Southern Brazil. Oils were obtained by hydrodistillation and were analyzed by gas chromatography–flame ionization detector (GC–FID) and GC–mass spectrometry (GC–MS). The dry leaf essential oil yield from *B. uncinella* was 0.65% (m/m), and 0.50% and 0.22% (m/m) for dry leaf and twig from *B. semiserrata*, respectively. The main components in the oil from *B. uncinella* were α-pinene (9.0%), β-pinene (9.2%), limonene (11.5%), β-caryophyllene (13.2%) and spathulenol (9.3%). The following major compounds were identified in the leaf essential oil from *B. semiserrata*: α-pinene (4.3%), β-pinene (11.4%), limonene (6.7%), β-caryophyllene (8.2%), γ-muurolene (7.3%), bicyclogermacrene (8.0%), *E*-nerolidol (9.6%) and spathulenol (9.8%). Similarly, in the essential oil from the twigs were α-pinene (3.3%), β-pinene (7.9%), limonene (9.1%), β-caryophyllene (3.9%), bicyclogermacrene (6.0%), spathulenol (25.1%), caryophyllene oxide (8.0%) and globulol (5.6%). The antibacterial activity was determined by microdilution method. *Baccharis uncinella* oil was inactive for all bacteria tested and *B. semiserrata* twigs oil presented moderate activity against *Staphylococcus aureus* and the leaves weak activity against *S. aureus* and *Bacillus cereus*. The antifungal activity was determinate by the agar dilution method. *Baccharis uncinella* oil was active against *M. gypseum*, *T. mentagrophytes*, *C. neoformans* and *M. canis*. Both, leaf and twig essential oils from *B. semiserrata* were active against *Microsporum gypseum*, *Candida albicans*, *Epidermophyton flocosum*, *Trichophyton mentagrophytes* and *Cryptococcus neoformans*. *Baccharis semiserrata* leaf essential oil was also active against *Trichophyton rubrum*. In the antiprotozoal assays, leaf essential oil from *B. uncinella* gave an IC_{50} of 223 μg/mL against *Tripanossoma cruzi*. Both leaf essential oils were active only at 500 μg/mL against *Leishmania braziliensis*.

Keywords: *Baccharis uncinella*; *Baccharis semiserrata*; essential oil composition; spathulenol; antibacterial; antifungal and antiprotozoal activities

Introduction

The Asteraceae family is the most numerous systematic group of the Angiospermae (1). It comprises the *Baccharis* genus, which is widely distributed in the Southeastern and Southern Regions of Brazil, mainly at tropical mountains (2, 3).

The *Baccharis* genus has a broad range of biological properties as summarized by Passero et al. (4) and Verdi et al. (3). Its economical potential has led to studies aiming to a possible large-scale essential oil production (5).

Baccharis uncinella D.C., popularly known as 'vassoura lageana', has been the subject of several works dealing mainly with its volatile chemical composition (5–10) but not exclusively (11). The essential oil variability associated with different populations and seasonality was investigated by means of Hierarchical Cluster Analysis (12). Physicochemical characterization was carried out by Fabiane et al. (8) with samples obtained from a population occurring in the Southwestern State of Paraná, Southern Brazil. Oils from the same population were assayed against Gram-negative and Gram-positive bacteria using the disk diffusion method (13). The leishmanicidal activity for this specie was investigated using fractions of the ethanolic extract from aerial parts previously treated with hexane, as well as with isolated compounds (4). Similarly, components obtained from the same extract exhibited significant anti-inflammatory activity (14). Furthermore, this plant has been used by the Xokleng Indians from

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the region of Ibirama in the State of Santa Catarina (SC), Southern Brazil, as tranquilizer and blood pressure regulator (10, 15).

Popularly known as 'tupixaba' or 'vassoura do campo', *B. semiserrata* D.C. is not mentioned in the Brazilian popular medicine and just two studies have been published concerning the chemical characterization of the volatile constituents (7, 16). Some biological activities were also examined using volatile and non-volatile extracts (16).

Due to the limited information about the antimicrobial activity of these species, the antibacterial, antifungal and antiprotozoal properties of essential oils from *B. uncinella* and *B. semiserrata* are investigated. The chemical characterization of the twig essential oil from *B. semiserrata* is here reported for the first time, as well as the evaluation of the antifungal and antiprotozoal activities of the essential oils from both species.

Experimental

Plant material

Aerial parts from *B. uncinella* were collected in Campo Alegre, SC-Brazil (26°12'13" S, 49°08'21" W, at an altitude of *c*. 870 m) and *B. semiserrata* was collected in Atalanta, SC-Brazil (27°28' S, 49°48' W, at an altitude of *c*. 548 m). Voucher specimens have been deposited at the Herbarium Dr. Roberto Miguel Klein of the Regional University of Blumenau, FURB, under the numbers 4500 and 8557 for *B. uncinella* and *B. semiserrata*, respectively.

Oil isolation

The plant material was dried in the shade at room temperature (*c*. 20°C), until constant mass. The essential oil was obtained through hydrodistillation in triplicate using a modified Clevenger-type apparatus, under a nitrogen atmosphere for 4 hours. The obtained oils were separated and dried over anhydrous sodium sulfate, stored in hermetically sealed in glass containers and kept under refrigeration at 4°C before analysis.

Analysis

The essential oils from *B. uncinella* and *B. semiserrata* were characterized by gas chromatography (GC) on Shimadzu 14B equipped with a flame ionization detector (FID) and capillary column of fused silica (Simplicity-1/Supelco, low polarity) with 30 m × 0.25 mm × 0.25 μm film thickness. Oven temperature was raised from 60° to 195°C with a rate of 3°C/minute and then from 195° to 235°C with a rate of 20°C/minute, remaining at 235°C for 30 minutes. Injector and detector were set at 220° and 240°C, respectively. Helium was used as carrier gas at a flow rate of 1 mL/minute and diluted samples (1% in hexane, m/v) of 0.5 μL were manually injected in the split mode (1:100). Peak area percentages were used for obtaining quantitative data, without taking into account relative response factors. The GC–MS analysis were performed on a Varian CP-3800 gas chromatograph coupled to a Saturn 2000 mass spectrometer fitted with the same column, conditions and temperature program as that for the GC experiments. The mass selective detector in the electron impact mode was operating at 70 eV and mass range of 40–400 amu. Retention indices (RI) for all the components were determined relative to a series of linear alkanes (C_8–C_{19}). Compounds identification was based on RI comparison and mass spectra computer matching (NIST 02 library) and the literature (17).

Antibacterial activity

Minimal inhibitory concentration (MIC) was evaluated by the microdilution method (18). The oils were individually tested against Gram-negative strains of *Acinetobacter baumanii* (ATCC 17978), *Escherichia coli* (ATCC 25922) and *Pseudomonas aeruginosa* (ATCC 27853), and Gram-positive strains of *Staphylococcus aureus* (ATCC 25923) and *Bacillus cereus* (ATCC 11778). Initially, the essential oils were dissolved in 20% aqueous DMSO to give a 16,000 μg/mL stock solution. One hundred microliters of the stock solution was transferred to a 96-well microplate and concentrations varying from 8,000 to 62.5 μg/mL were obtained by serial twofold dilution adding 100 μL of Müller–Hinton culture medium (MH). Five microliters containing 1.5×10^8 colony-forming units per mL (0.5 of the McFarland scale), was added to each well. Some wells were reserved in order to control the sterility of the medium (MH medium with DMSO 20%), the bacterial growth (MH medium DMSO 20% and bacterial inoculum), and also the action of the reference antibacterial drug (MH medium, DMSO 20%, bacterial inoculum and gentamicine) (19). The final volume in each well was 105 μL. Microplates were incubated in conditions of aerobiosis for 18–24 hours at 35°C. Then, 10 μL of 2,3,5-triphenyl tetrazolium chloride (TTC; 5 mg/mL in methanol) were added to each well for the detection of color change where TTC (colorless) to red, reflects the active bacterial metabolism. The MIC was defined as the lowest concentration of oil that visibly inhibited the bacterial growth (20). The assay was carried out in duplicate.

Antifungal activity

MIC was evaluated by the agar dilution method (21). The yeast fungi used were *Candida albicans* (ATCC 10231) and *Cryptococcus neoformans* (ATCC 32264) and the filamentous fungi were *Aspergillus flavus* (ATCC 9170), *Aspergillus fumigatus* (ATCC 26934), *Rhizopus sp*. (CL 35), *Epidermophyton floccosum*

(C114), *Microsporum canis* (C112), *Microsporum gypseum* (C115), *Trichophyton mentagrophytes* (ATCC 9972) and *Trichophyton rubrum* (C137). For the filamentous fungi, the inocula suspensions were obtained according to reported procedures and were adjusted to the range of 1.0×10^6 to 5.0×10^6 spores/mL by microscopic counting using a Neubauer chamber (22). The yeasts were prepared according to Espinel-Ingrof and Pfaller (23) and cell suspension was adjusted to a transmittance of a 0.5 McFarland standard scale at 530 nm.

The oils were dissolved in 40% DMSO solution in concentrations varying from 1,000 to 7.81 μg/mL, and added to 1 mL of dextrosed Sabouraud agar in a culture flask, immediately followed by homogenization of the mixture. After solidification of the respective culture media, the microorganisms, previously activated, were inoculated into the corresponding series and incubated at 37°C for 24–48 hours for the yeast fungi and at room temperature (25°C) from 5 to 15 days for the filamentous fungi. Controls of the culture medium and solvent were used. The result was considered valid only when there was microbial growth under the growth control, inhibition in the control of sterility and in the reference antifungal agent. The antifungal control used was ketoconazole (Sigma K-1003) and the assay was carried out in triplicate.

In vitro *evaluation of the leishmanicidal activity*

The essential oils were previously solubilized in DMSO to a concentration of 16 mg/mL. Promastigote forms of *Leishmania braziliensis* (strain H3), cultured in Schneider medium, at a concentration of 3×10^6 parasites/mL, were seeded and incubated with different concentrations (500, 100, 20, 4 and 0.8 μg/mL) for 48 hours at 26°C. As means of control, parasites were grown in the presence of DMSO (1%) and Amphotericin B (0.1 μM). The antiprotozoal activity was evaluated through the MTT technique, according to Sieuwerts et al. (24), with addition of 50 μL of MTT (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) solution and incubation at 26°C for 4 hours. The supernatant was removed and 100 μL of DMSO were added to solubilize the formazan crystals, while optical density was determined at 540 nm.

In vitro *evaluation of the trypanocidal activity*

Epimastigote forms of *Tripanossoma cruzi* (strain Y, at a concentration of 5×10^6 parasites/mL) cultured in liver infusion triptose (LIT medium) added with 10% bovine fetal serum (BFS), penicillin 10 U/mL and streptomycin 10 μg/mL at pH 7.2, were seeded and incubated with different oil concentrations (500, 100, 20, 4 and 0.8 μg/mL) for 48 hours at 26°C. As control, parasites were grown in the presence of DMSO (1%) and benznidazole (10 μM). The anti-parasitic activity was evaluated through the MTT technique, with addition of 50 μL of MTT solution and incubation at 26°C for 6 hours. The supernatant was removed and 20 μL of 10% sodium dodecyl sulfate (SDS) in HCl 0.01 M were added; after 1 hour under the same conditions of incubation, 100 μL of DMSO were added to solubilize the formazan crystals. The antiprotozoal activity of the oils was made based on the reading of the optical density determined at 540 nm, corresponding to the percentage of living cells.

Results and discussion

Chemical composition

The dry leaf essential oil yield from *B. uncinella*, Campo Alegre-SC, was 0.65% (m/m). *Baccharis semiserrata* from Atalanta-SC showed yields of 0.50% and 0.22% (m/m) for dry leaf and twig, respectively. The essential oils were subjected to detailed GC and GC–MS analysis in order to determine their chemical composition. Table 1 contains the 32 identified compounds, corresponding to 88.1–92.0% of the oil constituents.

In *B. uncinella*, the sesquiterpene hydrocarbons were the main chemical group, comprising 38.4% of the sample with α-pinene (9.0%), β-pinene (9.2%), limonene (11.5%), β-caryophyllene (13.2%), germacrene D (3.1%), viridiflorene (8.4%), spathulenol (9.3%) and caryophyllene oxide (3.3%) as its major compounds (Figure 1).

Both oils from *B. semiserrata* have high percentage of sesquiterpenes in their content. The leaf oil presented 33.8% of sesquiterpene hydrocarbons and 34.5% of oxygenated sesquiterpenes, while the twig oil showed 49.3% of oxygenated sesquiterpenes. The following major compounds were identified in the leaf essential oil from *B. semiserrata* (Figure 2): α-pinene (4.3%), β-pinene (11.4%), limonene (6.7%), β-caryophyllene (8.2%), γ-muurolene (7.3%), bicyclogermacrene (8.0%), *E*-nerolidol (9.6%) and spathulenol (9.8%).

Similarly, the main constituents in the twig oil were α-pinene (3.3%), β-pinene (7.9%), limonene (9.1%), β-caryophyllene (3.9%), bicyclogermacrene (6.0%), spathulenol (25.1%), caryophyllene oxide (8.0%) and globulol (5.6%) (Figure 3).

As it can easily be observed, five out of eight main compounds are present in all analyzed samples, namely α-pinene, β-pinene, limonene, β-caryophyllene and spathulenol.

The essential oil profile of *B. uncinella* from Campo Alegre-SC was similar to those reported in studies

Table 1. Chemical composition (%) of volatile oils from *Baccharis uncinella* and *Baccharis semiserrata*.

No.	Compounds	B. uncinella[a] Leaf	B. semiserrata[a] Leaf	B. semiserrata[a] Twig	$RI_{calc.}$	$RI_{lit.}$ (17)
1	α-Thujene	3.0±0.9	–	–	931	924
2	α-Pinene	9.0±0.9	4.3±1.4	3.3±0.8	934	932
3	Sabinene	1.3±0.3	–	–	974	969
4	β-Pinene	9.2±1.7	11.4±1.8	7.9±2.0	975	974
5	β-Myrcene	1.2±0.3	0.8±0.2	0.9±0.2	988	988
6	Limonene	11.5±2.4	6.7±1.4	9.1±1.6	1026	1024
7	(*E*)-β-Ocimene	–	0.5±0.1	–	1042	1044
8	γ-Terpinene	0.8±0.2	–	–	1057	1054
9	Terpinen-4-ol	1.1±0.2	–	–	1177	1174
10	α-Copaene	1.2±0.3	–	–	1376	1374
11	β-Elemene	–	1.1±0.6	0.8±0.0	1390	1389
12	β-Caryophyllene	13.2±2.2	8.2±0.5	3.9±0.6	1418	1417
13	Aromadendrene	0.9±0.2	0.7±0.3	0.9±0.0	1440	1439
14	α-Caryophyllene	2.3±0.3	1.3±0.2	0.7±0.1	1452	1452
15	Alloaromadendrene	1.0±0.2	0.8±0.4	0.6±0.0	1459	1458
16	*trans*-Cadina-1(6),4-diene	–	0.8±0.3	–	1472	1476
17	γ-Muurolene	0.8±0.1	7.3±1.6	2.5±1.0	1475	1478
18	Germacrene D	3.1±0.7	–	–	1483	1484
19	Viridiflorene	8.4±1.6	0.9±0.0	–	1498	1496
20	Bicyclogermacrene	–	8.0±0.9	6.0±0.8	1499	1500
21	α-Muurolene	1.0±0.2	0.9±0.2	–	1501	1500
22	γ-Cadinene	1.8±0.2	0.8±0.3	0.8±0.2	1514	1513
23	δ-Cadinene	3.0±0.5	3.0±0.7	1.4±0.8	1522	1522
24	α-Calacorene	1.7±0.9	–	–	1549	1544
25	(*E*)-Nerolidol	–	9.6±1.0	2.6±1.5	1564	1561
26	Spathulenol	9.3±2.1	9.8±2.2	25.1±2.7	1579	1577
27	Caryophyllene oxide	3.3±0.5	3.6±2.1	8.0±1.3	1584	1582
28	Globulol	0.9±0.2	2.9±1.4	5.6±0.9	1588	1590
29	Viridiflorol	–	3.4±0.6	1.9±0.2	1594	1592
30	τ-Cadinol	–	0.8±0.3	2.4±1.1	1636	1638
31	τ-Muurolol (epi-α)	–	1.8±0.6	1.5±0.8	1643	1640
32	α-Cadinol	–	2.6±0.8	2.2±1.3	1654	1652
Total		89.0	92.0	88.1	–	–
Monoterpene hydrocarbons		36.0	23.7	21.2	–	–
Oxygenated monoterpenes		1.1	0.0	0.0	–	–
Sesquiterpene hydrocarbons		38.4	33.8	17.6	–	–
Oxygenated sesquiterpenes		13.5	34.5	49.3	–	–

Notes: [a]Concentrations are expressed as mean ± standard deviation; $RI_{calc.}$, retention index calculated; $RI_{lit.}$, retention index literature reference (17).

where the plant material was collected in the State of Rio Grande do Sul, Southern Brazil (5, 12). The criteria of similarity used were the presence of α-pinene, β-pinene, limonene, β-caryophyllene and spathulenol among the major compounds and the absence of *E*-nerolidol.

Leaf and twig essential oils from *B. semiserrata*, Atalanta-SC, presented very similar qualitative chemical composition. The oxygenated sesquiterpene *E*-nerolidol, present in twig and leaf essential oils from *B. semiserrata*, Santa Catarina, was not detected in the same specie occurring in Rio Grande do Sul (7) and it is not mentioned by Mendes (16).

The combination of spathulenol and *E*-nerolidol in the essential oil of aerial parts from *B. semiserrata* show the superior quality of this species when compared with the essential oil of *B. uncinella*, since one may consider its use in perfumery and also as food flavoring (8, 25, 26).

Antimicrobial evaluation

The essential oil from *B. uncinella* and *B. semiserrata* were assayed against two Gram-positive and three Gram-negative bacteria as presented in Table 2.

The present study revealed that *B. uncinella* showed MIC superior to 1000 μg/mL for all the bacteria tested; therefore this oil can be considered inactive according to Ebrahimabadi et al. (27). Although leaf essential oil from *B. uncinella*, Southwestern Paraná, had been tested as antibacterial (13), a comparison with the present work is not appropriate since the disc

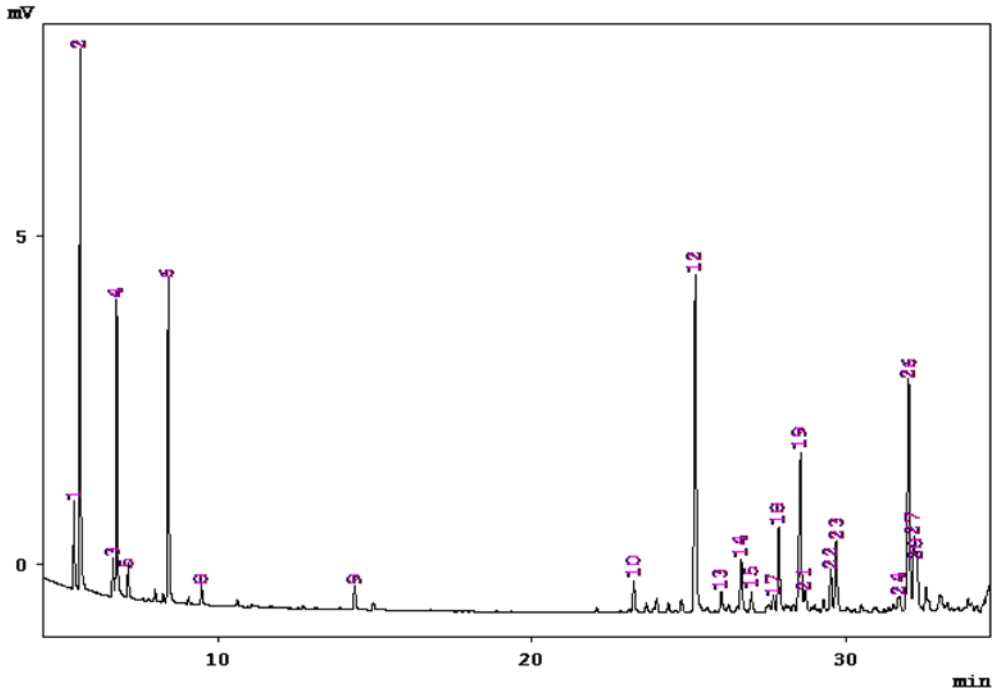

Figure 1. Typical gas chromatogram of the leaf essential oil from *Baccharis uncinella*. The numbered peaks are the identified compounds (Table 1).

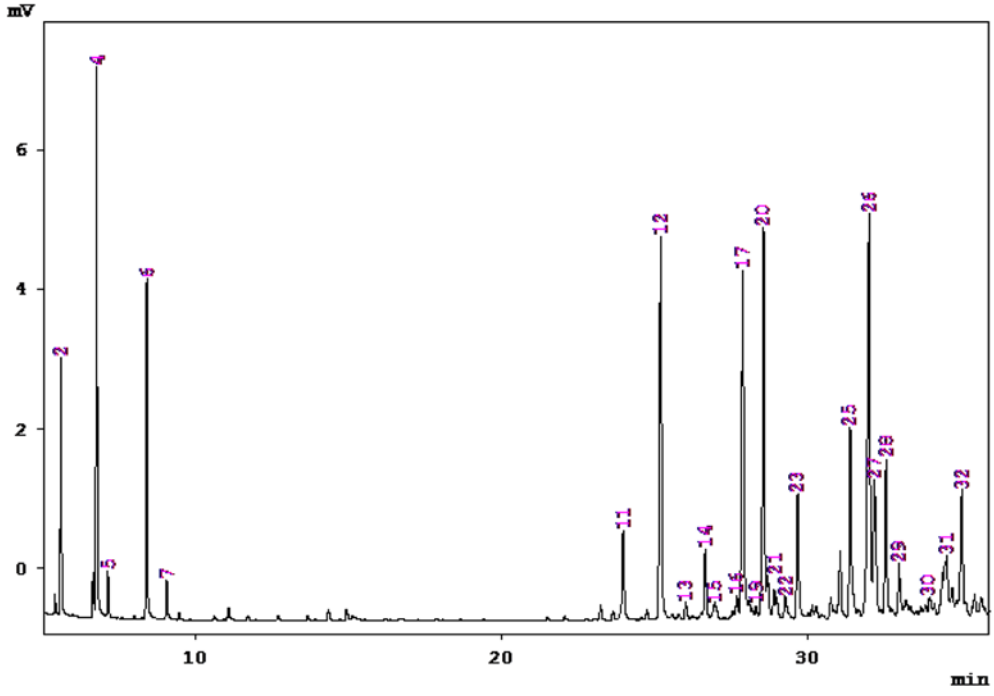

Figure 2. Typical gas chromatogram of the leaf essential oil from *Baccharis semiserrata*. The numbered peaks are the identified compounds (Table 1).

diffusion method, which should not be considered a quantitative screening (28), was used in the previous investigation.

Plant compounds are routinely classified as 'antimicrobials' on the basis of susceptibility tests that produce MICs in the range of 100–1000 µg/mL (29). Therefore,

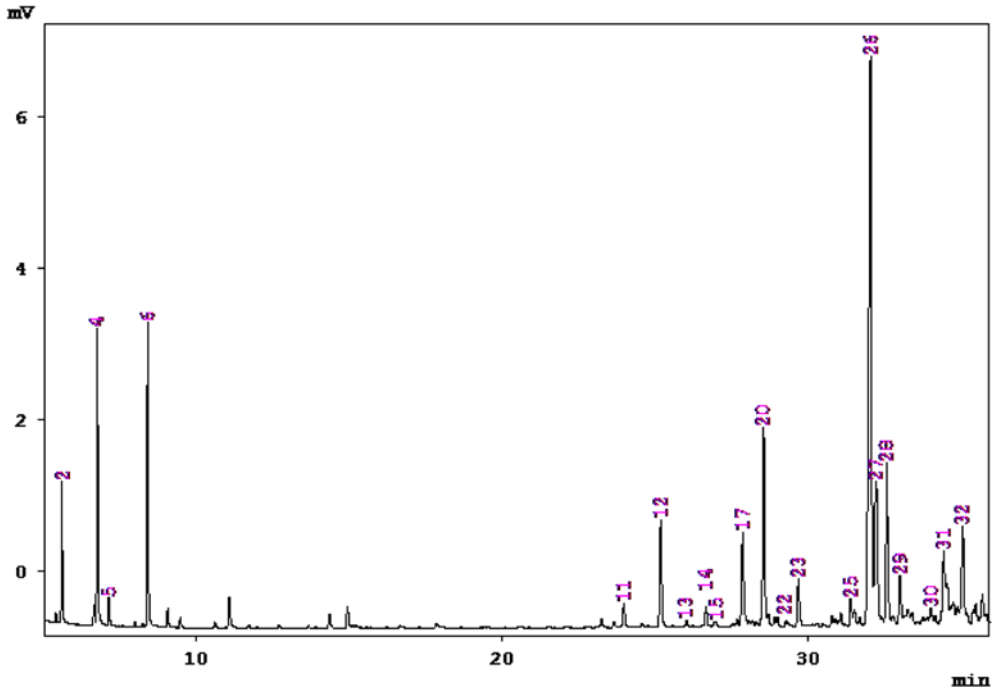

Figure 3. Typical gas chromatogram of the twig essential oil from *Baccharis semiserrata*. The numbered peaks are the identified compounds (Table 1).

based on antimicrobial activity classification (30, 31), *B. semiserrata* twig essential oil presented moderate activity against *S. aureus* and the leaf essential oil presented weak activity against *S. aureus* and *B. cereus*, both Gram-positive bacteria.

With regard to antifungal activity, the results are shown in Table 3.

The dermathophytes, and yeasts *C. albicans* and *C. neoformans*, were selectively inhibited by the essential oils of both plants. In contrast, they did not exert an inhibitory effect against hyaline hyphomycetes of the genus *Aspergillus* and the zygomycete *Rhizopus* spp., which are intrinsically more resistant than others. The leaf essential oil from *B. uncinella* was weak against *M. gypseum* and *T. mentagrophytes*, and moderate against *M. canis* and *C. neoforms*. Both leaf and twig essential oils from *B. semiserrata* showed weak activity against *M. gypseum* and *C. albicans* and moderate against *E. flocosum*, *T. mentagrophytes* and *C. neoformans*. Only the leaf essential oil from *B. semiserrata* was active against *T. rubrum* being this activity considered moderate.

This is the first work demonstrating the tripanocidal and leishmanicidal effects of volatile constituents from *B. uncinella* and *B. semiserrata*.

Parasite growth inhibition was only observed at 500 µg/mL for *T. cruzi* and *L. braziliensis*. For *T. cruzi*, the oil from *B. uncinella* showed an IC_{50} of 223 µg/mL. The flavonoid pectolineragenin isolated by Passero et al. (4) from aerial parts from *B. uncinella* was active against *L. braziliensis* with an IC_{50} of $110^{+}30$ µg/mL. On the other hand, cafeic acid and the binary mixture

Table 2. Antibacterial activity (minimal inhibitory concentration) of essential oils from *Baccharis uncinella* and *B Baccharis semiserrata*.

Bacteria	B. uncinella[a] Leaf	B. semiserrata[a] Leaf	B. semiserrata[a] Twig	Gentamicine
Staphylococcus aureus	>1000	1000	500	1
Bacillus cereus	>1000	1000	>1000	1
Acinetobacter baumanii	>1000	>1000	>1000	1
Escherichia coli	>1000	>1000	>1000	6
Pseudomonas aeruginosa	>1000	>1000	>1000	1

Notes: [a]Concentrations are expressed in µg/mL. All bacteria tested have shown positive results in bacterial growth control.

Table 3. Antifungal activity (minimal inhibitory concentration) of essential oils from *Baccharis uncinella* and *Baccharis semiserrata*.

Microorganisms	B. uncinella[a] Leaf	B. semiserrata[a] Leaf	B. semiserrata[a] Twig	Ketoconazole
Aspergillus flavus	>1000	>1000	>1000	8
Aspergillus fumigatus	>1000	>1000	>1000	7
Rhizopus sp.	>1000	>1000	>1000	4
Epidermophyton flocosum	>1000	500	250	0.5
Microsporum canis	500	>1000	>1000	8
Microsporum gypseum	1000	1000	1000	6
Trichophyton mentagrophytes	1000	500	250	8
Trichophyton rubrum	>1000	500	>1000	4
Candida albicans	>1000	1000	1000	0.3
Cryptococcus neoformans	500	250	250	6

oleanolic:ursolic acids, also isolated from the same plant extract, were inactive.

In general, the essential oils from *B. semiserrata* were more active than the oil from *B. uncinella* and this activity might be related to the higher oxygenated sesquiterpenes content (Table 1) in *B. semiserrata* (32) and it might also be attributed to the presence of minor components, such as τ-muurolol and α-cadinol (33), and caryophyllene oxide (33, 34). The study shows that the antimicrobial results are promising, especially towards fungi, which could justify further studies on the same subject.

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