Antimicrobial Activity of the Crude Extracts and Fractions of Three Baccharis Species

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Abstract

The antimicrobial activities of crude extracts and fractions from three Baccharis L. species were tested against Staphylococcus aureus (ATCC 6538), Escherichia coli (ATCC 8738), Pseudomonas aeruginosa (ATCC 9027), and Candida albicans (ATCC 10231) using the microdilution plate method. The results showed that the crude extract from female B. burchellii Baker had moderate activity against S. aureus (minimum inhibitory concentration [MIC], 0.9 mg.mL^{-1}). Among the fractions obtained from this extract, the dichloromethane fraction showed the highest activity against S. aureus (MIC, 0.4 mg.mL^{-1}). The ethyl acetate fractions from female B. burchellii (MIC, 0.6 mg.mL^{-1} and 1.2 mg.mL^{-1}, respectively) and B. aracatubaensis Malag (MIC, 1.1 mg.mL^{-1} for both) were moderately effective against S. aureus and P. aeruginosa. The extracts from B. organensis Baker showed no significant activity against any organism tested. None of the extracts from Baccharis species showed any activity against C. albicans. In addition, the chemical investigation of the dichloromethane and ethyl acetate fractions from female B. burchellii was carried out, resulting in the identification of trans-ferulic acid, ethyl caffeate, naringenin, and 7-hydroxy-benzaldehyde compounds. These phenolic compounds were found in other species of Baccharis and have been shown to possess antimicrobial activity. The results obtained in this work with respect to B. burchellii indicate that this species is a promising source of compounds with antimicrobial activities.

Keywords: Asteraceae; Baccharis; Antimicrobial; Phenolic compounds

Introduction

The Baccharis L. genus consists of about 500 species distributed exclusively in the Americas, found in the southern United States to southern Argentina and Chile [1,2]. There are about 178 described species in Brazil, mainly located in the southeastern and southern regions [3]. Species of this genus are well known for their use in folk medicine, especially in South America. These plants are used for the treatment of various diseases such as ulcers, gastritis, inflammatory, and diabetes, and skin infections [4-6]. Numerous biological activities have been attributed to essential oils, extracts, and compounds isolated from the Baccharis genus [7-9]. Campos et al. noted that several species of this genus have shown anti-inflammatory, anti-diabetic, anti-ulcer, or anti-microbial activities. However, there are very few reports on the antimicrobial activities of the genus Baccharis [10]. In this context, the aim of this study was to evaluate the antimicrobial activities of crude extracts (male and female specimens) and fractions (female specimens) from Baccharis organensis Baker, Baccharis burchellii Baker, and Baccharis aracatubaensis Malag, as well as to perform a chemical analysis of fractions obtained from female B. burchellii.

Materials and Methods

Chemicals and reagents

Dimethyl sulfoxide (DMSO), methanol (MeOH), ethyl acetate (EtOAc), and dichloromethane (CH2Cl2) were purchased from Tedia (Fairfield, OH, USA). Deuterium solvents (CDCl3, DMSO-d6, and CD3OD) (≥ 99.9% D) were purchased from Cambridge Isotope Laboratories, Inc. (Andover, MA, USA). Mass spectrometry (MS)-grade methanol and acetonitrile (ACN) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Distilled and deionized water was obtained using a Millipore system (Millipore Milli-RO plus, MA, USA). Tryptic soy broth (TSB), Mueller-Hinton broth (MHB), sabouraud dextrose broth (SDB), tryptic soy agar (TSA), sabouraud agar, chloramphenicol, soy broth (TSB), Mueller-Hinton broth (MHB), sabouraud dextrose agar, chloramphenicol, Mueller-Hinton agar and tryptic soy agar (TSA) were used for the cultivation of the bacteria. GC columns (60 m, 0.25 mm, 0.25 μm) were used for analytical thin layer chromatography (TLC). Gel plates were coated with precoated silica gel plates (60 F254, Merck, Darmstadt, Germany).

Chemical analysis

NMR data were acquired at 303 K in CDCl3 for all compounds by using a Bruker AVANCE 600 NMR spectrometer operating at 14.1 Tesla, and 1H and 13C spectra were recorded at 600.13 and 150.61 MHz, respectively. The spectrometer was equipped with a 5-mm quadrinuclear inverse detection probe with z-gradient. One-bond and long-range 1H-13C correlations from the HSQC and HMBC NMR experiments were obtained with average coupling constants J_{1H-13C} and J_{13C-13C} optimized for 140 and 8 Hz, respectively. The 1H and 13C NMR chemical shifts are given in ppm relative to the tetramethylsilane (TMS) signal as the internal reference, and the coupling constants (J) in Hz. Low-resolution electrospray ionization mass spectrometry (LC/ESIMS) experiments were performed on a Thermo LTQ XL Ion Trap, equipped with an ESI source. Silica gel 60 (70-230 mesh) and sephadex LH-20 (25-100 μm) were used for column chromatography (CC), and precoated silica gel plates (60 F254, Merck, 0.2 mm, aluminum) were used for analytical thin layer chromatography (TLC). Gel plates were sprayed with p-anisaldehyde and heated, followed by exposure to UV light for visualization of compounds.

Plant material collection

Botanical materials of male and female specimens of Baccharis were collected separately and randomly along a transect within the same population in November 2013 in the "Morro do Canal", Municipality of...
of Piranquara, Paraná State, Brazil. The *B. aracatubaensis* (leaves) and *B. organensis* (leaves) samples were collected at [25º30’52.48” S/48º59’10.41” O] and [25º30’52.39’’ S/48º59’10.78’’ O], respectively, at an elevation of 1200-1300 m. The *B. burchellii* (cladodes) samples were collected in the proximity of one river in [25º31’11.54” S/49º00’21.17” O] at an elevation of 906 m. The species were identified by Osmar dos Santos Ribas, Dr. Gustavo Heiden, and Dr. Angelo Alberto Schneider. The voucher specimens were deposited in the Botanical Museum of Curitiba (MBM), under the registration numbers: (MBM-286268/MBM-286267), (MBM-386275/MBM-386266), and (MBM-386257/MBM-386256), respectively.

The access to the botanical material was authorized and licensed by the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and the Conselho de Gestão do Patrimônio Genético (CGEN/MMA) and registered as N° 010304/2013-4.

### Collection and purification of the extracts

The air-dried botanical materials from the male and female specimens of *B. aracatubaensis* (0.996 kg and 1.0 kg, respectively), *B. burchellii* (1.1 kg and 1.2 kg, respectively), and *B. organensis* (0.490 kg and 0.560 kg, respectively) were extracted successively with a solution of ethanol:water (90:10, v/v), at room temperature. The solvent was removed from the extracts under reduced pressure to obtain the crude extracts Ba-M (129.1 g) and Ba-F (121.6 g), Bb-M (211.4 g) and Bb-F (230.4 g), and Bo-M (158.2 g) and Bo-F (184.7 g), respectively. The crude extracts from all the three female species were defatted with n-hexane; then, the crude extracts Bb-F and Bo-F were subjected to liquid-liquid partitioning with the solvents EtOAc (3 × 500 mL) to yield Bb-Ae (26.7 g) and Bo-Ae (20.8 g) fractions, respectively. The crude extract of *B. aracatubaensis* was subjected to liquid-liquid partitioning with the solvents EtOAc (3 × 500 mL) to yield Ba-Ae (6.2 g) and remaining aqueous residue to yield Ba-Aq (26.2 g) fractions.

Part of the Bb-Ae fraction (4.5 g) was subjected to silica gel CC and was eluted with increasing concentrations of CHCl3 in n-hexane (1000 to 1090, v/v), followed by EtOAc in CHCl3 (1000 to 370, v/v), and MeOH in EtOAc (100% to 30, v/v), affording 181 sub-fractions (30 mL each) that were pooled into 10 groups according to TLC analysis. Groups 2 (48.8 mg), 5 (131.8 mg), and 6 (171.9 mg) resulted from MeOH and 5% DMSO, and the EtOAc and aqueous fractions were solubilized in water.

In this assay, the crude extracts were used at concentrations between 0.78 µg.mL⁻¹ and 100 µg.mL⁻¹, and the fractions were used at concentrations between 0.39 µg.mL⁻¹ and 50 µg.mL⁻¹. In each well of the microplate, was added 100 µL MBH for bacterial strains or 100 µL of SDB for yeast strain. In the first well, was added 100 µL of extracts or essential oils, and then performed serial dilutions (1:1, v/v), followed by addition of 10 µL of the inoculum into each well, and incubated at 35°C for 20 h. After the incubation period, was added 20 µL of 0.125% TTC solution to all wells of the plates, followed by two hours of incubation. The absorbance was measured using a spectrophotometer at 540 nm. The minimal inhibitory concentration (MIC) was defined as the lowest concentration of the extract showing no visible bacterial growth after the incubation period.

### Results and Discussion

#### Identification of the compounds

The chromatographic fractionation was achieved only for the fractions from female *B. burchellii* (Bb-Ae and Bb-D), resulting in the identification of the four compounds by LRESIMS and 1D and 2D NMR and with comparison with previous literature. In the analysis of the Bb-D fraction from group 2 was identified, trans-ferulic acid (1) [12]; from group 5 resulted in isolation of caffeoate ethyl (2) [13]; from group 6, a mixture of caffeoate ethyl (2) and naringinin (3) [14]. In the analysis of the Bb-D fraction was identified from group 2, a mixture of caffeoate ethyl (2) and 7-hydroxy-benzaldehyde (4) [15]; from group 3 was identified caffeoate ethyl (2) and naringinin (3).
All compounds have been identified for the first time from the female cladodes of *B. burchellii* and are commonly found in this genus.

**Antimicrobial activity**

The antimicrobial activities of the samples from *B. organensis*, *B. burchellii*, and *B. aracatubaensis* were evaluated according to the microdilution method described by CLSI [11]. According to the results summarized in Table 1, the crude extract from female *B. burchellii* showed a MIC of 0.9 mg.mL\(^{-1}\) against *S. aureus*, which was the highest activity among all the crude extracts analyzed. This extract was fractionated and the dichloromethane fraction (Bb-D) showed the highest antimicrobial activity against *S. aureus*, with a MIC of 0.4 mg.mL\(^{-1}\) [16]. Caffeate ethyl, naringenin, and 7-hydroxy-benzaldehyde compounds were identified upon chemical analysis of this fraction. Furthermore, the ethyl acetate fractions from *B. burchellii* (Bb-Ae) and *B. aracatubaensis* showed moderate activity, with MIC values ranging between 0.6 and 1.2 mg.mL\(^{-1}\). Ethyl caffeate, naringenin, and trans-ferulic acid compounds were identified in the Bb-Ae extract. Campos et al. had reported that the extracts and/or fractions from this genus are constituted mainly of phenolic compounds such as flavonoids, phenolic acids, and terpenes, and these possess antimicrobial activity [10], corroborating with the results obtained in this work. According to a survey conducted by Coppo and Marchese, the antibacterial activity of polyphenols can be attributed mainly to flavonols, flavones, isoflavones, flavanones, and flavan-3-ol [17]. Among the compounds tested against *S. aureus*, naringenin demonstrated strong antimicrobial activity [18-20]. Rangel observed antimicrobial activity of the extracts from *Baccharis nitida* against *S. aureus* strains [21]. Other compound classes, such as diterpenes, identified from *Dracunculifolia*, *B. griesbachii*, *B. trimera*, *B. incarum*, and *B. dentata*, also showed activity against *S. aureus* strains [22-26].

In tests conducted using samples from *Baccharis* against *Pseudomonas aeruginosa*, MIC between 1.1 and 26.5 mg.mL\(^{-1}\) was obtained (Table 1). The fractions that showed moderate activities were Ba-Ae (MIC, 1.1 mg.mL\(^{-1}\)) and Bb-Ae (MIC, 1.2 mg.mL\(^{-1}\)), which were from *B. aracatubaensis* and *B. burchellii*, respectively [16]. Previous studies have reported antimicrobial activities against *P. aeruginosa* in other species of *Baccharis* such as *B. dracunculifolia*, *B. articulata* [27,28], and *B. nitida* [21]. In the plant kingdom, phenolic compounds are involved in the plant defense; and since they are synthesized in response to microbial infections [29], they can also be effective antimicrobials against a wide variety of microorganisms [30,31].

**Conclusion**

The crude extracts and fractions from *Baccharis aracatubaensis*, *B. burchellii*, and *B. organensis* showed significant antibacterial activity against tested strains. The highest activity against *S. aureus* was exhibited by the dichloromethane fraction from female *B. burchellii*, and moderate activity was observed in the crude extract from its male specimens and the ethyl acetate fraction from its female specimens. The ethyl acetate fractions from female *B. burchellii* and *B. aracatubaensis* showed moderate activity against *P. aeruginosa*. Extracts from *B. organensis* showed no significant activity against any organism tested. Neither the extracts nor fractions from *B. organensis*, *B. aracatubaensis*, or *B. burchellii* showed antifungal activity. Phenolic derivate compounds identified in the dichloromethane and ethyl acetate fractions from *B. burchellii* were the trans-ferulic, ethyl caffeate, naringinin, and 7-hydroxy-benzaldehyde compounds. These phenolic compounds were found in other species of the *Baccharis* and have been shown to possess antimicrobial activity. In this context, the results obtained in this work, with respect to *B. burchellii*, indicate that this species is a promising source of compounds with antimicrobial activity.

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| Species               | Extract and Fraction | S. aureus | E. coli | P. aeruginosa | C. albicans |
|-----------------------|----------------------|-----------|---------|---------------|-------------|
| *Baccharis aracatubaensis* | Ba-M | 8.0 | 16.0 | 8.0 | --- |
|                        | Ba-Ae | 9.8 | 19.7 | 19.7 | --- |
|                        | Ba-Aq | 1.1 | 4.5 | 1.1 | --- |
| *Baccharis burchellii*  | Bb-M | 4.0 | 14.8 | 3.7 | --- |
|                        | Bb-F | 0.9 | 8.1 | 4.0 | --- |
|                        | Bb-D | 0.4 | 2.9 | 2.9 | --- |
|                        | Bb-Ae | 0.6 | 4.9 | 1.2 | --- |
| *Baccharis organensis*  | Bo-M | 3.6 | 14.4 | 14.4 | --- |
|                        | Bo-F | 4.0 | 16.0 | 16.0 | --- |
|                        | Bo-D | 2.6 | 10.4 | 5.2 | --- |
|                        | Bo-Ae | 5.6 | 11.7 | 5.6 | --- |
|                        | Bo-Aq | 6.6 | 26.4 | 26.4 | --- |

Ba-M and Ba-F: crude extract from *B. aracatubaensis* male and female, respectively; Ba-Ae and Ba-Aq: fractions ethyl acetate and aqueous from *B. aracatubaensis* female, respectively; Bb-M and Bb-F: crude extract from *B. burchellii* male and female, respectively; Bb-D, Bb-Ae and Bb-Aq: fractions dichloromethane, ethyl acetate and aqueous from *B. burchellii* female, respectively; Bo-M and Bo-F: crude extract from *B. organensis* male and female, respectively; Bo-D, Bo-Ae and Bo-Aq: fractions dichloromethene, ethyl acetate and aqueous from *B. organensis* female, respectively; ---: Not activity; Positive control for antibacterial activity: cloramphenicol (100 µg.mL\(^{-1}\)); Negative control: Methanol/DMSO/H\(_2\)O (20:5:75, v/v) or H\(_2\)O.

**Table 1:** Antimicrobial activity of crude extracts and fractions from *Baccharis aracatubaensis*, *B. burchellii* and *B. organensis*.
