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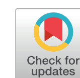
Original Article

Phytochemistry and antimicrobial activity of *Campomanesia adamantium*



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ABSTRACT

Campomanesia adamantium (Cambess.) O. Berg., Myrtaceae, is a plant popularly used for its anti-inflammatory, anti-diarrhoeal and urinary antiseptic activities. The aims of this study were to obtain the crude ethanolic extract and the hexane, dichloromethane, ethyl acetate, aqueous and concentrated aqueous tannin fractions from *C. adamantium* leaves, perform biomonitored fractionation to isolate and identify chemical compounds, study the chemical composition of the volatile oils of the leaves and flowers and test the antimicrobial activity of the ethanolic extract, fractions, isolated substances and volatile oils. Phytochemical screening and chromatographic and spectrometric techniques were used. Volatile oils were isolated by hydrodistillation in a Clevenger apparatus and analyzed by gas chromatography/mass spectrometry. The antimicrobial activity was tested by a broth microdilution test. The component stictane-3,22-diol was isolated and identified from the hexane fraction, while valoneic and gallic acid were isolated and identified from the concentrated aqueous tannin fraction. The major constituents of the volatile oils of the leaves were verbenene (13.91%), β-funebrene (12.05%) and limonene (10.32%), while those of the volatile oils of the flowers were sabinene (20.45%), limonene (19.33%), α-thujene (8.86%) and methyl salicylate (8.66%). Antibacterial activity was verified for the hexane fraction, while antifungal activity was observed for the aqueous fraction and concentrated aqueous tannin fraction and for vanoleic acid. These results may justify the popular use of *C. adamantium*.

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Introduction

In Brazil, the Myrtaceae family comprises 23 genera and 976 species, of which fourteen genera and 211 species exist in the Cerrado (Sobral et al., 2010). The genus *Campomanesia* has 36 species, 31 of which are species of the Brazilian flora (Sobral et al., 2010; Govaerts et al., 2014).

Campomanesia adamantium (Cambess.) O. Berg is a native fruit species of the Cerrado biome, popularly known as “guabiroba-do-campo”. This plant is a deciduous shrub ranging from 0.5 to 1.5 m in height (Lorenzi et al., 2008; Lima et al., 2011). The leaves of this plant have been used in the form of tea for their anti-inflammatory, anti-diarrhoeal, and urinary antiseptic activities and to treat stomach disorders (Piva, 2002; Lorenzi et al., 2008).

Terpenoids (Stefanello et al., 2008; Coutinho et al., 2008b, 2009), flavonoids (Ferreira et al., 2013) and chalcone derivatives (Pascoal et al., 2014) have been isolated and identified from *C. adamantium* leaves.

Several studies on *C. adamantium* have reported antioxidant activity of hexanic, chloroform and ethanolic extracts of the leaves and fruits of this plant (Vallilo et al., 2006; Ramos et al., 2008; Coutinho et al., 2008a, 2009; Alves et al., 2013). Antinociceptive and anti-inflammatory effects were observed with ethyl acetate fractions, aqueous fractions and flavonoids isolated from the hexanic fraction of the leaves (myricetin and myricitrin) (Ferreira et al., 2013); antimicrobial activity was observed with ethanolic extracts of the leaves and fruits (Coutinho et al., 2009; Pavan et al., 2009; Cardoso et al., 2010a,b). Cardamonin isolated from ethanolic

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fractions of the leaves exhibited cytotoxicity in prostate cells (Pascoal et al., 2014).

There are no data in the literature about the antimicrobial activity of substances isolated from *C. adamantium* leaves. Due to the resistance of microorganisms to commonly used antimicrobials, the search for new substances with antimicrobial activity is necessary (Holetz et al., 2002; Canton and Onofre, 2010; Mulyaningsih et al., 2011).

The aims of this paper were to determine the chemical composition of volatile oils from the leaves and flowers of *C. adamantium*; evaluate the antimicrobial activity of these volatile oils and of ethanolic, hexanic, dichloromethane, ethyl acetate and aqueous extracts of the leaves against gram-positive and gram-negative bacteria and fungi; perform biomonitored fractioning of fractions that presented high antimicrobial activity; and isolate and identify substances and test their antimicrobial activity.

Material and methods

Plant material

Leaves and flowers of *Campomanesia adamantium* (Cambess.) O. Berg., Myrtaceae, were collected in Bela Vista, Goiás, Brazil (17° 02′ 01.1″ S; 48° 49′ 00.3″ W; at an altitude of 847 m). The plant material was identified by Prof. Dr. José Realino de Paula. A voucher specimen has been deposited at the Herbarium of Federal University of Goiás under the code number UFG-243832.

To obtain the crude ethanolic extract (CEE), the leaves were dried at 40 °C in a drying oven under forced ventilation. The plant material was ground in a Wiley knife mill. The powdered material was subjected to maceration using an ethanol:water solution (95:5, v/v) as the solvent mixture. A mechanical shaker was employed for 4 h to perform the maceration using a ratio of 1:4. The extract was filtered and evaporated under reduced pressure on a rotary evaporator at 40 °C (Ferri, 1996).

Fractionation of the CEE was conducted according to the methodology described by Ferri (1996). CEE was diluted in methanol:water (7:3, v/v), and successive liquid–liquid extractions were performed with hexane, dichloromethane and ethyl acetate. The solvents from each fraction were evaporated on a rotary evaporator under reduced pressure, and the aqueous fractions were lyophilized. Four fractions were obtained: hexanic (HF), dichloromethane (DF), ethyl acetate (AcF) and aqueous (AqF). The yields of the fractions were calculated according to the following equation:

$$\text{Yield} \quad (\%) = \frac{(\text{fraction weight})}{\text{crude extract weight}} \times 100$$

ESI FT-ICR MS analysis

To identify the chemical compounds present in the CEE, HF, DF, AcF and AqF, an electrospray ionization source was used with Fourier-transform ion cyclotron resonance mass spectrometry (ESI FT-ICR MS) at atmospheric pressure. Fractions were diluted in approximately 0.25 mg/ml in water:methanol (1:1) with 0.1% ammonium hydroxide for analysis in negative ion mode and 0.1% acetic acid for analysis in positive ion mode. The resulting solution was infused directly into the electrospray source (for ESI) at a flow rate of 5 ml/min. The mass spectrometer (model 9.4 T solariX, Bruker Daltonics, Bremen, Germany) was configured to operate in a range of *m/z* 150–2000. The general ESI analysis conditions were as follows: pressure, 3.0 bar; capillary voltage, 4.5 kV; and temperature of capillary ion transfer, 220 °C. ESI-FT-ICR MS spectra were acquired and processed using the Bruker Compass Data Analysis software (Bremen, Germany). MS data were processed, and the molecular formulas of the compounds were determined by measuring the *m/z* values. Structures for each compound were putatively assigned using the ChemSpider (www.chemspider.com) database.

Obtaining sub-fractions

HF (4 g) was fractionated by column chromatography (CC) with Vetec silica gel G60 0.05–0.2 mm (1:40) and eluted with hexane-ethyl acetate (5–100%), ethyl acetate-methanol (1:1) and methanol. Ninety 20-ml fractions were collected. After evaporation of the solvent, these fractions were grouped into eleven additional fractions (HF1–HF11) based on their chromatographic profiles by analytical thin layer chromatography (TLC) on silica gel G60 F254 (Vetec, Brazil) with mobile phases consisting of hexane-ethyl acetate mixtures (10–30%). The TLC analysis was based on the retention factors (R_f) of the spots observed under 254/365-nm light, visualized with vanillin/sulfuric acid solution. HF1 was analyzed by gas chromatography coupled with mass spectrometry (GC/MS). HF2 was sub-fractionated using silica gel CC (1:40) and eluted with hexane-dichloromethane (9:1 and 8:2), resulting in 33 fractions. These fractions were pooled into six fractions based on their chromatographic profiles (HF2/1 to HF2/6). The fractions HF2/3 and HF2/6 were analyzed by GC/MS, and fraction HF2/5 was analyzed by nuclear magnetic resonance spectroscopy (^{13}H and ^{13}C NMR). HF9 was sub-fractionated by silica gel CC (1:40) and eluted with hexane-ethyl acetate as the mobile phase (8:2 and 7:3), resulting in nineteen fractions, which were pooled into four fractions based on their chromatographic profiles (HF9/1 to HF9/6). The HF9/3 fraction was sub-fractionated by preparative thin layer chromatography (PTLC) and eluted with a mobile phase of hexane-ethyl acetate (7:3), resulting in three fractions: HF9/3/1, HF9/3/2, and HF9/3/3. The HF2 and HF9 samples were fractioned by silica gel CC (1:40), and the sub-fractions were pooled according to their chromatographic profiles. The new sub-fractions were further fractionated by CC, TLC and PTLC.

The results obtained from fractions HF1, HF2/3, HF2/6 and HF9/3/1/2/1 were analyzed by GC/MS.

Obtaining concentrated aqueous tannin fractions (CAqTF): extraction and purification of tannins

Powdered *C. adamantium* leaves (500 g) were subjected to extraction with acetone/water at a ratio of 1:1 on a mechanical shaker for 3 h. Subsequently, liquid–liquid extractions with ethyl ether and ethyl acetate were performed. Three fractions were obtained: ethyl ether and ethyl acetate fractions and a concentrated aqueous tannin fraction (CAqTF). The ethyl ether and ethyl acetate fractions were not used in the tests; only the CAqTF was used.

The CAqTF was lyophilized, and 12 g of this sample was subjected to CC using the polymeric vinyl gel Diaion® HP-20 (Sigma, USA) as an adsorbent (column size 28 × 4 cm). The eluents used were water, methanol:water (20–100%), and methanol. At the end of the elution, 88 20-ml fractions were obtained (AqF1–AqF88). These fractions were lyophilized and analyzed by TLC with a mobile phase consisting of acetone/toluene/formic acid (3:3:1) based on the retention factors (R_f) of the spots observed under UV light at 254/365 nm and by staining with $FeCl_3$/HCl solution (0.01 M). After analysing the TLC profile, fractions containing only one or two spots on the chromatographic plate after visualization were selected for analysis by high-performance liquid chromatography (HPLC). After HPLC analysis, the samples AqF80 and 88 exhibited peaks indicative of pure substances and were subjected to ^{1}H and ^{13}C NMR analysis.

Nuclear magnetic resonance (NMR) analysis was used to identify the individual substances in the HF and AqF. ^{1}H and one-dimensional and two-dimensional ^{13}C NMR spectra (heteronuclear single quantum coherence (HSQC) and heteronuclear multiple

bond (HMBC)) were obtained on a Brüker Avance III 500 spectrometer operating at 500 MHz (^{1}H) and 125 MHz (^{13}C) using deuterated chloroform ($CDCl_3$) and deuterated methanol (methanol-d) as solvents for nonpolar and polar samples, respectively. Tetramethylsilane (TMS) (Sigma, USA) was used as an internal reference standard for chemical shifts (δ, ppm), and in some cases, the solvent signal was used as the reference. To obtain the chromatographic profile of the AqF subfractions, a HPLC instrument equipped with a quaternary pump and diode array detector (DAD) and the Empower 2.0 2998 data system was used with the following parameters: waters C18 column (250 × 4.6 mm); flow rate, 1.0 ml/min; injection volume, 10 μl; temperature, 25 °C; and peak detection at 254 nm. The mobile phase was a gradient of acetonitrile (A) and a 0.01 M H_3PO_4:0.01 M KH_2PO_4 solution (B) (starting at 8% A, 18% A over 20 min, 50% A over 35 min, 80% A over 45 min, and ending with 8% A over 50 min) (Okuda et al., 1989).

Analysis of volatile oils of the leaves and flowers and sub-fractions HF1, HF2/3, HF2/6 and HF9/3/1/2/1

The leaves and flowers of *C. adamantium* were dried at room temperature and ground in a knife mill. Different batches (100 g) of powdered leaves and flowers were subjected to hydrodistillation in a Clevenger-type apparatus for 2 h (Farmacopeia Brasileira, 2010). After drying with anhydrous Na_2SO_4, the oils were stored in glass vials at a temperature of −18 °C until further analysis. The volume of the volatile oils was measured in the graduated tube of the apparatus and was calculated as a percentage of the initial amount of dry plant material used in the extraction. Each experiment was performed in triplicate.

Volatile oils from leaves (EOl) and flowers (EOfl) and subfractions HF1, HF2/3, HF2/6 and HF9/3/1/2/1 were analyzed using a Shimadzu GC/MS-QP5050A fitted with a fused silica SBP-5 (30 m × 0.25 mm I.D.; 0.25 μm film thickness) capillary column (composed of 5% phenylmethylpolysiloxane). The following temperature program was used: the temperature was raised from 60 to 240 °C at 3 °C/min and then to 280 °C at 10 °C/min, ending with 10 min at 280 °C. The carrier gas had a flow rate of 1 ml/min, and the split mode had a ratio of 1:20. The injection port was set at 225 °C. The significant operating parameters for the quadrupole mass spectrometer were as follows: interface temperature, 240 °C; electron impact ionization at 70 eV with a scan mass range of 40–50 *m/z* at a sampling rate of 1 scan/s. Constituents were identified by an electronic search using digital libraries of mass spectral data (NIST, 1998), comparison of the retention indices of the constituents (Van Den Dool and Kratz, 1963) to those of C_8–C_{32} n-alkanes, and comparison of the mass spectra with literature data (Adams, 2007).

Evaluation of antimicrobial activity

The microorganisms used for susceptibility testing were from the American Type Culture Collection and were provided by the National Institute of Health Quality Control (INCQS), which is administratively associated with the Oswaldo Cruz Foundation (Fiocruz). Gram-positive bacteria: *Bacillus cereus* ATCC 14579, *Bacillus subtilis* ATCC 6633, *Listeria innocua* (CT) ATCC 33090, *Listeria monocytogenes* ATCC 19117, *L. monocytogenes* ATCC 7644, *Micrococcus luteus* ATCC 9341, *Micrococcus roseus* ATCC 1740, *Staphylococcus aureus* ATCC 6538, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12229, and *S. epidermidis* ATCC 12228. Gram-negative bacteria: *Enterobacter aerogenes* ATCC 13048, *Enterobacter cloacae* clinical isolate HMA/FT 502, *Escherichia coli* ATCC 8739, *E. coli* ATCC 11229, *Klebsiella pneumoniae* ATCC 700603, *Salmonella enterica* subsp. *enterica* serovar Typhi ATCC 10749, *Salmonella* spp. ATCC 19430, and *Pseudomonas aeruginosa* clinical isolate SPM1. Yeasts: *Candida krusei* ATCC 34135, *C. parapsilosis* ATCC 22019, *C. albicans* clinical isolate 02, *C. parapsilosis* clinical isolate 11-A, *C. tropicalis* ATCC 28707, *Cryptococcus neoformans* var. *neoformans* ATCC 28957, *C. neoformans* var. *neoformans* clinical isolate L2, and *C. neoformans* var. *gatti* clinical isolate 01. Filamentous fungi: *Trichophyton mentagrophytes* ATCC 11480, and *Trichophyton rubrum* ATCC 28189.

To determine antimicrobial activity, the bacteria were grown in Casoy broth (Himedia, India) for 18–24 h at 35 °C ± 2 °C and then transferred to Casoy agar (Himedia) for 18–24 h at 35 °C ± 2 °C. *L. innocua* and *L. monocytogenes* were grown in brain heart infusion broth (BHI; Oxoid, England) for 18–24 h at 35 °C ± 2 °C and then transferred to BHI agar (BHI supplemented with 1.5% bacteriological agar; Oxoid) for 18–24 h at 35 °C ± 2 °C. The fungi were grown on Sabouraud dextrose agar (Himedia) at 25 °C for 24–48 h (yeasts) or 48–72 h (other fungal species).

The minimum inhibitory concentrations (MIC) of the CEE, HF, DF, AcF, AqF, CAqTF, EOl, EOfl and isolated substances (valoneic acid and stictane-3,22-diol) were determined by microdilution techniques in Mueller-Hinton broth (Himedia, India) under aerobic conditions. Susceptibility tests were performed for bacteria (CLSI, 2009), yeasts (CLSI, 2008a) and filamentous fungi (CLSI, 2008b).

Initially, vegetal extracts were solubilized in 10% dimethylsulfoxide (DMSO; Vetec, Brazil) and Mueller-Hinton broth (Himedia) to obtain an initial concentration of 2000 μg/ml for bacteria. For fungi, the samples were solubilized in 10% DMSO and Roswell Park Memorial Institute (RPMI) 1640 broth (Himedia, India) to obtain an initial concentration of 1000 μg/ml. For bacterial susceptibility tests, 200 μl aliquots were added to microplate wells and subjected to serial dilutions in Mueller-Hinton broth until a final concentration of 1.95 μg/ml of each extract was attained. For fungal tests, dilutions were made in RPMI until a final concentration of 0.98 μg/ml was attained.

For *Listeria* strains, cation-adjusted Mueller-Hinton broth (Fluka, Sigma–Aldrich, India) with lysed horse blood (2.5–5%, v/v) was used (CLSI, 2005).

Bacterial suspensions were prepared in sterile 0.85% sodium chloride solution (w/v) within a transmittance range of 79.4–83.2% at 625 nm (Hinotek SP-2000UV UV–Vis spectrophotometer), corresponding to the 0.5 McFarland standard. Tenfold dilution was performed to obtain a cell concentration of 10^7 CFU/ml. Each microplate received 5 μl of microbial suspensions to obtain a final bacterial concentration of 10^4 CFU/ml. The plates were incubated at 35 °C for 18–24 h. After the incubation period, 20 μl of 0.5% triphenyl tetrazolium chloride (TTC; Vetec, Rio de Janeiro) was added to each well. The presence of red coloration after 30 min of incubation was considered to be an indicator of microbial growth, and the MIC was defined as the lowest concentration that was able to inhibit bacterial growth. Assays were performed in duplicate, with two repeats each.

The fungal suspension was prepared in sterile 0.85% sodium chloride solution (w/v) within a transmittance range of 79.4–83.2% at 530 nm (Hinotek SP-2000UV UV-Vis spectrophotometer), which was equivalent to the 0.5 McFarland standard. Tenfold dilution was performed to obtain a cell concentration of 1–5 × 10^3 CFU/ml. Each well received 100 μl of microbial suspension. The concentration of the vegetal extracts was reduced by half, and a final inoculum concentration of approximately 0.5–2.5 × 10^3 CFU/ml was obtained. The plates were incubated at 25 °C for 24–48 h (yeasts) or 48–72 h (filamentous fungi).

Control experiments were performed with 10% DMSO (w/v), extracts, fractions, bacteria and fungi. Vancomycin (250 μg/ml) (Sigma–Aldrich), gentamicin (2000 μg/ml) (Sigma-Aldrich), ciprofloxacin (2000 μg/ml) (Sigma-Aldrich) (bacteria) and itraconazole (Sigma) (16 μg/ml) (fungi) were used as positive controls.

The classification criteria for antimicrobial activity were: MIC < 100 μg/ml (good antimicrobial activity); MIC between 100 and 500 μg/ml (moderate antimicrobial activity); MIC between 500 and 1000 μg/ml (weak antimicrobial activity); and MIC above 1000 μg/ml (inactive) (Holetz et al., 2002).

Results

Yields of ethanolic extract and fractions of Campomanesia adamantium *leaves*

The yields from the *C. adamantium* leaves were as follows: 12.2% for the CEE, 0.9% for the HF, 1.0% for the DF, 1.3% for the AcF, 25% for the AqF and 2.8% for the CAqTF.

Phytochemical study

The fractions HF1, HF2/2, HF2/6 and HF9/3/1/2/1, obtained from the HF by silica gel G60 column chromatography (CC) fractionation, had an oily and viscous appearance and yellow coloration. In HF1, 32 compounds were identified by GC/MS (63% of the constituents), with 31.25% oxygenated sesquiterpenes, 53.12% non-oxygenated sesquiterpenes and 15.62% other hydrocarbons. In HF2/2, five compounds were identified (71% of the constituents); in HF2/6, twelve compounds were identified (67% of the constituents); and in HF9/3/1/2/1, one compound was identified (98.80% of the constituents). The major compounds of HF1 were caryophyllene oxide (19.09%), aromadendrene (10.73%), viridiflorene (7.49%) and spathulenol (7.35%); the major compounds of HF2/2 were isoaromadendrene (23.80%), bicyclo(3,2,2)nonane (17.20%) and tricycle(2,4)oct-5-ene (11.44%); the major compounds of HF2/6 were octadecanoic acid (12.68%), ethyl stearate (8.69%) and ethyl palmitate (7.94%); and the major compound of HF9/3/1/2/1 was cubenol (98%) (Table 1).

The presence of multiple singlets in the δ region from 0.7 to 1.8 ppm in the ^{1}H NMR spectrum of HF2/5, corresponding to methyl H, revealed a characteristic pattern of triterpenoids. The chemical shifts of 3.23 ppm and 3.38 ppm (double doublet, J = 11 and 3.9 Hz) is characteristic of the carbinolic hydrogen at the 3 and 22 triterpene positions. The HSQC and HMBC analyses suggest the existence of a triterpene structure with a stictane skeleton, containing carbinolic carbon signals at δ 79.0 ppm for C-3 and C-22, corresponding to stictane-3,22-diol (1).

The substances AqF80 and AqF88, obtained from the CAqTF by Diaion® HP-20 column chromatography (CC) fractionation, have structures related to ellagitannins. These substances were identified by ESI FT-ICR MS analysis. In the ^{1}H NMR spectrum of AqF80, a singlet was observed at δ 6.94 ppm in the region corresponding to aromatic hydrogens. The HMBC analysis indicated the presence of a quaternary carbon at δ 164.0 ppm associated with the carboxylic acid carbon coupled with the hydrogen chemical shift at 6.94 ppm. The HSQC analysis indicated that this hydrogen was directly bonded to a carbon with a chemical shift of 108.7 ppm, suggesting that the structure is a derivative of benzoic acid (C6-C1), and the structure was assigned as gallic acid (2).

The ^{1}H NMR spectrum of AqF88 exhibited 3 singlets, δ 7.49, 6.94, and 7.33 ppm, suggesting the presence of three gallic acid units. The NMR spectra showed correlations of the hydrogen signals at δH 7.49, 6.94, and 7.33 ppm with the signals from the carbonyl carbons at δC 159.0, 159.0 and 161.0 ppm, respectively, the first two being related to an ellagic acid residue. The signal δC 161.0 ppm was assigned to the carbonyl galloyl unit, which can be confirmed by the correlation of this signal in the HMBC spectrum with the singlet at δH 7.20 ppm, which was assigned to H-6. The chemical shifts of the signals observed in the AqF88 spectra were consistent with those of the dilactone valoneic acid (3).

Volatile oils from the leaves and flowers of Campomanesia adamantium

The volatile oil from the leaves was slightly yellow in color and had low volatility and a pleasant aroma. The yield was 1.41% (v/w). Thirty-seven compounds were identified: 10.81% oxygenated monoterpenes, 16.21% non-oxygenated monoterpenes, 35.13% oxygenated sesquiterpenes, 32.43% non-oxygenated sesquiterpenes and 5.40% other hydrocarbons. The major constituents were verbenene (13.91%), β-funebrene (12.05%), limonene (10.32%), α-guaiene (6.33%), linalool (4.91%), and spathulenol (3.86%) (Table 1).

The volatile oil of the flowers had a yellowish color, a pleasant citrus-like aroma, and a yield of 0.23% (v/w). A total of 23 compounds were identified: 17.39% oxygenated monoterpenes, 26.09% non-oxygenated monoterpenes, 34.78% oxygenated sesquiterpenes, 19.39% non-oxygenated sesquiterpenes and 4.34% other hydrocarbons. The major constituents were sabinene (20.45%), limonene (19.33%), α-thujene (8.86%), methyl salicylate (8.66%) and globulol (7.4%) (Table 1).

Antimicrobial activity

The CEE showed good inhibitory activity against *L. innocua* ATCC 33090, *C. tropicalis* ATCC 28707, *C. neoformans* L2, and *C. neoformans* L1 and moderate inhibitory activity against *B. cereus* ATCC 14579, *S. aureus* ATCC 25923, *L. innocua* ATCC 33090, *L. monocytogenes* ATCC 7644, *S. aureus* ATCC 6538, *C. albicans* 02, *C. krusei* ATCC 34135, *C. parapsilosis* (clinical isolate) 11-A, *C. parapsilosis* ATCC 22019 and *T. mentagrophytes* ATCC 11480 (Table 2).

The HF showed good inhibitory activity against *E. cloacae* HMA/FT 502, *S. aureus* ATCC 25923, *S. epidermidis* ATCC 12229 and *S. aureus* ATCC 6538 and moderate inhibitory activity against *B. cereus* ATCC 14579, *B. subtilis* ATCC 6633, *M. roseus* ATCC 1740, *M. luteus* ATCC 9341, *L. innocua* ATCC 33090, *C. tropicalis* ATCC 28707 and *T. mentagrophytes* ATCC 11480 (Table 2).

The DF had good inhibitory activity against *L. innocua* ATCC 33090 and moderate inhibitory activity against *L. monocytogenes* ATCC 19117, *B. cereus* ATCC 14579, *B. subtilis* ATCC 6633, *L. monocytogenes* ATCC 7644, *S. aureus* ATCC 6538, *S. aureus* ATCC 25923, *S. aureus* ATCC 29213, *S. epidermidis* ATCC 12229, *S. epidermidis* ATCC 12228, *E. aerogenes* ATCC 13048, *Salmonella enterica* subsp. *enterica* serovar Typhi ATCC 10749, and *T. mentagrophytes* ATCC 11480 (Table 2).

The AcF had good inhibitory activity against *C. krusei* ATCC 34135, *C. tropicalis* ATCC 28707, *C. neoformans* L2, and *C. neoformans* L1 and moderate inhibitory activity against *L. innocua* ATCC 33090, *L. monocytogenes* ATCC 19117, *L. monocytogenes* ATCC 7644, *C. albicans* 02, *C. parapsilosis* 11-A, *C. parapsilosis* ATCC 22019, *T. mentagrophytes* ATCC 11480 and *T. rubrum* ATCC 28189 (Table 2).

The AqF and CAqTF had good inhibitory activity against *C. albicans* 02, *C. krusei* ATCC 34135, *C. parapsilosis* 11-A, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 28707, *C. neoformans* L2 and *C. neoformans* L1 and moderate inhibitory activity against *L. innocua* ATCC 33090, and *T. mentagrophytes* ATCC 11480 (Table 2).

The EOl had good inhibitory activity against *L. monocytogenes* ATCC 7644 and *T. mentagrophytes ATCC* 11480 and moderate inhibitory against *L. monocytogenes* ATCC 19117 (Table 2).

The EOfl had good inhibitory activity against *T. mentagrophytes* ATCC 11480 and moderate inhibitory activity against *L. monocytogenes* ATCC 7644, *C. krusei* ATCC 34135, *C. tropicalis* ATCC 28707 and *T. rubrum* ATCC 28189 (Table 2).

The valoneic acid had good inhibitory activity against *C. neoformans* ATCC 28957, *C. tropicalis* ATCC 28707, *C. krusei* ATCC 34135,

Table 1
Chemical constituents of the hexane fractions (HF1, HF 2/2, HF 2/6, HF 9/3/1/2/1), essential oils from leaves (EOl) and essential oil from flowers (EOfl).

Constituents	KI	HF1, %	HF2/2, %	HF2/6, %	HF9/3/1/2/1, %	EOl, %	EOfl, %
Artemisia triene	929						0.25
Tricyclene	926					8.67	
α-Thujene	930						8.86
2-Methyl-pentanoic acid	933					0.22	
α-Pinene	939					0.28	
6-Methyl-heptan-2-ol	965					0.29	
Verbenene	967					13.91	
Sabinene	975						20.45
β-Pinene	979					1.52	
3-*ρ*-Menthene	987						3.93
α-Terpinene	1017					0.69	
ρ-Cymene	1024						0.98
Limonene	1029					10.32	19.33
1,8-Cineole	1031					0.96	3.75
Hexanoic acid	1032	–	–	5.05	–		
Linalool	1090					4.91	5.59
Terpinen-4-ol	1177					0.34	0.67
α-Terpineol	1188					1.40	4.58
Methyl salicylate	1191						8.66
α-Ylangeno	1375					0.77	
Isoledene	1376	0.13					
α-Copaiene	1376	0.51					
β-Funebrene	1414					12.05	
E-Caryophyllene	1419	3.46					3.13
α-Guaiene	1439					6.33	
β-Gurjunene	1433	1.00	–	–	–		
Aromadendrene	1441	10.73	–	–	–	0.28	1.23
cis-Prenyl limonene	1443						0.70
Myltayl-4(12)-ene	1447	0.42	–	–	–		
α-Humulene	1454	0.61	–	–	–	2.38	
allo-Aromadendrene	1460	5.89	–	–	–	1.77	
Isoaromadendrene	1461	–	23.80	–	–		
Dauca-5,8-diene	1472					0.77	
γ-Gurjunene	1477	0.22	–	–	–		
Widdra-2,4(14)-diene	1482					0.42	
γ-Muurolene	1479	4.03	–	–	–		
α-Amorphene	1484	0.80	–	–	–		1.05
cis-Eudesma-6,11-diene	1489	2.11	–	–	–	4.47	
β-Selinene	1490					0.45	
Viridiflorene	1496	7.49	–	–	–		
α-Muurolene	1500	3.10	–	–	–		
Epizonarene	1501					0.73	
γ-Cadinene	1513	3.32	–	–	–		
trans-Calamenene	1522	1.09	–	–	–		
Methyl docosanoate	1525	–	–	3.31	–		
α-Cadineno	1538	1.21	–	–	–		
Italicene epoxide	1548					0.60	
trans-Dauca-4(11),7-dieno	1557					0.53	
cis-Muurol-5-en-4-α-ol	1561						0.38
Maaliol	1567					1.21	
1α,10α-epoxy-Amorph-4-ene	1572					6.38	3.46
α-Cedrene epoxide	1575					5.65	
Spathulenol	1577	7.35	–	–	–	3.86	
Caryophyllene oxide	1583	19.09	–	–	–	2.29	
Thujopsan-2-α-ol	1587					1.28	
Globulol	1590					7.40	
β-Copaen-4-ol	1590					0.40	
Viridiflorol	1592						2.21
Cubeban-11-ol	1595						0.74
n-Hexadecane	1600	0.61	–	–	–		
Rosifoliol	1600					1.38	1.10
Ledol	1602					0.97	
5-*epi*-7-*epi*-α-Eudesmol	1607					0.38	0.71
Humulene epoxide II	1608	1.73	–	–	–		
Muurola-4,10(14)-dien-1-β-ol	1631					0.65	
Cubenol	1646	–	–	–	98.80	0.50	0.83
cis-Calamenen-10-ol	1661	1.17	–	–	–		
7-*epi*-α-Eudesmol	1663	0.92	–	–	–		
Amorpha-4,9-dien-2-ol	1700	0.78	–	–	–		
cis-Thujopsenal	1709	1.13	–	–	–		
Eremophilone	1736	0.57	–	–	–		
14-oxy-α-Muurolene	1768	0.54	–	–	–		
Squamulosone	1770	2.07	–	–	–		
n-Octadecane	1800	0.58	–	–	–		

Table 1
(*Continued*)

Constituents	KI	HF1, %	HF2/2, %	HF2/6, %	HF9/3/1/2/1, %	EOl, %	EOfl, %
Androstan-17-ano	1940	–	9.69	–	–		
Ethyl palmitate	1993	–	–	7.94	–		
n-Eicosane	2000	0.21	–	–	–		
Heptadecanoic acid	2022	–	–	1.20	–		
Ethyl stearate	2194	–	–	8.69	–		
Ethyl eicosanoate	2375	–	–	4.97	–		
Hexacosane	2600	2.33	–	–	–		
Heptacosane	2700	0.86	–	–	–		
Epiro-2,7-dec-4-ene	6066	–	10.40	–	–		
Triciclo(2,4)-oct-5-ene	6168	–	11.44	–	–		
Bicyclo(3,2,2)nonane	6761	–	17.20	–	–		
Ethyl tetracosanoate	21,432	–	–	6.83	–		
Octadecanoic acid	23,513	–	–	12.68	–		
Hexadecanal	24,097	–	–	1.82	–		
1-Octacosanol	25,624	–	–	3.72			

Table 2
Antimicrobial activity of the crude ethanolic extract (CEE) fractions: hexane (HF), dichloromethane (DF), ethyl acetate (AcF), aqueous (AqF), concentrated aqueous tannin (CAqTF), valoneic acid (VaAc), volatile oil of the leaves (EOl) and volatile oil of the flowers (EOfl) against gram-positive and gram-negative bacteria and fungi.

Microorganisms	CEE	HF	DF	AcF	AqF	CAqTF	VaAc	EOl	EOfl
Gram-positive bacteria									
B. cereus ATCC 14579	250	125	125	>1000	500	500	–	–	–
B. subtilis ATCC 6633	250	125	250	500	>1000	>1000	–	>1000	–
L. innocua (CT) ATCC 33090	125	125	31.25	125	250	250	–	1000	1000
L. monocytogenes ATCC 19117	500	1000	250	125	>1000	>1000	>1000	250	1000
L. monocytogenes ATCC 7644	250	500	125	125	>1000	>1000	>1000	31.25	250
M. luteus ATCC 9341	500	125	500	500	>1000	>1000	>1000	>1000	–
M. roseus ATCC 1740	500	125	500	500	>1000	>1000	>1000	>1000	–
S. aureus ATCC 6538	250	31.25	250	500	>1000	>1000	>1000	1000	–
S. aureus ATCC 25923	250	62.5	250	500	>1000	>1000	>1000	>1000	–
S. aureus ATCC 29213	1000	500	250	500	1000	1000	>1000	1000	500
S. epidermidis ATCC 12229	500	62.5	125	500	>1000	>1000	>1000	500	–
S. epidermidis ATCC 12228	1000	1000	250	1000	1000	1000	>1000	1000	500
Gram-negative bacteria									
E. coli ATCC 11229	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	–
E. coli ATCC 8739	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	–
E. aerogenes ATCC 13048	500	>1000	250	1000	>1000	>1000	>1000	–	–
E. cloacae (clinical isolate) HMA/FT 502	>1000	62.5	>1000	1000	>1000	>1000	>1000	>1000	–
K. pneumoniae ATCC 700603	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000	1000
P. aeruginosa (clinical isolate) SPM1	>1000	500	>1000	1000	>1000	>1000	>1000	–	–
Salmonella spp. ATCC 19430	>1000	>1000	>1000	500	>1000	>1000	>1000	>1000	–
S. enterica subsp. enterica serovar Typhi ATCC 10749	1000	1000	250	500	1000	1000	>1000	1000	1000
Fungi									
C. albicans (clinical isolate) 02	125	>1000	1000	250	62.5	62.5	–	–	–
C. krusei ATCC 34135	250	1000	1000	31.25	7.81	7.81	31.25	>1000	250
C. parapsilosis (clinical isolate) 11-A	125	>1000	1000	125	62.5	62.5		–	–
C. parapsilosis ATCC 22019	125	>1000	1000	125	62.5	62.5	62.5	–	–
C. tropicalis ATCC 28707	62.5	125	>1000	7.81	7.81	7.81	31.25	>1000	250
C. neoformans ATCC 28957	–	–	–	–	–	–	31.25	–	–
C. neoformans var. neoformans (clinical isolate) L2	15.62	1000	1000	15.62	31.25	31.25	–	–	–
C. neoformans var. gatti (clinical isolate) L1	15.62	1000	1000	15.62	31.25	31.25	–	–	–
T. mentagrophytes ATCC 11480	250	250	125	125	250	250	31.25	15.62	7.81
T. rubrum ATCC 28189	500	500	>1000	250	1000	1000	15.62	>1000	250

C. parapsilosis ATCC 22019, *T. mentagrophytes* ATCC 11480 and *T. rubrum* ATCC 28189 (Table 2).

Discussion

Silica gel G60 column chromatography (CC) was employed to fractionate and sub-fractionate the hexanic fraction. Diaion® HP-20 column chromatography (CC) was used to fractionate the CAqTF. Thin layer chromatography (TLC) was used to determine the groups of compounds in the fractions. In addition, preparative thin layer chromatography (PTLC) was used to isolate compounds for further analysis by high-performance liquid chromatography (HPLC). HPLC was used to evaluate the purified compounds, and then, ^{1}H and ^{13}C NMR analysis was performed to elucidate the molecular structures.

Among the fractions, the major compounds in HF1 were caryophyllene oxide (19.09%), aromadendrene (10.73%), viridiflorene (7.49%), and spathulenol (7.35%). The components of HF2/2 were isoaromadendrene (23.80%), bicyclo(3,2,2)nonane (17.20%), and tricyclo(2,4)oct-5-ene (11.44%). The components of HF2/6 were octadecanoic acid (12.68%), ethyl stearate (8.69%) and ethyl palmitate (7.94%), while the main component of HF9/3/1/2/1 was cubenol (98%). The compounds aromadendrene, α-humulene, *allo*-aromadendrene, *cis*-eudesma-4,11-diene and spathulenol, identified in HF1, and cubenol, identified in HF9/3/1/2/1, were also identified in the volatile oils of *C. adamantium* leaves. In the volatile oils of the flowers, the common compounds were aromadendrene and cubenol. In fraction HF2/6, the main components were free fatty acids. The ESI FT-ICR MS analysis revealed a wide

range of structural classes, such as triterpenoids, flavonoids and tannin derivatives. In the literature, flavonoids (Borges et al., 2013; Carvalho Junior et al., 2014; Imatomi et al., 2013; Lima et al., 2013), triterpenoids (Lima et al., 2014; Pai and Joshi, 2014; Shao et al., 2012; Topçu et al., 2011) and tannins (Azevedo et al., 2012; Celli et al., 2011; Tahara et al., 2014) have been described in other species of Myrtaceae.

The major constituents of the volatile oils of the leaves were verbena (13.91%), β-funebrene (12.05%), limonene (10.32%), α-guaiene (6.33%), linalool (4.91%) and spathulenol (3.86%). The major compounds of *C. adamantium* leaves described in the literature are as follows: geraniol (18.1%), spathulenol (7.8%), globulol (5.6%) (Stefanello et al., 2008), limonene (21.9%) (Coutinho et al., 2008a,b), bicyclogermacrene (16.17%) and globulol (11.05%) (Coutinho et al., 2009). Limberger et al. (2001) identified the following major components: spathulenol (27.7%) and β-caryophyllene oxide (29.0%) in *Campomanesia guazumifolia* (Cambess.) O. Berg; bicyclogermacrene (13.6%) and globulol (10.8%) in *C. rhombea* O. Berg; *E*-nerolidol (28.8%) in *C. xanthocarpa* O. Berg; α-pinene (16.5%) and myrcene (11.5%) in *C. aurea* O. Berg (40.3%); and linalool in *C. rhombea* (9.7%) and in *C. xanthocarpa* (17.2%). Adati and Ferro (2006) identified linalool (11.1%), caryophyllene oxide (11.8%), β-caryophyllene (6.3%), β-selinene (6.9%) and α-cadinol (7.5%) in *C. phaea* (O. Berg.) L.; Paschal et al. (2011) identified myrtenal (27.0%), myrtenol (24.7%), and *trans*-pinocarveol (15.7%) in *C. guabiroba* (DC.) Maersk; Cardoso et al. (2010a) identified bicyclogermacrene (22.4%), spathulenol (15.9%) and germacrene D (14.6%) as the major compounds in *C. sessiliflora* (O. Berg) Mattos. Linalool has also been found in other species of the genus and has anti-inflammatory and antinociceptive activities in mice (Henriques et al., 2009).

The major components of the volatile oil of *C. adamantium* flowers were sabinene (20.45%), limonene (19.33%), α-thujene (8.86%), methyl salicylate (8.66%) and globulol (7.4%). Coutinho et al. (2008b) identified ledol (20.9%), globulol (9.3%) and α-cadinol (7.5%) in the volatile oil of *C. adamantium* flowers, and Coutinho et al. (2009) identified limonene (22.24%) and α-pinene (13.23%) as the major constituents. Another species of the genus, *C. pubescens* (DC.) O. Berg, contained ledol (19.8%), globulol (9.2%), α-cadinol (7.3%) and *epi*-α-muurolol (5.0%) as major compounds in the volatile oil of its flowers (Cardoso et al., 2008). According to Liu et al. (2013), methyl salicylate has anti-inflammatory and analgesic activities and has been reported as a chemical compound in the volatile oil of other species with these activities.

The variations between the chemical compositions of the volatile oils of the *C. adamantium* leaves and flowers reported in this study and those reported in the literature can be explained by genetic and climatic factors and by the vegetative cycle. According to Gobbo-Neto and Lopes (2007), the production of secondary metabolites can be influenced by environmental factors such as seasons, rainfall, circadian rhythms, altitude, temperature, vegetative cycle and soil type. According to Barros et al. (2009), climatic conditions may influence the enzymatic activity in certain plant species and may consequently interfere with the biosynthesis of certain secondary metabolites, including terpenoids.

In the present study, the hexanic fraction exhibited substantial antibacterial potential via antimicrobial activity evaluation, and the remaining extracts and fractions exhibited better antimicrobial activity against gram-positive bacteria. According to Cardoso et al. (2010b), hexanic extracts of the fruit of *C. adamantium* showed good inhibitory activity (MIC 5–20 mg/ml) against *S. aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *E. coli* ATCC 11103 and *Salmonella setubal* ATCC 19796. Pavan et al. (2009) evaluated the antimicrobial activity against *Mycobacterium tuberculosis* of the fruits of *C. adamantium* and observed good activity (MIC 62.5 μg/ml) with the ethyl acetate fraction. Coutinho et al. (2009) reported that the volatile oil obtained from the flowers and fruits showed good activity against *S. aureus* and *P. aeruginosa* and moderate activity against *Escherichia coli*. According to Cardoso et al. (2010b), the hexanic extract of the fruit of *C. adamantium* had good activity (15 and 5 mg/ml) against *Saccharomyces cerevisiae* and *C. albicans*, and this activity was associated with a majority of the volatile compounds (β-pinene and β-caryophyllene) found in the hexane extract of the fruit. In studies with other *Campomanesia* species, Adati (2001) showed that the crude extract of *C. phaea* inhibited the growth of *C. albicans* at a concentration of 250 μg/ml. Desoti et al. (2011) evaluated the methanol, hexane and ethyl acetate extracts of *C. xanthocarpa* leaves, and these extracts exhibited good antifungal activity against *C. albicans* and *Saccharomyces cerevisiae*. Moura-Costa et al. (2012) reported that the leaf extracts of *C. eugenioides* (Cambess.) D. Legrand ex L. R. inhibited the growth of three species of *Candida*.

Gallic acid, isolated in this study, is widely distributed in plants and has several activities, such as anti-diarrhoeal, antifungal, antibacterial, anti-inflammatory, antiviral, antioxidant and antineoplastic activities (Monteiro et al., 2005; Namkung et al., 2010; Sameermahmood et al., 2010). According to a review by Lima et al. (2005), the antifungal activity of gallic acid (FAD fraction 80) could be linked to the high antioxidant activity of this compound.

Valoneic acid, a hydrolysable tannin isolated from the CAqTF, showed good activity against all fungi used in the tests. The antifungal activity of the AqF and CAqTF is possibly associated with this compound. There are no reports in the literature regarding the antimicrobial activity of valoneic acid. Valoneic acid has been reported in *Epilobium hirsutum*, Onagraceae; *Shorea laevifolia*, Dipterocarpaceae; *Mallotus japonicas*, Euphorbiaceae; *Quercus*, Fagaceae, and *Lagerstroemia speciosa* (L.) Pers., Lythraceae. This substance has inhibitory activity toward 5α-reductase enzymes and xanthine oxidase (Hatano et al., 1990; Hirano et al., 2003; Unno et al., 2004).

The antifungal activities observed in the volatile oils of flowers and leaves are possibly associated with the major compounds identified (verbenene, sabinene and limonene), as described in the literature (Shimizu et al., 2006; Santos et al., 2010; Dambolena et al., 2011). Santos et al. (2010) suggest that the relative level of biological activity of volatile oils depends on particular chemical constituents (citral, α-pinene, 1,8-cineole, *trans*-caryophyllene, furanodiene, limonene, eugenol and carvacrol). However, due to the complexity of the chemical composition of volatile oils, it is difficult to link the biological activity to specific substances.

The major constituents of the volatile oils of the leaves were verbenene (13.91%), β-funebrene (12.05%), limonene (10.32%), α-guaiene (6.33%), linalool (4.91%) and spathulenol (3.86%) and those of the volatile oils of the flowers were sabinene (20.45%), limonene (19.33%), α-thujene (8.86%), methyl salicylate (8.66%) and globulol (7.4%). Starting from a phytochemical study, novel compounds were identified in *C. adamantium*, namely, stictane-3,22-diol (**1**), gallic acid (**2**) and valoneic acid (**3**). Evaluation of the antimicrobial activity revealed high antibacterial (hexane fraction) and antifungal (aqueous fraction, concentrated aqueous fraction of tannins and valoneic acid) potential. Antifungal activity of the CAqTF is associated with valoneic acid. These results may explain the popular use of *C. adamantium*.

Author contributions

SS contributed to the design and implementation of the experiment. LTC contributed to implementation of the experiment. VFA contributed to the design of the experiment. TSF contributed to a critical reading of the manuscript. LMFT contributed to a critical reading of the manuscript. BGV contributed to the mass spectrometric analysis. PHF contributed to the chromatographic analysis.

LLB contributed to a critical reading of the manuscript. Professor JRP was responsible for overall planning and experimental design.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Conflicts of interest

The authors declare no conflicts of interest.

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References

Adams, R.P., 2007. Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry, 4th ed. Allured Publishing Corporation, Illinois.

Adati, R.T., Dissertação (Mestrado em Farmaco e Medicamentos) 2001. Estudo biofarmacognóstico de *Campomanesia phaea* (O. Berg) Landrum, Myrtaceae. Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 125 pp.

Adati, R.T., Ferro, V.O., 2006. Volatile oil constituents of *Campomanesia phaea* (O Berg) Landrum (Myrtaceae). J. Essent. Oil Res. 18, 691–692.

Alves, A.M., Alves, M.S.O., Fernandes, T.O., Naves, R.V., Naves, M.M.V., 2013. Caracterização física e química, fenólicos totais e atividade antioxidante da polpa e resíduo de gabiroba. Rev. Bras. Frutic. 35, 837–844.

Azevedo, P.R., Silva, L.C.N., Gomes-Silva, A., Macedo, A.J., Araújo, J.M., Silva, M.V., 2012. Antimicrobial activity and phytochemical screening of branches, fruits and leaves of *Eugenia brejoensis*. Sci. Plena 26, 76–80.

Barros, F.M.C., Zambarda, E.O., Heinzmann, B.M., Carlos, A., Mallmann, C.A., 2009. Variabilidade sazonal e biossíntese de terpenóides presentes no óleo essencial de *Lippia alba* (Mill.) N E. Brown (Verbenaceae). Quim. Nova 32, 861–867.

Borges, L.L., Alves, S.F., Sampaio, B.L., Conceição, E.C., Bara, M.T.F., Paula, J.R., 2013. Environmental factors affecting the concentration of phenolic compounds in *Myrcia tomentosa* leaves. Rev. Bras. Farmacog. 23, 230–238.

2010. Farmacopeia Brasileira, 5th ed. Agência Nacional de Vigilância Sanitária, Ministério da Saúde, Brasília, DF.

Canton, M., Onofre, S.B., 2010. Interferência de extratos da *Baccharis dracunculifolia* DC., Asteraceae, sobre a atividade de antibióticos usados na clínica. Rev. Bras. Farmacog. 20, 348–354.

Cardoso, C.A.L., Silva, J.R.M., Kataoka, V.M.F., Brum, C.S., Poppi, N.R., 2008. Avaliação da atividade antioxidante, toxicidade e composição química por CG-EM do extrato hexânico das folhas de *Campomanesia pubescens*. Rev. Cien. Farm. Bas. Aplic. 29, 297–301.

Cardoso, C., Kataoka, V.M.F., Ré-Poppi, N., 2010a. Leaf oil of *Campomanesia sessiliflora* O. Berg. J. Essent. Oil 135, 303–304.

Cardoso, C., Salmazzo, G., Honda, N.K., Prates, C.B., Vieira, M., Coelho, C.R.G., 2010b. Antimicrobial activity of the extracts and fractions of hexanic fruits of *Campomanesia* species (Myrtaceae). J. Med. Food. 13, 1273–1276.

Carvalho Junior, A.R., Gomes, G.A., Ferreira, R.O., Carvalho, M.G., 2014. Chemical constituents and antioxidant activity of leaves and branches of *Eugenia copacabanensis* Kiaersk (Myrtaceae). Quim. Nova 37, 477–482.

Celli, G., Pereira-Netto, A., Beta, T., 2011. Comparative analysis of total phenolic content, antioxidant activity, and flavonoids profile of fruits from two varieties of Brazilian cherry (*Eugenia uniflora* L.) throughout the fruit developmental stages. Food Res. Int. 44, 2442–2451.

CLSI, 2005. M45-P: Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria. Approved Guideline, pp. 25, 27.

CLSI/NCCLS, 2008a. Método de referência para testes de diluição em caldo para determinação da sensibilidade de leveduras à terapia antifúngica: Documento M27-A3. Clinical and Laboratory Standards Institute, 22 (2).

CLSI/NCCLS, 2008b. Reference Method for Broth Dilution Antifungal Susceptibility Testing of Filamentous Fungi: M38-A2. Clinical and Laboratory Standards Institute, 28 (2).

CLSI/NCCLS, 2009. Performance standards for antimicrobial susceptibility testing; 19th informational supplement. Document M100-S19. Clinical Laboratory Standards Institute, Wayne, PA, USA.

Coutinho, I.D., Coelho, R.G., Kataoka, V.M.F., Honda, N.K., Silva, J.R.M., Vilegas, W., Cardoso, C.A.L., 2008a. Determination of phenolic compounds and evaluation of antioxidant capacity of *Campomanesia adamantium* leaves. Eclet. Quim. 33, 53–60.

Coutinho, I.D., Poppi, N.R., Cardoso, C., 2008b. Identification of the volatile compounds of leaves and flowers in Guavira (*Campomanesia adamantium* O. Berg.). J. Essent. Oil. 20, 405–407.

Coutinho, I.D., Cardoso, C.A.L., Ré-Poppi, Melo, A.M., Vieira, M.C., Honda, N.K., Roberta Gomes Coelho, 2009. Gas chromatography–mass spectrometry (GC–MS) and evaluation of antioxidant and antimicrobial activities of essential oil of *Campomanesia adamantium* (Cambess.) O. Berg (Guavira). Braz. J. Pharm. Sci. 45, 767–776.

Dambolena, J.S., Meriles, J.M., López, A.G., Gallucci, M.N., González, S.B., Guerra, P.E., Bruno, A., Zunino, M.P., 2011. Actividad antifúngica del aceite esencial de cinco especies de Juniperus de Argentina. Boletín Latinoamericano y del Caribe de Plantas Medicinales y Aromáticas 10, 104–115.

Desoti, V.C., Maldaner, C.L., Carletto, M.S., Heinz, A.A., Coelho, M.S., Piati, D., Tiuman, T.S., 2011. Triagem fitoquímica e avaliação das atividades antimicrobiana e citotóxica de plantas medicinais nativas da região oeste do estado do Paraná. Arq. Cienc. Saude Unipar 15, 3–13.

Ferreira, L.C., Grabe-Guimarães, A., Michel, M.C., Guimarães, R.G., Rezende, S.A., 2013. Anti-inflammatory and antinociceptive activities of *Campomanesia adamantium*. J. Ethnopharmacol. 145, 100–108.

Ferri, P.H., 1996. Química de produtos naturais: métodos gerais. Editora da Universidade Estadual Paulista, São Paulo.

Gobbo-Neto, L., Lopes, N.P., 2007. Plantas medicinais: fatores de influência no conteúdo de metabólitos secundários. Quim. Nova 30, 374–381.

Govaerts, R., Sobral, M., Ashton, P.F., 2014. World Checklist of Myrtaceae. The Board of Trustees of the Royal Botanic Gardens, Kew. http://www.kew.org/wcsp (accessed 23.07.14).

Hatano, T., Yasuhara, T., Yoshihara, R., Agata, I., Noro, T., Okuda, T., 1990. Effects of interaction of tannins with co-existing substances VII. Inhibitory effects of tannins and related polyphenols on xanthine oxidase. Chem. Pharm. Bull. 38, 1224–1229.

Henriques, A.T., Simões-Pires, C.A., Apel, M.A., 2009. In: Yunes, F. (Ed.), Óleos essenciais: importância e perspectivas terapêuticas. Editora Univali, Ijataí, pp. 221–250.

Hirano, Y., Kondo, R., Sakai, K., 2003. 5-alfa-Reductase inhibitory tannin-related compounds isolated from *Shorea laeviforia*. J. Wood Sci. 49, 339–343.

Holetz, F.B., Pessini, G.L., Sanches, N.R., Cortez, D.A.G., Nakamura, C.V., Dias Filho, B.P., 2002. Screening of some plants used in the Brazilian folk medicine for the treatment of infectious diseases. Mem. I. Oswaldo Cruz 97, 1027–1031.

Imatomi, M., Novaes, P., Gualtieri, S.C.J., 2013. Interspecific variation in the allelopathic potential of the family Myrtaceae. Acta Bot. Bras. 27, 54–61.

Lima, C.P., Queiroz, J.E., Santana, D.P., Ribeiro, E.L., Menezes, A.C.S., Naves, P.L.F., 2005. Atividade do ácido gálico sobre o crescimento e a formação de biofilme por *Candida albicans*. Rev. Biotecnol. Cienc. 2, 87.

Lima, D.F., Goldenberg, R., Sobral, M., 2011. O gênero *Campomanesia* (Myrtaceae) no estado do Paraná, Brasil. Rodriguésia 62, 683–693.

Lima, A.M.B., D'Avila, L.A., Siani, A.C., 2014. Comparison between methyl and trimethylsilyl ester derivatives in the separation and GC quantification of triterpene acids in *Eugenia brasiliensis* leaf extract. Chromatographia 77, 629–635.

Lima, C.A., Faleiro, F.G., Junqueira, N.T.V., Cohen, K.O., Guimarães, T.G., 2013. Características físico-químicas, polifenóis e flavonoides amarelos em frutos de espécies de pitaias comerciais e nativas do Cerrado. Rev. Bras. Frutic. 35, 565–570.

Limberger, R.P., Apel, M.A., Sobral, M., Menut, L.C., 2001. Chemical composition of essential oils from some *Campomanesia* species (Myrtaceae). J. Essent. Oil Res. 13, 113–115.

Liu, W.R., Qiao, W.L., Liu, Z.Z., Wang, X.H., Jiang, R., Li, S.Y., Ren-Bing Shi, R.B., She, G.M., 2013. Gaulteria: phytochemical and pharmacological characteristics. Molecules 18, 12071–12108.

Lorenzi, H., Lacerda, M., Bacheret, L., 2008. Frutas brasileiras e exóticas cultivadas. Instituto Plantarum, São Paulo.

Monteiro, J.M., Albuquerque, U.P., Araújo, E.L., Amorin, E.L.C., 2005. Taninos: uma abordagem da química à ecologia. Quim. Nova 28, 892–896.

Moura-Costa, G.F., Nocchi, S.R., Ceole, L.F., Mello, J.C.P., Nakamura, C.V., Dias Filho, B.P., Temponi, L.G., Ueda-Nakamura, T., 2012. Antimicrobial activity of plants used as medicinals on an indigenous reserve in Rio das Cobras Paraná, Brazil. J. Ethnopharmacol. 143, 631–638.

Mulyaningsih, S., Sporer, F., Reichling, J., Wink, M., 2011. Antibacterial activity of essential oils from *Eucalyptus* and of selected components against multidrug-resistant bacterial pathogens. Pharm. Biol. 49, 893–899.

Namkung, W., Thiagarajah, J.R., Phuan, P.W., Verkman, A.S., 2010. Inhibition of Ca^{2+} activated Cl^{-} channels by gallotannins as a possible molecular basis for health benefits of red wine and green tea. FASEB J. 24, 4178–4186.

NIST, 1998. National Institute of Standards and Technology. PC Version of the NIST/EPA/NIH Mass Spectral Database. U.S. Department of Commerce, Gaithersburg.

Okuda, T., Yoshida, T., Hatano, T., 1989. New methods of analyzing tannins. J. Nat. Prod. 52, 1–31.

Pai, S.R., Joshi, R.K., 2014. Distribution of betulinic acid in plant kingdom. Plant Sci. Today 1, 103–107.

Pascoal, A.C.R.F., Ehrenfried, C.A., Lopez, B.G.C., Araujo, T.M., Pascoal, V.D.B., Gilioli, R., Anhê, G.F., Ruiz, A.L.T.G., Carvalho, J.E., Stefanello, M.E.A., Salvador, M.J., 2014. Antiproliferative activity and induction of apoptosis in PC-3 cells by the chalcone cardamonin from *Campomanesia adamantium* (Myrtaceae) in a bioactivity-guided study. Molecules 19, 1843–1855.

Pavan, F.R., Leite, C.Q.F., Coelho, R.G., Coutinho, I.D., Honda, N.K., Cardoso, C.A.L., Vilegas, W., Leite, S.R.A., Sato, D.N., 2009. Evaluation of anti-*Mycobacterium tuberculosis* activity of *Campomanesia adamantium* (Myrtaceae). Quim. Nova 32, 1222–1226.

Piva, M.G., 2002. O Caminho das plantas medicinais: estudo etnobotânico, vol. 1. Editora Mondrian, Rio de Janeiro.

Ramos, D.D., Cardoso, C.A.L., Yamamoto, N.T., 2008. Avaliação do potencial citotóxico e atividade antioxidante em *Campomanesia adamantium* (Cambess.) O. Berg (Myrtaceae). Rev. Bras. Biociênc. 5, 774–776.

Sameermahmood, Z., Raji, L., Saravanan, T., Vaidya, A., Mohan, V., Balasubramanyam, M., 2010. Gallic acid protects RINm5F beta-cells from glucolipotoxicity by its antiapoptotic and insulin-secretagogue actions. Phytother. Res. 24, 83–94.

Santos, A.C.A., Rossato, M., Serafini, L.A., Bueno, M., Liziane, B., Crippa, L.B., Sartori, V.C., Dellacassa, E., Moyna, P., 2010. Efeito fungicida dos óleos essenciais de *Schinus molle* L. e *Schinus terebinthifolius* Raddi Anacardiaceae, do Rio Grande do Sul. Rev. Bras. Farmacog. 20, 154–159.

Shao, M., Wang, Y., Huang, X.J., Fan, C.L., Zhang, Q.W., Zhang, X.Q., Ye, W.C., 2012. Four new triterpenoids from the leaves of *Psidium guajava*. J. Asian Nat. Prod. Res. 14, 348–354.

Shimizu, M.T., Bueno, L.J.F., Rodrigues, R.F.O., Sallowicz, F.A., Sawaya, A.C.H.F., Marques, M.O.M., 2006. Essential oil of *Lithraea molleoides* (Vell.): chemical composition and antimicrobial activity. Braz. J. Microbiol. 37, 556–560.

Sobral, M., et al., 2010. Myrtaceae. Lista de espécies da flora do Brasil. http://www.floradobrasil.jbrj.gov.br/ (accessed July 2018).

Stefanello, M.E.A., Cervi, A.C., Wisniewski Jr., A., Simionatto, E.L., 2008. Essential oil composition of *Campomanesia adamantium* (Camb) O. Berg. J. Essent. Oil Res. 20, 424–425.

Tahara, K., Hashida, K., Otsuka, Y., Ohara, S., Kojima, K., Shinohara, K., 2014. Identification of a hydrolyzable tannin, oenothein B, as an aluminum-detoxifying ligand in a highly aluminum-resistant tree, *Eucalyptus camaldulensis*. Plant Physiol. 164, 683–693.

Topçu, G., Yapar, G., Türkmen, Z., Gören, A.C., Öksüz, S., Schilling, J.K., Kingston, D.G.I., 2011. Ovarian antiproliferative activity directed isolation of triterpenoids from fruits of *Eucalyptus camaldulensis* Dehnh. Phytochem. Lett. 4, 421–425.

Unno, T., Sugimoto, A., Kakuda, T., 2004. Xanthine oxidase inhibitors from the leaves of *Lagerstroemia speciosa* (L.) Pers. J. Ethnopharmacol. 93, 391–395.

Vallilo, M.I., Lamardo, L.C.A., Gaberlotti, M.L., Oliveira, E., Moreno, P.R.H., 2006. Composição química dos frutos de *Campomanesia adamantium*. Cienc. Tecnol. Aliment. 26, 805–810.

Van Den Dool, H., Kratz, P.D., 1963. Generalization of the retention index system including linear temperature programmed gas-liquid partition chromatography. J. Chromatogr. 11, 463–471.