CONSTITUENTS FROM ROOTS OF *Maytenus distichophylla*, ANTIMICROBIAL ACTIVITY AND TOXICITY FOR CELLS AND *Caenorhabditis elegans*

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*Maytenus distichophylla* is a medicinal species used in Northeast of Brazil. The hexane (HE), chloroform (CE), ethyl acetate (EAE) and methanol (ME) extracts and compounds from its roots were evaluated for their protective activity against *Staphylococcus aureus* and *Candida albicans*. The cytotoxicity for chronic myeloid leukemia (K562), acute monocytic leukemia (THP-1), and peripheral blood mononuclear cells of normal individuals (PBMC) cells was established by MTT method using etoposide as standard. The *in vivo* toxicity of samples was determined using *Caenorhabditis elegans* model. From HE and CE were isolated: friedelan-3-one (1), 3-oxo-olean-9(11),12-diene (3), a mixture of pristimerin (4) and 11α-hydroxyfriedel-20(29)-en-3-one (5), 30-hydroxyfriedel-20(29)-en-3-one (6), friedelane-3,7-dione (7), tingenone (8) and tricylglycerol (9). The structures of 1-9 were confirmed by spectral data. All samples reduced the viability of *S. aureus* and present no effect against *C. albicans*. The HE, CE, mixture of 4/5 and 8 reduced 75% of *S. aureus* viability. Cytotoxic effect for K562 and THP-1 cells was caused by compounds 1, 2 and 8. All samples displayed selectivity for leukemic cells and low toxicity to PBMC cells, suggesting their potential as anticancer agents. Extracts and compounds were non-toxic to L1 larvae of *C. elegans*. However, most of them reduced significantly young adult worm’s survival, being considered as potential nematicides.

Keywords: Celastraceae; pentacyclic triterpenes; toxicity; K562 cells; THP-1 cells.

INTRODUCTION

Brazilian plant biodiversity, only having around 55,000 species catalogued of an estimated 550,000, represents an important research area. An example of plant biodiversity is the Celastraceae family, which comprises approximately 100 genera and more than 1200 species worldwide, many of them found mainly in South America, North Africa, and East Asia. The most abundant secondary metabolites isolated from species of the Celastraceae family, including those of the genus *Maytenus*, are the pentacyclic triterpenes (PCTT). Evidence has been gathered on the potential activities of PCTT, such as antiulcer and analgesic, antiviral, antibacterial, antiparasitic, anti-inflammatory, antinociceptive, antiangiogenic, and cytotoxicity. According to Saleem et al., the mechanism of action of PCTT on viruses, bacteria, and fungi is associated with cellular membrane rupture.

In Brazil, Celastraceae family is mainly represented by five genera: *Maytenus* Juss, *Salacia* Mart, *Gouppia* Reiss, *Austrolepia* Lund, and *Francrheura* Mart. The genus *Maytenus*, one of the largest of the Celastraceae family, comprises 225 species, 76 of them recognized in Brazil, distributed in a variety of locations such as Amazonian and tropical forests, altitude fields, and the Cerrado, a vast tropical Brazilian savanna ecoregion. Plants of this genus are popularly used in Brazil to treat a diversity of illnesses, mainly gastrointestinal disorders. Among these species, *Maytenus distichophylla* (Figure 1S), popularly known as “pau-culher”, is a medium-sized tree up to 12 meters high. In traditional medicine, the infusion of *M. distichophylla* leaves and steam bark is used for the treatment of symptoms of chronic kidney disease. The compounds 3,16,21-trioxo-6β,12α-dihydroxyfriedel-1-ene, 3-oxofriedelane, 3,12-dioxofriedelane, 3β-hydroxyfriedelane, 3-oxo-29-hydroxyfriedelane, 3-oxo-12α-hydroxyfriedelane, and a new triterpene named maytensifolone were previously described as constituents of *M. distichophylla* leaves.

As a continuation of our research on bioactive metabolites of the Celastraceae species used in folk medicine, in the present work, we describe the obtention of the hexane (HE), chloroform (CE), ethyl acetate (EAE), and methanol (ME) extracts from *M. distichophylla* roots. Aiming the isolation of non-polar constituents, HE and CE were fractioned by chromatography following methodology suggested by Matos and Wagner & Bladt. Nine compounds were isolated. The HE, CE, EAE and ME root extracts and the isolated compounds 1 to 8 were evaluated against *Candida albicans* and *Staphylococcus aureus*, their cytotoxic activity was assayed using the human cell lines K562 (chronic myeloid leukemia), THP-1 (acute monocytic leukemia), and PBMC (peripheral blood mononuclear cells of normal individuals), and their toxicity activity was evaluated *in vivo* using *Caenorhabditis elegans*.
EXPERIMENTAL

General experimental procedures

The Fourier-transform infrared (FT-IR) spectra were recorded on a Perkin Elmer Frontier Single-Range MIR spectrometer with attenuated total reflection (ATR). The $^1$H and $^13$C (400.129 and 100.613 MHz, respectively) NMR spectra were performed on a Bruker DRX400 or DRX200 AVANCE spectrometers, using pure CDCl$_3$. The chemical shifts were registered in $\delta$, TMS was used as an internal standard, and the coupling constants ($J$) were registered in Hertz (Hz). Chromatographic columns (CC) were packed with silica gel 60 (70–230 Mesh). Thin layer chromatography (TLC) processes were developed using Merck silica gel 60 precoated plates.

Plant material

*M. distichophylla* roots were collected in Brejo Novo (12° 50’ 0.0’’ S, 40° 48’ 0.0’’ W), Jequié, Bahia, in July 2011. The species was identified by Dr. Guadalupe Edilma Lincona de Macedo, and a voucher specimen (Nº HUESB 2093) of the collected material was deposited in the Herbarium of the Department of Botany of UESB. The botanical material accessed was registered at Conselho de Gestão do Patrimônio Genético (CGEN/SISGEN), under number AFBF625.

Extraction and isolation

After drying at room temperature, the roots (1722 g) were ground on a mill. The resultant powder was subjected to continuous maceration with 3L of hexane for 1 week and then filtered. This procedure was repeated two more times. These three filtrates were subjected to maceration using 3L of chloroform, ethyl acetate, and methanol. Thus, hexane (HE, 11.4 g), chloroform (CE, 28.4 g), ethyl acetate (EAE, 56.3 g), and methanol (ME, 181.5 g) extracts were obtained. Part of the HE (9.4 g) was chromatographed using silica gel (500 g) CC as eluents, hexane, chloroform, ethyl acetate and methanol, pure or in a mixture in increasing order of polarity, which produced 211 fractions (200 mL each). The following sequence was used for elution: hexane (fr 1-8); hexane:chloroform 9:1 (fr 9-48); hexane:chloroform 1:1 (fr 49-117); chloroform (fr 118-142); chloroform:ethyl acetate 1:1 (fr 143-171); ethyl acetate (fr 172-207) and methanol (fr 208-211). In accordance with their similar TLC profiles, the fractions were pooled to yield 15 groups. Thus, compound 1 (3.0 mg) was isolated from group 4 (fr 75-82); hexane:chloroform 1:1; 28.3 mg) after separation through a new CC, using as eluents, hexane, chloroform, ethyl acetate and methanol, pure or in a mixture in increasing order of polarity (145 fr, 50 mL each), which furnished an additional amount of 2 (8.0 mg), 3 (21.0 mg), a mixture of 4 and 5 (8.8 mg), 5 (3.0 mg), and 6 (13.0 mg). Group 9 (fr 153-155; chloroform:ethyl acetate 1:1; 960.0 mg), after separation through a new CC as eluents, hexane, chloroform, ethyl acetate and methanol, pure or in a mixture in increasing order of polarity (140 fr, 10 mL each), yielded 7 (5.0 mg). By means of similar procedure, part of the CE (24.4 g) was chromatographed using silica gel (500 g) producing 85 fractions (200 mL each). The fraction 47 (chloroform:ethyl acetate 7:3; 4.2 g) was subjected to a new CC using as eluents, hexane, chloroform, ethyl acetate and methanol, pure or in a mixture in increasing order of polarity (fr 118, 10 mL each) resulting in the isolation of compound 2 (fr 21-23; chloroform; 17.0 mg) and 8 (fr 42-47; chloroform:ethyl acetate 8:2; 28.0 mg). The ethyl acetate and methanol extracts were not fractionated, but they were submitted to biological assays.

Antimicrobial activity

To evaluate a potential antimicrobial activity of samples from *M. distichophylla* roots against *Staphylococcus aureus* MRSA (ATCC 5923) and *Candida albicans* (SC5314), solutions (µg mL$^{-1}$) of HE (27.4), CE (27.8), EAE (40.8), and ME (40.8) extracts and of the compounds 1 (11.0), 2 (16.1), 3 (20.0), the mixture of 4 and 5 (54.8), 5 (29.6), 6 (32.0), 7 (45.6), and 8 (56.0) were prepared using dimethyl sulfoxide (DMSO, Merck) as solvent. *S. aureus* MRSA was maintained in solid Luria-Bertani (LB) medium, and for assays, a colony was isolated and cultured for 18 h in liquid LB medium. *C. albicans* was maintained in brain heart infusion (BHI) medium and for the assays and isolated colony grown in a liquid BHI medium at 28 °C, during 18 h. The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) (Sigma) tetrazolium reduction assay was used to evaluate microbial viability. 52 *S. aureus* or *C. albicans* (2 x 10$^6$ UFC mL$^{-1}$) were prepared in M9 buffer and added to a 96-well Microtiter plate wells (final volume of 200 µL). To each well 2% (v/v) of *M. distichophylla* extracts or compounds were added, the mixture was incubated for 24 h at 28 °C, and 20 µL of MTT (5.0 mg mL$^{-1}$) were added to the suspension. This mixture was incubated for 18 hours at 28°C. Culture medium was removed, 100 µL of DMSO were added and the absorbance (500 nm) was determined to measure the cellular viability in comparison with negative control (microbial suspension not exposed to the tested substances).

Cytotoxicity assay against leukemic cells

Cytotoxic activity of extracts and compounds from *M. distichophylla* roots was carried out in vitro using the human cancer cell lines K562 (chronic myeloid leukemia, ATCC CCL-243) and THP-1 (acute monocytic leukemia, ATCC TIB-202), and PBMC (peripheral blood mononuclear cells) of healthy individuals. The in vitro activity was evaluated by plating 1 x 10$^5$ cells per well. After cell plating, microplates were incubated for at least 24 h at 37 °C in the presence of 5% CO$_2$. Then, the wells were washed with culture medium (RPMI-1640 + 20% inactivated fetal bovine serum + 2 mmol L$^{-1}$ L-glutamine + 100 U mL$^{-1}$ penicillin) and incubated with samples at concentrations of 0.1, 1.0, 10.0, and 100.0 µg mL$^{-1}$. Untreated cells were used as negative controls. After incubation for 48 h, the medium in each well was removed and discarded, and 100µL of 5% MTT were added to determine the cell viability. Colorimetric measurements were performed at 550 nm using a Spectramax M5e microplate reader. All experiments were performed in triplicate. The optical density of MTT-formazan formed in untreated control cells was taken as 100% viability. The results were expressed as the mean of the IC$_{50}$ (the lethal drug concentration that reduced cell viability by 50%). IC$_{50}$ values were calculated using OriginPro 8.0 software. The selectivity index (SI), which corresponds to the ratio between the cytotoxic activities to PBMC and the tumor cell lines, was also calculated.
Toxicity evaluation for Caenorhabditis elegans

Caenorhabditis elegans N2 wild-type Bristol was purchased from Caenorhabditis Genetics Center (CGC), University of Minnesota, USA. The worms were maintained at 20 °C in nematode growth medium (NGM) seeded with Escherichia coli OP50, as described by Brenner.35

For the sample toxicity evaluation, the nematodes were maintained in M9 buffer (KH₂PO₄ 3.0 g, Na₂PO₄ 6.0 g, NaCl 5.0 g in 1 L of water and 1 mL of MgSO₄ 1 mol L⁻¹) following a modified standard protocol.36,37 Then C. elegans larvae were exposed to extracts or compounds from M. distichophylla in a 96-well Microtiter plate in M9 buffer (200 μL) at 25 °C, by means of two systems: L1 larvae in M9 buffer for 24 h (acute exposure of L1) and L4 larvae with normal E. coli OP50 feed for 12 days. The exposures were realized at 25 °C in the absence of normal feed with E. coli OP50 and DMSO was used as a negative control. After acute and chronic exposure of L1, the MTMT method was used to evaluate the viability of C. elegans.38

The viability and survival evaluation were performed in 96-well plates using young adult C. elegans (L4) (n = 10-15) in M9 buffer containing extract or compound in defined concentration, nystatin (streptomycin (50 μg mL⁻¹), Sigma) and 5-fluoro-2'-deoxyuridine [FUDR (80 μg mL⁻¹), Sigma]. This inhibitor of DNA synthesis was previously added to M9 buffer aiming to prevent C. elegans progeny production.39 The maximal sample concentration was used in the assays. After exposure of L4 larvae, the percentage of live larvae was used to evaluate survival. The C. elegans larvae were examined at 24h intervals with a stereomicroscope and those not responding to mechanical stimulus with a platinum wire were considered dead.39,40

Statistical analysis

The IC₅₀ values were calculated using the OriginPro 8.0 software and analyzed using the program Sigma Stat version 2.03. The Shapiro-Wilk test was used to test the data for the normal distribution. Normally distributed data were analyzed by analysis of variance (ANOVA), followed by the Holm-Sidak multiple comparison test. In the case the data did not present a normal distribution, between-group comparisons were performed with ANOVA followed by the nonparametric Kruskal-Wallis test. The significance level was p ≤ 0.05 for all statistical tests. S. aureus and C. albicans viability after extract or compound exposure were analyzed in GraphPad Prism® software 5.01. C. elegans survival curves were plotted, and estimation of differences (log-rank and Wilcoxon tests) in survival analyzed with the Kaplan-Meier method also using GraphPad Prism 5.01 software. A p-value of 0.05 was considered significant.

RESULTS AND DISCUSSION

Phytochemical study

Through phytochemical methods, eight compounds were isolated from HE and CE extracts of the M. distichophylla roots, being friedelan-3-one (1), β-sitosterol (2), 3-oxo-olean-9(11),12-diene (3), 11β-hydroxylup-20(29)-en-3-one (rigidenol) (5), 30-hydroxylup-20(29)-en-3-one (6), friedelan-3,7-dione (7), tingenone (8), triacylglycerol (9) and a mixture of compounds 4 (pristimerin) and 5 (Figure 1).

The chemical structures of 1 to 9 (Figure 1) were established comparing the obtained spectral data (Figure 2S to 25S and Table 1S) with those found in literature.41-49

The ¹H NMR spectra of compound 1 and 7 showed seven singlets between δH 0.77 to 1.19 referring to seven methyl and one doublet referring to methyl H-23, at the range δH 0.87-0.89, which are characteristic of friedelan skeleton triterpenes.41 Compound 1 was identified as friedelan-3-one and 7 as friedelane-3,7-dione, mainly due to carbonyl groups present in its structures (at δC 213.2 for 1 and δC 211.0 and δC 210.5 for 7). The compound 2 presented signal at δC 5.35 (IH), characteristic of olefinic hydrogen and signal at δC 3.52 (1H) that corresponds to methine hydrogen attached to hydroxylated carbon atom. In the ¹³C NMR spectrum of 2, signals were observed at δC 140.8 (C-5) and at δC 121.7 (C-6) correspondent to olefinic carbons and signal at δC 71.8 (C-3) associated to hydroxylated carbon, consistent with the steroid β-sitosterol. The compounds 3, 5 and 6 presented two signals between δH 5.62 to δH 4.60, characteristic of double bond hydrogen. Additionally, compounds 5 and 6 presented signals of hydroxylated carbon hydrogen at δH 3.90 (IH) and δH 4.12 (2H), respectively. The ¹³C NMR spectrum of compound 3 showed four signals of olefinic carbon, between δC 152.3 and δC 117.5, and the spectra of compounds 5 and 6 showed two characteristic signals of olefinic carbon (δC 154.8 to δC 107.0). The spectra of 3, 5 and 6 also showed a carbonyl signal between δC 217.8 and δC 218.7 which was attributed to carbon C-3. These NMR signals are characteristic of lupane and oleanane skeleton, which allowed the identification of 3, 5 and 6 as 3-oxo-olean-9(11),12-diene, 11β-hydroxylup-20(29)-en-3-one and 30-hydroxylup-20(29)-en-3-one, respectively. The ¹H NMR spectra of compounds 4 and 8 displayed signals at δH 7.0 (d, J=7.1 Hz, 1H), δH 6.5 (s, 1H) and δH 6.3 (d, J=7.1 Hz, 1H), characteristic of quinonemethide triterpenes. Compounds 4 and 8 presented carbon signals between δC 213.5 and δC 117.2 corresponding to olefinic and carbonyl carbons. The presence of these signals allowed the identification of 4 and 8 as pristimerin and tingenone, respectively. Based on the signals at δC 173.3 and δC 172.9 (C=O), δC 68.9 (CH), δC 62.1 (CH₂), δC 14.1 (CH) and the signals between δC 34.3 to δC 22.7 (CH₃), compound 9 was characterized as triacylglycerol. After integration of the hydrogen signals (Figure 11S), and by the intensity of the signals of carbon atoms (Figure 12S) it was established that the 4 and 5 mixture consisted of 60% pristimerin (4) and 40% rigidenol (5).

Among the isolated compounds, the six triterpenes (3 to 8) and triacylglycerol (9) are reported for the first time for the M. distichophylla
species. Friedelan-3-one (1) is abundant in leaves of species of the genus Maytenus, such as M. ilicifolia,53-55 M. salicifolia,52 M. undata,5 and even in leaves of M. distichophylla.29 However, this compound is rare in roots of higher plants. On the other side, triacylglycerol (9) is uncommon in species of Maytenus. Mixtures of fatty acid short-chain-alkyl esters, such as 9, have been found in fruits of species of the Celastraceae family.55 β-sitosterol (2) is a steroid commonly found in species of the Celastraceae family. There are works reporting 2 as recurrent in roots of Maytenus species, such as M. ilicifolia,51 M. gonoclada,28 M. acanthophylla55 and in the leaves of M. distichophylla.29 The triterpene 3-oxo-olean-9(11),12-diene (3) was previously isolated from species of the Celastraceae family,56 as well as the triterpene 6 was previously reported for the stems and branches of M. imbricata.53

Pristimerin (4) is a quinonemethide naturally found in species of the Celastraceae family that induces cytotoxicity in different cancer cell lines.38-60 Rigidenol (5) was previously reported as constituent of M. acanthophylla roots,64 of M. chiapensis leaves64 and of M. cuzzoina root bark.62 Rigidenol isolated from M. cuzzoina and M. chiapensis exhibited anti-inflammatory activity and caused inhibitory effects on nitric oxide and prostaglandin E2 production in mouse macrophages (RAW 264.7) stimulated with bacterial endotoxin.61,62 In the present work, for the first time, rigidenol (5) is reported as a constituent of M. distichophylla roots. Rigidenol and 16β-hydroxypristimerin from M. salicifolia exhibited activity against Helicobacter pylori, and non-polar and polar extracts of this plant showed in vitro activity against oral pathogens such as Candida albicans, Streptococcus mutans, Streptococcus sanguinis, and Staphylococcus aureus.63 For these reasons, the mixture compounds 4 and 5 was also subjected to the biological assays in parallel with extracts and compounds isolated from M. distichophylla roots.

Antimicrobial evaluation

The activity of samples from M. distichophylla roots against S. aureus MRSA and C. albicans (SC5314) viability was evaluated by the MTT method. In this work, it was not observed significant antifungal activity against C. albicans of tested compound at maximal concentration when compared to DMSO used as control (Figure 2). It is important to highlight that it was not discarded possible activity against other pathogenic yeast and filamentous fungi.

Activity against S. aureus MRSA was observed at maximal tested concentrations and all samples demonstrating significant activity against S. aureus (Figure 2). The HE and CE extracts induced greater activity than ethyl acetate (EAE) and methanol (ME) extracts. HE and the mixture of 4 and 5 (Figure 2A), CE extract, compound 5 and 8 (Figure 2B) were the most active samples reducing 75% of S. aureus viability. Compounds 4 and 5 were isolated from HE and CE extracts, respectively. The HE and CE extracts induced greater activity than ethyl acetate (EAE) and methanol (ME) extracts. These results may be associated with the presence of pentacyclic triterpenes in the HE and CE extracts, for which antibacterial activity has been reported.10 Tingenone (8) isolated from CE extract induced similar activity to CE extract suggesting that the activity of this crude extract may be associated with the presence of 8 (Figure 2B). There was no significant difference in S. aureus susceptibility between the more active compounds (Figure 2). Thus, the more active samples can be considered as potential candidates for in vivo assays involving S. aureus infections aiming to establish its efficacy.

Cytotoxicity evaluation

Due to the demand for new resources against diseases that involve complicated, invasive, and expensive treatments such as anti-carcinogenic64 or antiretroviral agents, different compounds from plants have been used as a starting point for development of new drugs with social and economic potential. In this context, the cytotoxic activities of compounds and extracts from M. distichophylla roots were evaluated against leukemic cells through the IC50 value calculated by the MTT assay. The samples exhibited in vitro cytotoxicity against K562 chronic myeloid leukemia cells (1: 14.70 µg mL⁻¹; 2: 15.08 µg mL⁻¹; and 8: 12.56 µg mL⁻¹) and THP-1 acute leukemia cell lines (1: 10.95 µg mL⁻¹; 2: 13.25 µg mL⁻¹; and 8: 9.80 µg mL⁻¹) (Table 1). According to the results, the cytotoxic activities of compounds 1, 2 and 8 induced activity against the THP-1 cell, similar to etoposide, which is an inhibitor of DNA synthesis that acts through stabilization of type II topoisomerase-DNA complexes, preventing joining of single- and double-strand DNA breaks.65,66 On the other hand, the compound 8 showed similar cytotoxic activity (p > 0.05) to that of the etoposide in the K562 line (Table 1).

In addition to low cytotoxic activities, the compounds must show a higher selectivity index than the etoposide (Table 2). It may be noted that compounds 1 to 8 have better selectivity index than the etoposide, which is considered very cytotoxic to the studied cells. Therefore, the compounds with better cytotoxic activity and better SI are the compounds 1, 2, and 8 (THP-1 cells) and 2 and 8 (K562 cells) (Table 2).

Compounds 1, 2 and 8 showed cytotoxic activity and selectivity against THP-1 and K562 cells, being considered as candidates for new studies to elucidate its mechanism of inhibition.

Figure 2. Percent viability of S. aureus MRSA (ATCC 25923) and C. albicans (ATCC SC5314) treated with substances from Maytenus distichophylla roots. A) HE = hexane extract, 1 = friedelan-3-one, 2 = β-sitosterol, 3 = 3-oxo-olean-9(11),12-diene, 4 + 5 = [mixture of pristimerin (4) + rigidenol (5)], 5 = Rigidenol. B) 6 = 30-hydroxylap-20(29)-en-3-one, 7 = friedelan-3,7-dione, 8 = tingenone, CE = chloroform, EAE = ethyl acetate and ME = methanol extracts. DMSO = positive control
Table 1. Cytotoxic activity of *M. distichophylla* compounds, extracts and etoposide against THP-1, K562 and PBMC cell lines

| Sample | IC<sub>50</sub> (µg mL<sup>-1</sup>) ± SD * | THP-1 | K562 | PBMC |
|--------|----------------------------------------|-------|------|------|
| 1      | 10.95 ± 1.04                          | 14.70 ± 1.86 | 55.68 ± 2.87 |
| 2      | 13.25 ± 1.55                          | 15.08 ± 1.92 | 67.90 ± 2.51 |
| 3      | 25.55 ± 2.05                          | 29.01 ± 2.38 | 88.50 ± 3.11 |
| 4±5    | 51.02 ± 2.15                          | 42.89 ± 2.88 | 92.58 ± 3.78 |
| 5      | 66.37 ± 3.02                          | 78.09 ± 3.92 | 121.11 ± 5.02 |
| 6      | 31.04 ± 2.30                          | 33.60 ± 2.10 | 76.22 ± 3.58 |
| 7      | 25.44 ± 1.55                          | 30.88 ± 2.45 | 80.20 ± 4.11 |
| 8      | 9.80 ± 1.10                           | 12.56 ± 58  | 50.12 ± 3.57 |
| HE     | 88.20 ± 3.90                          | 92.50 ± 3.20 | 110.08 ± 4.12 |
| CE     | 29.20 ± 2.08                          | 34.50 ± 2.44 | 76.62 ± 3.50 |
| EAE    | 50.15 ± 3.29                          | 68.80 ± 3.56 | 80.65 ± 4.02 |
| ME     | 45.70 ± 3.17                          | 52.15 ± 3.01 | 78.70 ± 4.76 |
| Etoposide | 12.04 ± 2.32                      | 9.11 ± 1.33 | 8.63 ± 0.13 |
| P value | <0.05<sup>a</sup>                     | <0.05<sup>b</sup> | <0.05<sup>c</sup> |
|        | >0.05<sup>b</sup>                     | >0.05<sup>c</sup> |        |

*Values presented as average ± standard deviation. a: THP-1, Etoposide versus HE, 3-8, CE, EAE and ME; b: THP-1, Etoposide vs 1, 2 and 8; c: K562, Etoposide vs HE-7, CE, EAE and ME; d: K562, Etoposide vs 8, e: PBMC. Etoposide vs all compounds tested. 1 = friedelan-3-one, 2 = β-sitosterol, 3 = 3-oxo-olean-9(11),12-diene, 4±5 = [(mixture of pristimerin (4) + rigidenol (5)), 5 = rigidenol, 6 = 30-hydroxy lup-20(29)-en-3-one, 7 = friedelane-3,7-dione, 8 = tingenone. HE = hexane, CE = chloroform, EAE = ethyl acetate and ME = methanol extracts.

Table 2. Selectivity index determined for compounds and extracts from *M. distichophylla* for THP-1 and K562 cells

| Sample | Selectivity index for cells |
|--------|-----------------------------|
|        | THP-1 | K562 |
| 1      | 5.08  | 3.79 |
| 2      | 5.12  | 4.50 |
| 3      | 3.46  | 3.05 |
| 4±5    | 1.81  | 2.16 |
| 5      | 1.82  | 1.55 |
| 6      | 2.45  | 2.27 |
| 7      | 3.15  | 2.60 |
| 8      | 5.12  | 4.00 |
| HE     | 1.25  | 1.19 |
| CE     | 2.62  | 2.22 |
| EAE    | 1.61  | 1.17 |
| ME     | 1.72  | 1.51 |
| Etoposide | 0.72  | 0.95 |

*1 = friedelan-3-one, 2 = β-sitosterol, 3 = 3-oxo-olean-9(11),12-diene, 4±5 = [(mixture of pristimerin (4) + rigidenol (5)), 5 = rigidenol, 6 = 30-hydroxy lup-20(29)-en-3-one, 7 = friedelane-3,7-dione, 8 = tingenone. HE = hexane, CE = chloroform, EAE = ethyl acetate and ME = methanol extracts.

Toxicity to *Caenorhabditis elegans*

In the present work, extracts and compounds of *M. distichophylla* roots were tested using the *C. elegans* model. Firstly, the samples were tested to verify if are toxic to the L1 larvae, that is the most vulnerable development stage of the *C. elegans*. Thus, the acute (24 h exposition) toxicity of samples from *M. distichophylla* roots vs *C. elegans* L1 larvae was evaluated by the MTT method. For some samples from *M. distichophylla*, especially for 6 and 8, an increase in the blue color of MTT-formazan was observed, suggesting proliferation of *C. elegans*. However, as L1 larvae do not multiply, values higher than 100% indicate a larger number of nematodes in the wells, leading to overestimation of the spectrophotometric reading in acute toxicity evaluation of the compounds on *C. elegans*. Thus, no toxicity induced by the samples on L1 larva was detected under the experimental conditions used (Figure 3).

The chronic toxicity and survival of adult (L4) *C. elegans* can be evaluated through the median lethal time (LT<sub>50</sub>), which represents 50% lethality induced by a specific concentration of a substance, observed after specific period of time. So, the LT<sub>50</sub> of *C. elegans* observed after its exposure to extracts and compounds from *Maytenus distichophylla* roots was determined (Figure 4). Compounds which show high LT<sub>50</sub> values after 5 days are considered to possess therapeutic potential. This property can be assigned to hexane extract (HE) (8 days) and to 4±5 mixture (6 days) (Figure 4). The other samples from *M. distichophylla* roots showed values of time until death LT<sub>50</sub> ≤ 5 days at the tested concentrations, indicating that chronic exposure to them affects the viability of *C. elegans*. Therefore, these substances have nematicide properties that make them suitable for parasitology applications.

In the chronic trials, adult (L4) *C. elegans* were used to verify if toxic effects are induced by the samples of *M. distichophylla*, after an exposure time. Thus, survival curves of *C. elegans* (L4 larvae) normally fed with *E. coli* OP50 and in the presence of samples at the maximum concentration tested were established (Figure 5). It was observed that all extracts and compounds, after a chronic exposure, reduced significantly the *C. elegans* (L4 larvae) survival (Figure 5).
Constituents from roots of *Maytenus distichophylla*, antimicrobial activity and toxicity for cells

### Compound 1 (friedelan-3-one), 2 (β-sitosterol), 3 (3-oxo-olean-9(11),12-diene), 4+5 = [(mixture of pristimerin (4) + rigidenol (5)], 5 = rigidenol, 6 = 30-hydroxyulp-20(29)-en-3-one, 7 = friedelane-3,7-dione, 8 = tingenone, HE = hexane, CE = chloroform, EAE = ethyl acetate and ME = methanol extracts. DMSO = positive control

Compound 1 (friedelan-3-one), 2 (β-sitosterol), 3 (3-oxo-olean-9(11),12-diene), 5 (rigidenol), 6 (30-hydroxyulp-20(29)-en-3-one), 7 (friedelane-3,7-dione) and 8 (tingenone), and the HE, CE, EAE and ME extracts showed a potent nematicidal activity, reducing more than 60% the LT50 of *C. elegans* and thus decreasing worm’s survival (Figure 5). More relevant are rigidenol (5), 30-hydroxyulp-20(29)-en-3-one (6) and ethyl acetate extract (EAE) of *M. distichophylla* that killed more than 80% of the worm population and thus, these samples are interesting candidates to more detailed studies related to mechanisms of nematicide action. The nematicide potential found could be explored in parasitological studies.

### CONCLUSIONS

Nine compounds were isolated from *M. distichophylla* roots, being seven pentacyclic triterpenes, one steroid and one triacylglycerol. Among the isolated compounds, seven (3 to 9) were described for the first time from *M. distichophylla*. The effects of the compounds on the viability of *S. aureus* together with the non-toxicity of the compounds for *C. elegans* larvae constitute good prediction for the use of hexane extract and the mixture of 4+5 [pristimerin (4) + rigidenol (5)], in researches involving in vivo bacterial infections. Compounds 1, 2 and 8 showed potential cytotoxic activity and selectivity against THP-1 and K562 cells.

### SUPPLEMENTARY MATERIAL

The Figures 1S–25S present spectral data of compounds isolated of hexane and chloroform extracts of the roots of *Maytenus distichophylla*. This supplementary material is available free of charge at http://quimicanova.sbq.org.br, as PDF file.

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