Article

Leishmanicidal and Antimicrobial Activities of 4-Quinolone Alkaloids from Stems of the Medicinal Plant *Waltheria indica* (Malvaceae) and Their Chemotaxonomic Significance

Raquel M. Silva,[a] Levy T. S. Domingos,[b] Gabriellen M. M. de Castro,[b] Marinella S. Laport,[b] Antônio Ferreira-Pereira, [b] Murilo M. C. Lima[c] and Fernando Cotinguiba *,[a]

[a]*Instituto de Pesquisas de Produtos Naturais Walter Mors (IPPN), Centro de Ciências da Saúde (CCS), Universidade Federal do Rio de Janeiro (UFRJ), Avenida Carlos Chagas Filho, 373, Bloco H, Ilha do Fundão, 21941-901 Rio de Janeiro-RJ, Brazil*

[b]*Instituto de Microbiologia Paulo de Góes (IMPG), Centro de Ciências da Saúde (CCS), Universidade Federal do Rio de Janeiro (UFRJ), Avenida Carlos Chagas Filho, 373, Bloco J, Ilha do Fundão, 21941-901 Rio de Janeiro-RJ, Brazil*

[c]*Instituto Federal de Educação, Ciência e Tecnologia do Rio de Janeiro (IFRJ), Campus Realengo, Rua Professor Carlos Wenceslau, 343, Realengo, 21710-240 Rio de Janeiro-RJ, Brazil*

A chemical investigation of the stem of *Waltheria indica* (Malvaceae) yielded twelve 4-quinolone alkaloids, which were primarily waltheriones. These were waltherione A (**1**), waltherione B (**2**), waltherione C (**3**), waltherione G (**4**), waltherione H (**5**), waltherione J (**6**), waltherione L (**7**), waltherione P (**8**), chamaedrone (**9**), 8-deoxy-antidesmone (**10**), antidesmone (**11**), and the previously unreported alkaloid *N*-methoxy-waltherione A (**12**). These alkaloids belong to an unusual class of 4-quinolones and therefore, have chemosystematic significance for distinguishing the *Waltheria* and *Melochia* genera from the rest of the Malvaceae family. The ability of the alkaloid isolates to reverse the phenotypic expression of fluconazole-resistance was tested by using a mutant strain of *Saccharomyces cerevisiae* that expressed a *Candida albicans* transporter. Of the isolates tested, waltherione G afforded a positive result. Leishmanicidal activity and bactericidal tests were also performed using the isolated alkaloids, which showed promising results.

Keywords: *Waltheria*, *Melochia,* waltheriones, antidesmone, fungicide

Introduction

Waltheria indica L. (synonym *Waltheria americana*) is a shrub that belongs to the Malvaceae family. The shrub is widespread in tropical and subtropical regions of the world, where it occurs in small urban areas and is considered a weed on some plantations. In Brazil, *Waltheria indica* is commonly known as "*vassourinha*", which is a Portuguese term that means "small broom". As a part of traditional medicine in several countries, it is used for the treatment of inflammatory diseases such as wounds, skin ulcers, rheumatism, sore throat, gingivitis, diarrhea, and conjunctivitis. There are also reports of the use of *W. indica* to treat anemia, malaria, seizures, asthma, hemorrhoids, leprosy, infertility, erectile dysfunction, and impotence.[1]

Previous chemical investigations of the genus *Waltheria* have indicated high similarity with the chemical composition of *Melochia*, which is another genus of Hermannieae, an order of the Malvaceae family.[2-5] Both genera are potential sources of bioactive alkaloids, cyclic peptides, and an unusual class of 4-quinolone alkaloids called waltheriones.[2,3,6-8]

Waltheriones are structurally characterized by a 4-quinolone moiety with a methyl group at the C-2 position and a methoxy group at the C-3 position. These compounds are divided into open-chain (*exempli gratia*, alkaloids **4-11**) and cyclized compounds (*exempli gratia*, alkaloids **1-3** and **12**). The open-chain waltheriones include the alkaloid antidesmone and its analogs. These compounds are chemosystematic markers of the Antidesmeae

*e-mail: fernando@ippn.ufrj.br
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order of the Euphorbiaceae family.[9,10] For example, antidesmone has been reported in *Waltheria douradinha*,[7] *Melochia chamaedrys*,[11] and *Waltheria indica*.[2,12,13] Other characteristic compounds such as open-chain waltheriones E-Q and cyclized waltheriones A and C have also been isolated from *Waltheria indica*.[2,3,12,13] More recently, the new open chain waltheriones R-T, novel derivatives of open chain waltheriones, two derivatives of waltherione C, and the new cyclized waltheriones U-V and 13-methoxy-waltherione V have been reported from *Waltheria indica*.[14] To date, only flavonoids and four cyclic peptide alkaloids, adoutines X, Y, Y1, and Z, have been isolated and identified from *W. indica*.[15-18]

Cyclized waltheriones typically contain a benzofused oxabicyclo [3.2.1] octene moiety, where the epoxide bridge of the bicycle can be between carbons C-13 and C-10, for example, in waltheriones A and B. Additionally, this epoxide bridge can exist between carbons C-13 and C-9, for example, in waltherione C. The precursor of the cyclized waltheriones is the alkaloid melochinone.[19] This alkaloid has been reported from *Melochia tomentosa* and has a 7-membered ring that is fused to the quinolinone system, which replaces the characteristic oxabicyclo [3.2.1] moiety.[5] Another example of a compound with this structure is the recently reported alkaloid waltherione U,[14] which is the most structurally similar to melochinone.

Among the cyclized waltheriones, the alkaloid waltherione A was first reported in *Waltheria douradinha*,[6] and was further reported in *Waltheria brachypetala*,[8] *Melochia chamaedrys*,[11] *Melochia odorata*,[4,20] and *Waltheria indica*,[2] while waltherione B was only reported in *Waltheria douradinha*.[7] Waltherione C has been reported in *Melochia odorata*,[4] *Melochia umbellata*,[5] and *Waltheria indica*.[2]

Several biological activities have been attributed to waltheriones. These include anticancer,[21] antifungal,[3,20,22] anti-inflammatory,[23] antichagasic,[14] anti-human immunodeficiency virus (anti-HIV),[4] and botanical nematicide activities.[24] Their inhibition of acetylcholinesterase (AChE),[8] and their potential use as larvicides for the *Aedes aegypti* mosquito[25] have also been reported.

Herein, the chemical composition of the stem of *Waltheria indica* is identified. The ability of the isolated alkaloids to reverse fluconazole-resistance that is expressed phenotypically, as well as their leishmanicidal activity and bactericidal activity, is investigated. The determination of these bioactivities is meaningful in the treatment of medical conditions related to parasitic and bacterial infection and antibiotic resistance.

Experimental

Plant material

The plant material was collected in June 2018 at Piripá (Bahia State, Brazil) by Prof Murilo Marinho de Castro Lima, and was identified by Prof Cássia Mônica Sakuragui (Institute of Biology, IB/UFRJ). A voucher specimen (RFA 40614) was deposited in the Herbarium RFA (IB/UFRJ) for reference and registered (AB50421) in the Sistema Nacional de Gestão do Patrimônio Genético e do Conhecimento Tradicional Associado (SisGen).

Extraction and isolation

The dried and subsequently powdered stems (901.3 g) of *Waltheria indica* were extracted by static maceration with ethanol (EtOH) (Dinâmica, Indaiatuba, Brazil) (3 × 48 h each, at room temperature). The *Waltheria indica* ethanolic stem extract (WISE) (55.08 g) was then subjected to liquid-liquid partition after being solubilized. Separately, using a mixture of water:methanol (H_2O:MeOH) (Dinâmica, Indaiatuba, Brazil) (3:1), subsequent funnel partitioning was performed with hexane (Dinâmica, Indaiatuba, Brazil), dichloromethane (CH_2Cl_2) (Isofar, Duque de Caxias, Brazil), and ethyl acetate (EtOAc) (Química Moderna, Barueri, Brazil) to obtain the respective hexane (WISH) (4.11 g), dichloromethane (WISD) (0.66 g), and ethyl acetate (WISEA) (3.71 g) phases. The hexane and dichloromethane fractions were analyzed by thin layer chromatography (TLC) (Merck, Darmstadt, Germany) in an eluent system of chloroform:methanol ($CHCl_3$:MeOH) (Dinâmica, Indaiatuba, Brazil) (97:3). This system was also developed with Dragendorff's reagent to indicate the presence of alkaloids. The hexane phase (WISH) was subjected to silica gel (230-400 mesh) chromatographic column (CC) (35 × 4 cm) (Merck, Darmstadt, Germany) eluted with hexane-EtOAc mixtures (90:10, 80:20 and 70:30, v/v) and CH_2Cl_2-MeOH mixtures (97:3, 95:5, 90:10, 80:20, 60:40, 50:50 and 20:40, v/v) to give 16 fractions. Fraction 1 (381.2 mg) was subjected to silica gel (230-400 mesh) CC (25 × 4 cm, hexane:MeOH, 60:40, v/v) to give **10** (2.5 mg). Fraction 3 (421.5 mg) was subjected to silica gel (230-400 mesh) CC (25 × 4 cm, hexane:MeOH, 80:20, v/v) to give 3 sub-fractions. Sub-fraction 3-2 was subjected to silica gel (230-400 mesh) CC (17 × 1.5 cm, $CHCl_3$:MeOH, 80:20 and 70:30, v/v) to give 4 sub-fractions. Sub-fraction 3-2-2 was subjected to silica gel (230-400 mesh) CC (17 × 1.5 cm, $CHCl_3$:MeOH, 90:10, 80:20, 70:30 and MeOH, v/v) to give **12** (15 mg). Fraction 10 (385.3 mg) was subjected to silica gel (230-400 mesh) CC (25 × 2 cm,

CH$_2$Cl$_2$:MeOH, 50:50 and MeOH, v/v) to give 7 sub-fractions. Sub-fraction 10-2 (203.8 mg) was subjected to silica gel (230-400 mesh) CC (25 × 2 cm, hexane:EtOAc, 1:1, CH$_2$Cl$_2$, CH$_2$Cl$_2$:MeOH, 97:3, 5:5, and MeOH, v/v) to give 7 sub-fractions. Sub-fraction 10-2-5 (154.4 mg) was subjected to silica gel (230-400 mesh) CC (25 × 2 cm, CH$_2$Cl$_2$, CH$_2$Cl$_2$:MeOH, 99:1, 98:2, 97:3, 95:5, 5:5, and MeOH, v/v) to give 7 sub-fractions. Sub-fraction 10-2-5-5 (27 mg) was subjected to silica gel (230-400 mesh) CC (15 × 2 cm, hexane, hexane:EtOAc, 90:10, 80:20, 70:30, 60:40, CH$_2$Cl$_2$, CH$_2$Cl$_2$:MeOH, 99:1, 98:2, 97:3, 95:5, 5:5, and MeOH, v/v) to give **9** and **7** (10 mg). The dichloromethane phase (WISD) was subjected to silica gel (230-400 mesh) CC (35 × 4 cm) eluted with CH$_2$Cl$_2$-MeOH mixtures (90:10, 80:20, 60:40, 50:50 and MeOH, v/v) to give 11 fractions. Fraction 1 (151.1 mg) was subjected to silica gel (230-400 mesh) CC (25 × 2 cm, CH$_2$Cl$_2$, CH$_2$Cl$_2$:MeOH, 97:3 and MeOH, v/v) to give 7 sub-fractions. Sub-fraction 1-5 was subjected to silica gel (230-400 mesh) CC (28 × 2 cm, CH$_2$Cl$_2$:MeOH, 99:1, 98:2, 95:5, 50:50 and MeOH, v/v) to give **3** (6 mg). Fraction 2 (99.2 mg) was subjected to silica gel (230-400 mesh) CC (25 × 4 cm, CH$_2$Cl$_2$, CH$_2$Cl$_2$:MeOH, 97:3 and MeOH, v/v) to give **1** (30 mg). Fraction 3 (45.6 mg) was subjected to silica gel (230-400 mesh) CC (25 × 2 cm, CH$_2$Cl$_2$:MeOH, 97:3, 90:10, 50:50 and MeOH, v/v) to give **2** (5 mg). The sub-fraction WISH 10-2-5-3 (45.5 mg, 15 mg mL^{-1}) was purified in semi-preparative mode using the high performance liquid chromatography (HPLC) semi-preparative equipment (Shimadzu, Kyoto, Japan), semi-preparative column (Phenomenex Luna, Torrance, USA) (C-18 (10 μm); diameter (d) = 1 cm; length (l) = 25 cm) at a flow rate of 2.8 mL min^{-1}, with detection at 220 nm using water:acetonitrile (Tedia, Fairfield, USA) (6:4), resulting in the isolation of five of the twelve 4-quinolone alkaloids: alkaloids **4** (retention time-t_R: 31.468 min), **5** (t_R: 60.129 min), **6** (t_R: 55.658 min), **8** (t_R: 29.770 min) and **11** (t_R: 46.955 min).

Nuclear magnetic resonance (NMR), mass spectrometry (MS) analysis and optical rotations

The structures of the alkaloids were confirmed from the ^{1}H and ^{13}C NMR spectral data, and the chemical shifts obtained were subsequently compared to those documented in the literature. ^{1}H and ^{13}C NMR experiments were conducted using a Varian 9.4 T instrument (Varian, Palo Alto, USA) at 500 and 400 MHz, respectively. The samples were dissolved in approximately 0.6 mL aliquots of deuterated solvent: chloroform (CDCl$_3$), methanol (MeOD) and dimethyl sulfoxide (DMSO-d_6) (CIL-Cambridge

Isotope Laboratories, Tewksbury, USA), with 99.8% deuterium for CDCl$_3$ and MeOD and 99.9% deuterium for DMSO-d_6. Tetramethylsilane (TMS) (CIL-Cambridge Isotope Laboratories, Tewksbury, USA) was used as an internal standard. *N*-Methoxy-waltherione A was analyzed by high-resolution mass spectrometry equipment (Bruker Daltonics, Billerica, USA), model UltrOTOFQ-ESI-TOF (time of flight), in positive and negative mode at the Mass Spectrometry Laboratory at Faculdade de Ciências Farmacêuticas de Ribeirão Preto, USP, Brazil. Optical rotations were taken on a PerkinElmer 341 digital Polarimeter (Norwalk, USA). Ultrapure dichloromethane and methanol (Tedia, Fairfield, USA) were used as solvents and concentrations were determined from models in the literature.

Chemosensitization assays

Chemosensitization tests were carried out at the Microbial Biochemistry Laboratory (Institute of Microbiology Paulo de Góes/UFRJ). For this assay, mutant strains of *Saccharomyces cerevisiae*, which overexpress proteins of *Candida albicans* (that confer resistance to xenobiotics), were used and considered as positive controls. The CaCDR1 and CaCDR2 strains overexpress the CaCdr1p and CaCdr2p ABC (ATP-binding cassette) transporters, respectively, and the CaMDR1 strain overexpresses the CaMDR1p major superfamily (MSF) transporter.[26] All strains were kindly provided by Dr Richard Cannon and Brian Monk (University of Otago, New Zealand). The resistant strain cells (2.5 × 10^6 cells mL^{-1}) were inoculated onto the surface of plates with Yeast Extract Peptone Dextrose (YPD) medium (1% yeast extract, 2% peptone, and 2% glucose) in the presence or absence of sub-inhibitory concentrations of fluconazole. Sterile disks (6 mm diameter) of Whatman 3MM paper (Merck, Darmstadt, Germany) were then placed on the surface of the medium, and 5 μL of each compound (10 mg mL^{-1}) was added. These plates were then incubated at 30 °C for 48 h to observe the growth of inhibition zones around the disks.[27]

Leishmanicidal activity test

Leishmanicidal activity assays were carried out at the Microbial Biochemistry Laboratory (Institute of Microbiology Paulo de Góes/UFRJ). Promastigotes of *Leishmania infantum* were first harvested and then counted in a Neubauer chamber. The promastigotes were finally seeded in Schneider's Insect medium, which was supplemented with 10% fetal bovine serum (FBS) at a concentration of 2 × 10^6 parasites mL^{-1} in 96-well plates. Amphotericin B was used as a positive control. Afterwards,

the cells were incubated in the absence or presence of serial dilutions of the compounds (100 to 6.25 µg mL^{-1}), for 72 h at 26 °C. Cells in the absence of compounds were used as controls. The cell viability was verified by the colorimetric reduction of methyl-thiazolyl-tetrazolium (MTT) (5 mg mL^{-1}) using a SpectraMax i3x microplate reader (Molecular Devices, San José, USA).[28]

Antibacterial activity assay

The antibacterial activity of the alkaloids was evaluated at the Molecular and Marine Bacteriology Laboratory (Institute of Microbiology Paulo de Góes/UFRJ). The antibacterial activities of the samples (1 mg mL^{-1}) were determined by the agar diffusion method, which involved the growth of bacteria.[29] Briefly, 50 µL of each sample was spotted onto brain heart infusion (BHI) agar (Bacto BD, Sparks, USA), and 10^5 cells mL^{-1} of each indicator strain (in 3 mL of BHI soft agar) was poured over the plates. The plates were then incubated at 37 °C for 18 h, and the occurrence of inhibition zones around the spotted samples was considered to be indicative of inhibitory activity. The assay was performed for initial screening using the reference strain *Staphylococcus aureus* ATCC 29213 as an indicator. The bioactive samples were tested against clinically important strains, namely *Staphylococcus epidermidis* ATCC 12228 and *Escherichia coli* ATCC 25922; multidrug-resistant strains, namely *Enterococcus faecalis* V583 (resistant to vancomycin (VRE), erythromycin,

chloramphenicol, and kanamycin), *S. aureus* (resistant to methicillin (MRSA)), *Klebsiella pneumoniae* (resistant to carbapenem, KPC), and *Citrobacter freundii* (resistant to aminoglycosides, quinolones, macrolides). The dilution method was used to determine the minimum inhibitory concentration (MIC). This method was evaluated using BHI medium.[30] *S. aureus* ATCC 29213 cells (10^4 colony forming unit (CFU) mL^{-1}) were inoculated in BHI soft agar and then poured over the plates containing the active samples (100-1.56 µg mL^{-1}). The plates were then incubated at 37 °C for 18 h. An isolated compound was considered to have significant antimicrobial activity for MIC ≤ 10 µg mL^{-1}, moderate for 100 µg mL^{-1} ≤ MIC > 10 µg mL^{-1}, and weak for MIC > 100 µg mL^{-1}. All the tests were performed in duplicate or triplicate when necessary, and organic solvents were used as negative controls.

Results and Discussion

Determination of structure of alkaloids

The structures of the isolated compounds (Figure 1) were first positively revealed with Dragendorff's reagent in TLC, suggesting the presence of nitrogen in their molecular structure. Subsequently their shifts were compared (^{1}H and ^{13}C NMR (1D and 2D), MS, and specific rotation) with the literature values for waltherione A (**1**),[6] waltherione B (**2**),[7] waltherione C (**3**),[4] waltherione G (**4**),[13] waltherione H (**5**),[13] waltherione J (**6**),[13] waltherione L (**7**),[13] waltherione P (**8**),[3]
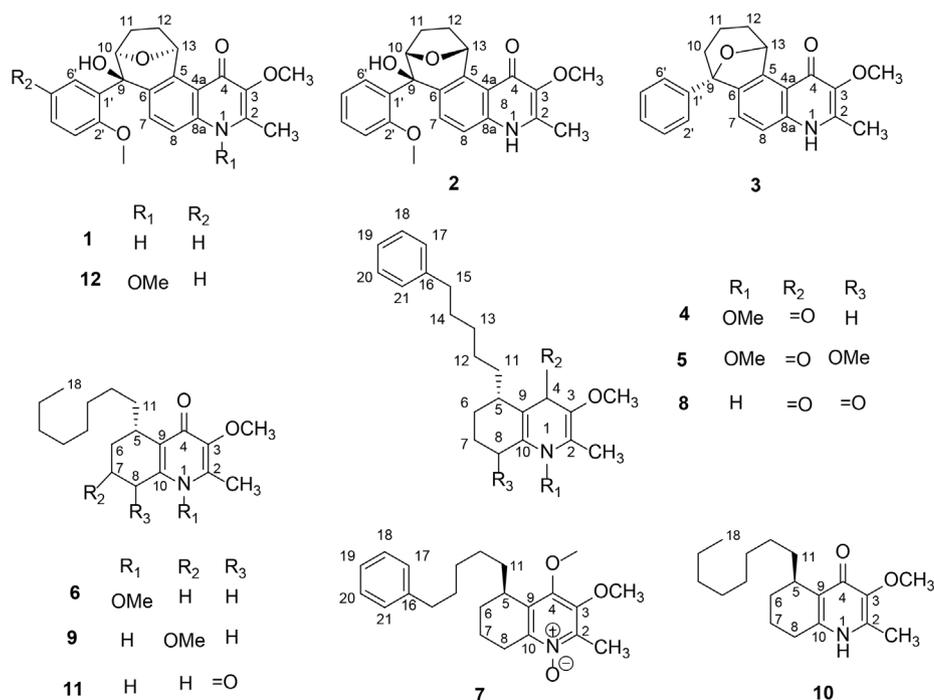
Figure 1. 4-Quinolone alkaloids isolated from *Waltheria indica*.

chamaedrone (**9**),[11] 8-deoxy-antidesmone (**10**),[13] antidesmone (**11**),[9] and *N*-methoxy-waltherione A (**12**).

The alkaloid (**12**) (15 mg) was obtained as an off-white solid. The ^{1}H NMR spectrum (Figure S1, Supplementary Information (SI) section) revealed the presence of characteristic signals of the cyclized waltheriones, such as those of the two oximethine hydrogens of the epoxide bridge. These oximethine hydrogens were between C-10 and C-13, and had shifts at δ 4.69 ppm (dd, *J* 7.6, 1.5 Hz, H-10) and δ 6.75 ppm (d, *J* 6.0 Hz, H-13), respectively. Additionally, diasterotopic methylene hydrogens that are characteristic of cyclized waltheriones were present. This is supported by the characteristic shifts at δ 2.10 ppm (m, H-11a), δ 2.38 ppm (m, H-11b), δ 2.05 ppm (m, H-12a), and δ 2.45 ppm (m, H-12b). The presence of these diasterotopic methylene hydrogens was confirmed by homonuclear correlated spectroscopy (COSY) ([1]H × [1]H) (Figure S2, SI section) and heteronuclear single quantum coherence (HSQC) ([1]H × [13]C) (Figure S3, SI section) correlations. The characteristic C-2 methyl and C-3 methoxy signals of the 4-quinolone moiety were verified by shifts at δ 2.41 ppm (s) and δ 3.87 ppm (s), respectively. The hydrogens that participated in *ortho* coupling to the benzofused oxabicyclo group were verified by shifts at δ 7.26 ppm (d, *J* 8.8 Hz, H-8) and δ 7.57 ppm (d, *J* 8.8 Hz, H-7). In addition, the ^{1}H NMR spectrum indicated the presence of an *ortho*-disubstituted benzene spin system with four hydrogens signals at δ 6.4 ppm (dd, H-6'), δ 6.73 ppm (m, H-5'), δ 7.22 ppm (m, H-4') and δ 6.96 ppm (dd, H-3'). These connectivities were confirmed by the homonuclear COSY ([1]H × [1]H) spectrum. The heteronuclear multiple bond correlation (HMBC) ([1]H × [13]C) (Figure S4, SI section) association between H-6' and C-9 (δ 78.20 ppm), as well as that between the methoxyl group, with a shift of δ 3.87 ppm (6H, C3-OMe and C2'-OMe), and C2' (δ 156.22 ppm) confirmed that an *ortho*-disubstituted aromatic ring was attached to the oxabicyclo moiety. The additional signal at δ 4.01 ppm (s, 3H), which had HSQC associations with the ^{13}C signal at δ 65.12 ppm, is attributed to the *N*-methoxy group. The ^{13}C NMR spectrum (Figure S5, SI section) revealed the presence of 24 carbons, highlighting the carbonyl group of a 4-quinolone moiety with a signal at δ 174.90 ppm and the oxygenated carbons C-10 (δ 80.12 ppm), C-13 (δ 75.68 ppm) of the oxabicyclo moiety. The ^{1}H, ^{13}C, COSY, HSQC, and HMBC NMR data and specific rotation of alkaloid **12** were similar to the literature values for waltherione A (**1**)[6] (see Table S1, SI section); however, alkaloid **12** had an additional signal, which was associated with a methoxyl group that was attached to a nitrogen. This was confirmed by ^{1}H and ^{13}C NMR and high-resolution electrospray ionization mass spectrometry (HRESIMS). Based on this data, the alkaloid **12** was elucidated to be a previously unreported *N*-methoxy-waltherione A. The ^{1}H and ^{13}C spectra, along with the HREISMS data for *N*-methoxy-waltherione are presented in the SI section. The HREISMS profile of compound **12** (Figure S6, SI section) showed a pseudomolecular ion peak at *m/z* 424.1756 Da [M + H^{+}] (calcd. for $C_{24}H_{25}NO_6$, 424.1760; calculate mass accuracy error: 0.94 ppm). Optical rotation was determined by method of polarimetry, $[\alpha]^{25}_D$: −4.5 (*c* 0.01 CHCl$_3$), and is in agreement with the previous literature, based on the structure of alkaloid **1**.[6] For $[\alpha]^{25}_D$ values of the other alkaloids, see Table S2, SI section.

Chemosystematics significance

The family of 4-quinolone alkaloids, which is also referred to as waltheriones, include the alkaloids antidesmone, chamaedrone,[11] melochinone, and melovinone.[19,31] These alkaloids are characteristic compounds that are often found in the genus *Waltheria* and the genus *Melochia* that belong to the Malvaceae family. However, to the best of our knowledge, very few chemical studies have been performed on plants of these genera; thus, it is impossible to distinguish between the *Waltheria* and *Melochia* genera based on these characteristic waltheriones. After the isolation of waltherione A,[6] studies on different species of these genera have cited the isolation of new waltheriones or the re-isolation of waltheriones.

Among the waltheriones reported herein, waltheriones A and C and antidesmone were previously reported in species of both *Waltheria* and *Melochia*.[2-6,8,11,12,20] The alkaloid waltherione B was only reported in *W. douradinha*;[7] thus, this is the second report of its isolation. Considering that the isolated compounds antidesmone and 8-deoxoantidesmone are chemical markers of the Antidesmeae order of the Euphorbiaceae family, there is likely a relationship among *Waltheria*, *Melochia*, and species of the Antidesmeae order of the Euphorbiaceae family. Additionally, to the best of our knowledge, this is the first report of the alkaloid *N*-methoxy-waltherione A (**12**).

Chemosensitization assays

To determine the chemoreversing action of the alkaloids isolated from *Waltheria indica*, they were screened against mutant cells that overexpress ABC or MSF transporters and are phenotypically fluconazole-resistant. *Saccharomyces cerevisiae* is a versatile experimental model, and mutant strains that express heterologous proteins of pathogenic yeast, such as

Candida albicans, are important alternatives for the discovery of new resistance modulators.[32]

In the absence of fluconazole, only substance **4** inhibited the growth of CaMDR1, which is suggestive of antifungal activity. In contrast, in the presence of fluconazole, the activity of compound **4** increased, and substances **3**, **5**, **6**, **7 + 9**, and **11** also developed an inhibition zone. These results suggest that there is a synergistic effect or an effect that is associated with an additive interaction between these compounds and fluconazole, which led to the inhibition of yeast growth. This is the first report on the chemoreversing action of *W. indica* alkaloids against fluconazole-resistant cells. None of the alkaloids showed activity, alone or in combination with fluconazole, against CaCDR1 and CaCDR2 (data not shown). This observation is suggestive of a specific effect against the CaMDR1 MFS transporter.

Leishmanicidal activity

Some waltheriones have shown potent *in vitro* inhibitory activity against the growth of *Trypanosoma cruzi*.[14,33]

Compounds **4**, **5**, **7 + 9**, and **11** were excellent inhibitors, affording almost 100% inhibition of *L. infantum* growth at low concentrations (Figure S8, SI section). Compound **1** (Table 1) was the least effective because it was not able to affect leishmania growth even at 100 µg mL⁻¹, and afforded only 40% growth inhibition. Compounds **3** and **8** showed slightly better activity than **1** but were also required in high concentrations to inhibit the growth of promastigotes.

Table 1. Chemosensitizing and leishmanicidal potential of alkaloids isolated from *W. indica*

Alkaloid	Chemosensitization assays without fluconazole[a]	Chemosensitization assays with fluconazole[a]	Leishmanicidal activity IC_{50} values[b] / (µg mL⁻¹)
1	–	–	> 100
2	–	–	–
3	–	+	36
4	++	+++	< 4.5
5	–	++	< 9.8
6	–	+	8.4
7 + 9	–	++	< 12.3
8	–	–	37
10	–	–	–
11	–	++	< 8.0
12	–	–	–

– not tested or activity not observed; + weak activity; ++ medium activity; +++ strong activity; positive control: amphotericin B (half maximal inhibitory concentration (IC_{50}) = 0.5 µg mL⁻¹). [a]Figure S7, SI section; [b]Figure S8, SI section.

Antibacterial activity assay

The discovery of new antibiotic compounds is important because of the increasing occurrence of pathogenic microorganisms that exhibit multiple resistance to drugs that are currently in clinical use.[34] The antibacterial activity of the alkaloids against *S. aureus* ATCC 29213 was determined, where this bacterium is a reference strain that is used as a control for testing antimicrobial activity. Alkaloids **3**, **5-9** and **11** all had antibacterial activity. The best MIC values were obtained for alkaloid **8** (3.13 µg mL⁻¹) and alkaloid **11** (6.25 µg mL⁻¹). The alkaloids **3**, **4**, **5**, **6**, **7 + 9**, and **12** had a moderate activity of 25, 100, 100, 25, 50, and 25 µg mL⁻¹, respectively.

These alkaloids were selected for evaluating their action against clinically important strains. In this assay, only the biofilm-producing strain *S. epidermidis* ATCC 12228 was inhibited by alkaloids **3**, **5**, **6**, **7 + 9**, **8**, and **11** (Table 2) (Figure S9, SI section). *Staphylococci* are currently the most common cause of nosocomial infections. This is mainly related to orthopedic devices, where bacteria can form biofilms. *S. aureus* strains that cause implant infections show high rates of antimicrobial resistance. Additionally, there is an alarming increase in the antimicrobial resistance in other species such as *S. epidermidis*.[35]

Table 2. Spectrum of action against strains of clinical importance

Alkaloid	Clinical strain					
	SA	EF	KP	CF	EC	SE
3	–	–	–	–	–	+
4	–	–	–	–	–	–
5	–	–	–	–	–	+
6	–	–	–	–	–	+
7 + 9	–	–	–	–	–	+
8	–	–	–	–	–	+
11	–	–	–	–	–	+
12	–	–	–	–	–	–

Clinical strains: *S. aureus* MRSA (SA), *E. faecalis* VRE (EF), *K. pneumoniae* KPC (KP), *C. freundii* (CF), *E. coli* (EC), and *S. epidermidis* (SE). Inhibitory activity: negative (–) and positive (+).

Conclusions

This study demonstrated the isolation and identification of twelve 4-quinolonic alkaloids from the species of *W. indica* from Brazil. These alkaloids are waltherione A (**1**), waltherione B (**2**), waltherione C (**3**), waltherione G (**4**), waltherione H (**5**), waltherione J (**6**), waltherione L (**7**), waltherione P (**8**), chamaedrone (**9**), 8-deoxy-antidesmone (**10**), antidesmone (**11**), and *N*-methoxy-waltherione A (**12**); the latter has been described for the first time.

The isolation and identification of these alkaloids from *W. indica* advances knowledge related to the chemosystemic characterization of the Hermannieae order (Malvaceae). Previous reports have already identified melamine quinolone alkaloids, such as melochinone, waltherione A (**1**), waltherione B (**2**), and waltherione C (**3**) in *Waltheria* and *Melochia* species, as potential chemotaxonomic markers for the Hermannieae order.

Chemosensitization assay confirmed that the growth inhibition of *Candida albicans* was amplified by a combination of fluconazole and waltherione G (**4**). This is the first report that details this type of inhibition by the alkaloids isolated in this study. The alkaloids waltherione G (**4**), waltherione H (**5**), waltherione L (**7**) + chamaedrone (**9**), and antidesmone (**11**) have excellent inhibitory activities as they afforded almost complete inhibition of the activity of *L. infantum* at low concentrations.

In the bactericidal tests, waltherione P (**8**) and antidesmone had the best MIC values against *S. aureus*, and only *S. epidermidis* was inhibited by the alkaloids waltherione C (**3**), waltherione H (**5**), waltherione J (**6**), waltherione L (**7**) + chamaedrone (**9**), waltherione P (**8**), and antidesmone (**11**).

Supplementary Information

Supplementary data are available free of charge at http://jbcs.sbq.org.br as PDF file.

Acknowledgments

Author Contributions

Raquel de M. Silva was responsible for the extraction, isolation, elucidation of the alkaloids found, data analysis and writing of the article, supervised by Murilo M. C. Lima and Fernando Cotinguiba who elaborated the experimental scheme. The plant material was collected by Murilo M. C. Lima. The chemosensitization assays were carried out by Levy T. S. Domingos and the leishmanicidal activity tests were carried out by Gabriellen M. M. de Castro, both from the Microbial Biochemistry Laboratory supervised by Antônio Ferreira-Pereira. The antibacterial activity of alkaloids was evaluated in the Laboratory of Molecular and Marine Bacteriology by the group of Marinella S. Laport. All authors revised the manuscript.

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