Original Article

**In vitro antileishmanial and antityrpanosomal activity of compounds isolated from the roots of Zanthoxylum tingoassuiba**

Rafael S. Costa\(^a, b, c\), Otávio P. Souza Filho\(^b\), Otávio C.S. Dias Júnior\(^c\), Jaqueline J. Silva\(^c\), Mireille Le Hyaric\(^d\), Marcos A.V. Santos\(^c, 1\), Eudes S. Velozo\(^a, b\)

\(^a\) Departamento do Medicamento, Faculdade de Farmácia, Universidade Federal da Bahia, Salvador, BA, Brazil  
\(^b\) Instituto de Química, Universidade Federal da Bahia, Salvador, BA, Brazil  
\(^c\) Laboratório de Biologia Parasitária, Instituto Gonçalo Moniz, Fundação Oswaldo Cruz, Salvador, BA, Brazil  
\(^d\) Departamento de Química, Instituto de Ciências Exatas, Universidade Federal de Juiz de Fora, Juiz de Fora, MG, Brazil

**A R T I C L E   I N F O**

Article history:
Received 13 September 2017  
Accepted 25 April 2018  
Available online 1 August 2018

Keywords: 
Leishmaniasis  
Trypanosomiasis  
Coumarins  
Furoquinoine alkaloids  
Syringaresinol

**A B S T R A C T**

Five coumarins (5,7,8-trimethoxycoumarin (1), sabandin (2), cubreuba lactone (3), 5,7-dimethoxycoumarin (5) and braylin (6)), seven furoquinoline alkaloids (isopimpinelin (4), pteleine (7), maculine (8), skimianine (10), robustine (11), y-fagarine (12) and dictamine (13) and the furofuran type lignin syringaresinol (9)) have been identified for the first time in the roots of Zanthoxylum tingoassuiba A. St.-Hil., Rutaceae. Pure compounds 1, 6, 9, 12 were tested against Leishmania amazonensis parasites and epimastigotes forms of Trypanosoma cruzi. All the tested products displayed an antiparasitic activity similar to that of the positive controls (benzimidazole and amphotericin B). Compound 9 was the most active against both parasites with IC\(_{50}\) values of 11.98 \(\mu\)M and 7.55 \(\mu\)M against L. amazonensis and T. cruzi, respectively.

© 2018 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

---

**Introduction**

Leishmaniasis and American trypanosomiasis (Chagas disease) are protozoal parasitic infections with worldwide distribution, which affect mostly sub-developed and developing countries. Both are among the most prevalent neglected tropical diseases (WHO, 2012). Chagas disease is caused by the parasite *Trypanosoma cruzi* and affects approximately 5.7 million people, mainly in Latin America countries (WHO, 2015a). Thousands of new cases are reported causing circa 7000 deaths annually, with a significant increase in North America, Europe and Asia (WHO, 2015b). The only drugs used for the treatment of trypanosomiasis are benzimidazole and nifurtimox. However, the treatment remains unsatisfactory due to the severe side effects and refractory cases (Paucar et al., 2016).

The three different forms of Leishmaniasis (cutaneous, mucocutaneous and visceral) are caused by a parasite of the genus *Leishmania*.

About 12 million people are infected worldwide, with approximately 1 million new cases and 20,000 to 30,000 deaths reported each year (WHO, 2016). The available treatments include pentavalent antimonials, amphotericin B, pentamidine, miltefosine, and paromomycin (Iqbal et al., 2016). The emergence of drug resistance, high costs, availability, and toxicity limit the use of these drugs.

New efficient antiparasitic agents are needed, and a great number of works has been developed to discover new bioactive natural compounds (Tagboto and Townson, 2001; Kayser et al., 2003; Wink, 2012; Cragg and Newman, 2013; Annang et al., 2016; Jain et al., 2016). Brazil is known for its high diversity of plant (Mesquita et al., 2005; Tempone et al., 2005; Braga et al., 2007; Muzitano et al., 2009; Cechinel Filho et al., 2013; Bou et al., 2014).

*Zanthoxylum* genus has been reported to have various bioactivities such as allelopathic, analgesic, anticonvulsant, anti-inflammatory, antimicrobial, antinociceptive, antioxidant, anti-helminthic, antiparasitary and antiviral activities, among others. These activities have been related to different chemical constituents: isouquinoline alkaloids, lignans, coumarins, flavonoids and terpenes (Patíño et al., 2012).

*Zanthoxylum tingoassuiba* A. St.-Hil., Rutaceae, also known as tinguaiba, is one of the 25 species that are endemic to Brazil. The plant is used in folk medicine and had been commercialized in Brazil since 1923 as an active component of a phytotherapeutic formulation.

---

* Corresponding author.  
E-mail: rafael.costa@ufba.br (R.S. Costa).  
1 Present address: Laboratório de Toxoplasmose e outras Protozooses, Instituto Oswaldo Cruz, Fundação Oswaldo Cruz; Focruz/RJ, Brazil.

https://doi.org/10.1016/j.bjp.2018.04.013  
0102-695X © 2018 Sociedade Brasileira de Farmacognosia. Published by Elsevier Editora Ltda. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).
prescribed for muscle cramps and spasms (Silva et al., 2008) and is also marketed as an herbal tea. Our group recently reported its antibacterial activity associated to the presence of benzophenanthridine alkaloids (Costa et al., 2017).

Considering the diversity of metabolites produced by plants of the genus Zanthoxylum and the need for the discovery of new antityranosomal and antileishmanial agents, this work aimed to evaluate the antiparasitic activity of compounds isolated from \textit{Z. tinoasa} against \textit{L. amazonensis} and \textit{T. cruzi}.

Materials and methods

Plant materials

\textit{Zanthoxylum tinoasa} A. St.-Hill., Rutaceae, was collected in August 2009 in Itabaí - Feira de Santana, Bahia, Brazil (12° 12′ 52.560″ S; 38° 52′ 46.205″ W). The voucher specimens were identified and deposited at the ACLB – Herbario Alexandre Leal Costa, Instituto de Biologia – UFBA (voucher 88005).

Chemicals

Analytical grade solvents were purchased from Sigma–Aldrich Co. (St. Louis, MO, USA) and used without further purification. HPLC solvents were obtained from Merck (Darmstadt, Germany). Deuterated water for NMR analysis were obtained from CIL (Cambridge Isotope Laboratories Inc., USA).

Instruments

HPLC analysis were performed on a Shimadzu (Kyoto, Japan) Prominence 20A system consisting of a CBM-20A controller, LC-6 AD parallel-flow pumps, DGU-20A degasser, SPD-M20A photo-diode array detector (DAD) and a FRC-20A fraction collector. Data were processed by LC Solution Version software (Shimadzu).

The separations were done on Kromasil C18 (250 × 4.6 mm; 5 μm and 250 × 21 mm; 5 μm) and Phenomenex Luna C18 (250 × 4.6 mm; 5 μm) columns.

HPLC-MS measurements were carried out on a Shimadzu Prominance 20A system consisting in a CBM-20A controller, LC-20 AD parallel-flow pumps, DGU-20A degasser, CTO-20A column oven, SIL-20A autosampler, and DAD-20A detector. The HPLC system was coupled to a microTOF-II mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany).

\textsuperscript{1}H and \textsuperscript{13}C NMR nuclear magnetic resonance (NMR) spectra were acquired on: Varian Gemini 500 MHz (\textsuperscript{1}H: 500 MHz and \textsuperscript{13}C: 125 MHz), Bruker DRX400 – Ultra Shield (\textsuperscript{1}H: 400 MHz and \textsuperscript{13}C: 100 MHz) and Bruker DXP300 (\textsuperscript{1}H: 300 MHz and \textsuperscript{13}C: 75 MHz) spectrometers, in pyridine-d₅, chloroform-d, acetone-d₆, or methanol-d₄ and trimethylsilane (TMS) as an internal standard.

Preparation of the extracts

Root bark (RB) (76.42 g) and of root heartwood (RH) (1.19 g) obtained from \textit{Z. tinoasa} were separated, grounded and extracted four times with methanol (500 ml) by maceration for 7 days and filtered. The filtrates were concentrated under reduced pressure to obtain the root bark methanol extract (RB, 12.72 g) and the root heartwood methanol extract (RH, 54.30 g). The RB extract (11.47 g) was partitioned into 11 of a mixture CHCl₃-MeOH:HCl 5% (5:5:3). The organic phase was separated and evaporated under reduced pressure (RBE1, 3.251 g) and the aqueous phase was basified with ammonium hydroxide until pH 10 was reached. The basic mixture was extracted with chloroform (3 × 300 ml). The organic phase was concentrated under reduced pressure (RBE2, 0.525 g).

The RH methanol extract (30 g) was dissolved in acetic acid 3% (650 ml) and extracted three times with chloroform (3 × 250 ml). The organic phase HR1 was concentrated under reduced pressure (935 mg).

HPLC analysis

RBE1, RBE2 and HR1 were analyzed by HPLC using the following conditions: C₁₈ column (4.6 × 250 mm, 100 Å, 5 μm particle size, Kromasil\textsuperscript{®}), guard column (4.6 × 20 mm, 5 μm particle size), isocratic flow of MeOH:H₂O (1:1) at a rate of 0.6 ml min⁻¹, injection volume of 20 μl. The HPLC fractions were monitored with the UV detector at 254 nm.

Preparative HPLC

The RBE1, RBE2 and HR1 fractions were solubilized in methanol (saturated solutions 313.1, 220.1 and 638.0 mg/ml for, respectively) and submitted to preparative HPLC using a C₁₈ column (21.2 × 250 mm, 100 Å, 5 μm particle size, Kromasil\textsuperscript{®}), an isocratic flow MeOH:H₂O 1:1. Injections were performed with a 100 μl loop. The flow rate was 10 ml/min for RBE1, RBE2 and 6 ml/min for HR1.

Parasites

\textit{Leishmania amazonensis} parasites (MHOM/BR88/BA-125) were isolated from infected C57BL/6 mice lesions and cultivated at 26 °C on NNN (Novy, McNeal, Nicolle) medium for 15 days. The promastigote forms were isolated and maintained at 26 °C in Warren's medium (brain–heart infusion 37 g/l plus haemin 10 mg/l and folic acid 10 mg/l) supplemented with 10% fetal bovine serum.

\textit{Trypanosoma cruzi} epimastigote forms (strain Y) were maintained at 26 °C in LIT (liver infusion tryptose) medium, supplemented with 10% fetal calf serum. For both strains the cell population was monitored and the transplantation was made when they reached the growth stationary phase.

Antileishmanial and antityranosomal susceptibility test

\textit{Leishmania amazonensis} promastigotes (5 × 10⁵ parasites/ml) and \textit{T. cruzi} (5 × 10⁵ parasites/ml) were inoculated in a 24-well plate containing Warren’s medium supplemented with 10% inactivated FBS with different concentrations (0.75–200 μM, obtained by three serial dilutions in DMSO) of the tested compounds (1, 6, 9 or 12).

The final volume was 2 ml/well, with a final concentration of DMSO less than 0.1%. The plates were incubated 26 °C for 72 h. The cells were fixed with 3.8% paraformaldehyde and the density was determined using a Neubauer counting chamber.

All the experiments were realized in triplicate. The antileishmanial and anti-trypanosomal activities were expressed as the percentage of growth inhibition in compound tested cells and IC₅₀ values were calculated.

Results and discussion

The preparative HPLC separation performed on the RBE1 and RBE2 extracts allowed the isolation of eight fractions (F2-F5 and H2-H5, respectively, Fig. 1). All the fractions were analyzed by HPLC/MS and NMR spectroscopy, allowing the identification of eight compounds.

Pure 5,7,8-trimethoxycoumarin (1) (7 mg) was obtained from F2. Sabandin (2) and cubreua lactone (3) eluted together (13.3 mg) in F3. A mixture of isopimpinelin (4) and 5,7-dimethoxycoumarin (5) (8.6 mg) was recovered from F4. Braylin (6) (51.10 mg) was purified from F5.
The chromatographic analysis of the extracts obtained from RBE2 allowed the identification of compounds 2 and 3 in fraction H2; 4 and 5 in fraction H3; 6 in fraction H4. Pteleine (7) could not be separated from maculine (8) (5.6 mg) in fraction H5.

The preparative scale HPLC analysis of fraction HR1 (Fig. 2) allowed the isolation of pure syringaresinol (9) (R1, 20.9 mg), a mixture of skimianine (10) and robustine (11) (R5, 3.9 mg), pure y-fagarine (12) (R6, 8.7 mg) and dictamine (13) (R7, 10 mg). Other peaks were observed on the HPLC chromatograms, but the amounts of these materials were too small and did not allow the identification of the compounds.

The identification of the chemical structures of the compounds was based on the comparison of the spectroscopic data of the isolated fractions to literature data (Puisset et al., 1991; Cuca et al., 1998; Chakravarty et al., 1999; Leong et al., 1999; Yoo et al., 2002; Bhoga et al., 2004; Randrianarivelosojia et al., 2005; Maes et al., 2005, 2008; Nunes et al., 2005; Chlouchi et al., 2006; Kutubi et al., 2011).

The 1H NMR spectrum of compound 1 show three singlets at 3.91, 3.92 and 3.97 ppm, characteristic shifts of methoxy groups on an aromatic ring, and a single aromatic hydrogen at 6.17 ppm. Two signals at 6.17 and 7.98 ppm indicate the presence of a conjugated double bond. The signal of the lactone carbonyl group is shown on the 13C NMR at 160.98 ppm. The structure of 5,7,8-trimethoxycoumarin (1) was confirmed by comparison with literature data (Maes et al., 2008). The analytical HPLC analysis of fractions F3 and H2 showed similar retention times, suggesting a similar composition. The analysis of the 1H NMR spectra of F2 reveals the presence of protons with characteristic shifts found in coumarins. Signals at 4.12, 4.02 and 3.99 ppm suggest the presence of a dimethoxy and a methoxy coumarin with a 5:1 ratio. Only one aromatic proton is present on the spectra (6.48 ppm), which was assigned to the minor compound, a methoxy coumarin. Signals at 5.99 and 6.01 ppm were attributed to methylenedioxy protons present in both structures. Duplicated signals are also observed on the 13C NMR spectra. The comparison with literature data (Maes et al., 2005) confirmed the structures of coumarins cubreuvia lactone 2 (minor compound) and sabandine 3 (major compound). The chromatograms of F4 and H3 fractions show two overlapping peaks in analytical chromatogram (tR = 33.3 min and 39.7 min) which could not be separated in these preparative conditions. The 1H NMR analysis of F4 reveals the presence of two dimethoxycoumarins, with a 1:2 ratio. Duplets at 7.00 ppm and 7.63 ppm (minor product) and at 6.28 and 6.41 ppm were attributed to aromatic or furanic protons. The comparison of the 13C NMR data with literature (Yoo et al., 2002; Kutubi et al., 2011) along with the MS spectra, allowed the identification of 5,7-dimethoxycoumarin 4 ([M+H]⁺ = 207,06779 Da) and isopimpinellin 5 ([M+H]⁺ = 247,06251 Da). The chromatograms of F5 and H4 show the presence of one compound ([M+Na]⁺ = 281,08164). The 1H NMR spectra shows the characteristic signals of the benzopyrone nucleus of the coumarins, carrying one methoxy group. One peak at 1.52 ppm was attributed to two methyl groups belonging to a 2,2-dimethylpyran cycle. The structure of braylin (6) was confirmed by comparison with literature data (Randrianarivelosojia et al., 2005). A mixture of compounds 7 and 8 was isolated from the H5 fraction. The analysis of 1H and 13C NMR spectra is consistent with methoxylated fururoquinoline alkaloids. The chemical structures were established by comparison with literature data as maculine (7) (Nunes et al., 2005) and pteleine (8) (Puisset et al., 1991). Pure (+)-seringaresinol (9) was isolated from the R1 fraction and identified by comparison with published data (Leong et al., 1999). Compounds 10 and 11 could not be separated from fraction R5 and were isolated as a mixture (31:69 ratio). The NMR spectra revealed characteristic signals assigned to furoquinoline alkaloids. The compounds were identified as skimianine (10) (Chakravarty et al., 1999) and robustine (11) (Chlouchi et al., 2006). Finally, y-fagarine (12) and dictamine (13) were obtained as pure compounds.

**Fig. 1.** Analytical and preparative HPLC chromatograms for RBE1 and RBE2 fractions. Analytical conditions: C18 column (4.6 × 250 mm, 100 Å, 5 µm particle size, Kromasil®), guard column (4.6 × 20 mm, 5 µm particle size), isocratic flow of MeOH:H2O (1:1) at a rate of 0.6 ml/min, injection volume of 20 µL. Preparative conditions: C18 column (21.2 × 250 mm, 5 µm, 100 Å), an isocratic flow MeOH:H2O 1:1 and injection volume of 100 µL. The flow rate was 10 ml/min, DAD® 254 nm.
in fraction R6 and R7, respectively, and comparison with published data (Bhoga et al., 2004; Cuca et al., 1998) allowed the confirmation of their chemical structures.

Cumarins, lignans and alkaloids are commonly found in plants belonging to the Rutaceae family. The group of alkaloid most frequently reported in the genus Zanthoxylum is isoquinoline, mainly benzopinanthenidines (Fish and Waterman, 1973; Hohlemwerger et al., 2012). Previous chemical studies of Z. tingoaussiba have shown the presence of this class of compounds, along with aporphine and protoberine alkaloids, as well as a N-methylanthranilate derivative (Silva et al., 2008; Hohlemwerger et al., 2012; Costa et al., 2017). Furanocumarins (7-oxygenated coumarins) are frequently found in the Rutaceae family and recently three new substances have been identified in Z. avicennae (Chen et al., 2015). This report describes for the first time the presence of five coumarins in Z. tingoaussiba: 5,7,8-trimethoxy coumarin (1), sabandin (2), cubreusa lactone (3), 5,7-dimethoxy coumarin (5) and braylin (6).

The present work also reports for the first time the occurrence of furuquinolone alkaloids in Z. tingoaussiba species. Seven different chemical structures were identified: isopimpinellin (4), pteleine (7), maculine (8), skimianine (10), robustine (11), y-fagarine (12) and dictamine (13). Additionally, syringaresinol (9) a furuuran type lignan is isolated for the first time from Z. tingoaussiba in the present study.

Spectral data

5,7,8-trimethoxy coumarin (1): (500 MHz, CDCl3): 6.16 (1, d, J = 9.7, H3), 7.98 (1H, d, J = 9.7, H4), 6.35 (1H, s, H6), 3.91 (3H, s, –OCH3), 3.92 (3H, s, –OCH3), 3.97 (3H, s, –OCH3), [M+H]+: 237.07917 Da, C12H12O5, UV λmax: 260.26 nm; 323.38 nm.

Sabadin (2): (500 MHz, CDCl3): 6.21 (1H, d, J = 10.0 Hz), 7.92 (1H, d, J = 10.0 Hz), 6.02 (2H, s, –OCH2O–), 4.02 (3H, s, –OCH3), 4.05 (3H, s, –OCH3), [M+H]+: 251.005834 Da, C12H10O6. UV λmax: 239.25 nm; 327.15 nm.

Cubreusa lactone (3): (500 MHz, CDCl3): 6.19 (1H, d, J = 9.6 Hz), 7.93 (1H, d, J = 9.5 Hz), 6.49 (1H, s, H8), 6.00 (2H, s, –OCH2O–), 4.12 (3H, s, –OCH3), [M+H]+: 221.04723 Da, C11H13O5.

Isopimpinellin (4): (500 MHz, CDCl3): 6.30 (1H, d, J = 9.8 Hz), 8.13 (1H, d, J = 9.8 Hz), 7.63 (1H, d, J = 2.3 Hz'), 7.00 (1H, d, J = 2.3 Hz'), 4.16 (3H, s, –OCH3), 4.17 (3H, s, –OCH3), [M+H]+: 247.06251 Da, C11H13O5, UV λmax: 221.75 nm; 267.34 nm; 313.09 nm.

5,7-dimethoxy coumarin (5): (500 MHz, CDCl3): 6.16 (1H, d, J = 9.7 Hz), 7.97 (1H, d, J = 9.7 Hz), 6.29 (1H, d, J = 2.1 Hz), 6.42 (1H, d, J = 2.1 Hz), 3.89 (3H, s, –OCH3), 3.86 (3H, s, –OCH3), [M+H]+: 207.06779 Da, C11H13O4, UV λmax: 250.51 nm; 327.67 nm.
Braylin (6): (500 MHz, CDCl₃): 6.26 (1H, d, J = 9.5 Hz), 7.59 (1H, d, J = 9.5 Hz), 6.80 (1H, s, H6), 6.89 (1H, d, J = 10.05 Hz), 5.76 (1H, d, J = 10.05 Hz), 1.53 (6H, s, -CH3 4' and 5'). 3.89 (3H, s, -OCH3). [M+H]⁺: 259.09914 Da, C₁₅H₁₅NO₄, UV λmax: 225.63 nm; 350.81 nm.

Pteleine (7): (500 MHz, CDCl₃): 8.07 (1H, d, J = 9.2 Hz), 7.40 (1H, dd, J = 9.2, J = 2.9 Hz), 7.55 (1H, d, J = 2.9 Hz), 7.66 (1H, d, J = 2.7 Hz), 7.11 (1H, d, J = 2.7 Hz), 3.96 (3H, s, -OCH3). 4.50 (3H, s, -OCH3). [M+H]⁺: 230.08254 Da, C₁₃H₁₃NO₃. UV λmax: 236.78 nm; 306.71 nm.

Maculine (8): (500 MHz, CDCl₃): 7.53 (1H, s, H5), 7.51 (1H, s, H8), 7.61 (1H, d, J = 2.7 Hz), 7.08 (1H, d, J = 2.7 Hz), 6.00 (2H, s, -OCH2O-), 4.46 (3H, s, -OCH3). [M+H]⁺: 244.06135 Da, C₁₃H₁₃NO₄. UV λmax: 243.70 nm; 307.20 nm.

Syringaresinol (9): (500 MHz, CDCl₃): 3.10 (1H, m, H1 and H5), 4.74 (2H, d, J = 4.1 Hz and H6), 4.29 (2H, dd, J = 6.8 and 9.1 Hz and H8eq), 3.91 (2H, m, H4ax and H8ax), 6.59 (4H, s, H2', 2'' and H6', 6''), 3.90 (12H, -OCH3), 5.51 (2H, s, -OH). [M+H]⁺: 419.17267 Da, C₂₂H₂₂O₈. UV λmax: 206.29 nm.

Skimmianine (10): (400 MHz, CDCl₃): 8.04 (1H, d, J = 9.4 Hz), 7.26 (1H, d, J = 9.4 Hz), 7.59 (1H, d, J = 2.8 Hz), 7.06 (1H, d, J = 2.8 Hz), 4.04 (3H, s, -OCH3), 4.11 (3H, s, -OCH3). [M+H]⁺: 260.09963 Da, C₁₄H₁₃NO₄. UV λmax: 248.02 nm; 320.99 nm.

Robustine (11): (400 MHz, CDCl₃): 7.75 (1H, d, J = 8.6, J = 1.2 Hz), 7.35 (1H, dd, J = 7.5, J = 7.5 Hz), 7.20 (1H, dd, J = 7.5, J = 1.2 Hz), 7.65 (1H, d, J = 2.8 Hz), 7.13 (1H, d, J = 2.8 Hz), 4.47 (3H, s, -OCH3). [M+H]⁺: 216.06782 Da, C₁₂H₁₄NO₃. UV λmax: 243.76 nm; 311.01 nm.

γ-Fagarine (12): (500 MHz, CDCl₃): 7.87 (1H, dd, J = 8.6 and 1.0 Hz), 7.37 (1H, dd, J = 7.7 and 8.6 Hz), 7.08 (1H, dd, J = 7.7 and 1.0 Hz), 7.67 (1H, d, J = 2.8 Hz), 7.10 (1H, d, J = 2.8 Hz), 4.10 (3H, s, -OCH3), 4.47 (3H, s, -OCH3). [M+H]⁺: 230.08335 Da, C₁₃H₁₁NO₃. UV λmax: 243.09 nm; 310.46 nm.

Dictamine (13): (300 MHz, CDCl₃): 8.28 (1H, ddd, J = 8.5, 1.5, 0.6 Hz), 7.45 (1H, ddd, J = 8.1, 8.1, 1.2 Hz), 7.69 (1H, dd, J = 8.4, 8.5, 1.5 Hz), 8.02 (1H, ddd, J = 8.5, 1.2, 0.3 Hz), 7.63 (1H, d, J = 2.8 Hz), 7.08 (1H, d, J = 2.8 Hz), 4.44 (3H, s, -OCH3). [M+H]⁺: 200.07320 Da, C₁₂H₁₃NO₂. UV λmax: 236.18 nm; 309.71 nm.

**Antiparasitic activity**

Pure compounds 1, 6, 9, 12 were tested against epimastigote forms of T. cruzi, Y-strain. The results are detailed in Fig. 3 and Table 1.

Compounds 6, 9 and 12 significantly reduced the proliferation of the parasite at low concentrations (6.2, 1.2 and 0.75 µM, respectively). The highest levels of inhibition were obtained at concentrations of 100 µM (γ-fagarine, 77%), 78 µM (syringaresinol, 80%), 200 µM (braylin 75% respectively) and 100 µM (5,7,8-trimethoxycoumarin, 75%). The most active substances were syringaresinol (IC₅₀ = 7.55 µM) and 5,7,8-trimethoxycoumarin (IC₅₀ = 25.5 µM), while γ-fagarine (12) and braylin (6) displayed moderate activities (IC₅₀ = 33.35 and 59.8 µM, respectively).

The four compounds also demonstrated a significant capacity to inhibit the growth of promastigote forms of L. amazonensis (Fig. 4) at concentrations of 78 µM (syringaresinol, 98%), 100 µM (γ-fagarine, 100%) and 5,7,8-trimethoxycoumarin 91%, and 200 µM (braylin 100%). At lower concentrations, 5,7,8-trimethoxycoumarin 1, braylin 6 and γ-fagarine 12 were more active than the positive control. Braylin and 5,7,8-trimethoxycoumarin stimulated the proliferation of the parasite at the concentration of 12.5 µM.

The most active substances were syringaresinol (IC₅₀ = 12.0 µM) and γ-fagarine (IC₅₀ = 31.3 µM) followed by 5,7,8-trimethoxycoumarin (IC₅₀ = 57.7 µM) and braylin (IC₅₀ = 70.0 µM).

Interestingly the antiparasitic activity of the natural products approached here displayed effectivity levels similar to the positive controls benznidazole and amphotericin B, used for trypanocidal and leishmanicidal activities, respectively.

Coumarins are known for their antityrpanosomal effects (Guíñez et al., 2013; Chen et al., 2015) and antileishmanial activities (Reyes-Chilpa et al., 2008; Mandlik et al., 2016). Previous studies have shown that they act on an enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) of the glycolytic pathway of the trypanosomatids (Freitas et al., 2009) and that they inhibit the growth...
of *L. amazonensis* and altered the parasite structure (Brenzan et al., 2007). A survey of the literature showed that there is no report on the antitrypanosomal activities of the coumarins 1 (5,7,8-trimethoxycoumarin) and 6 (braylin) isolated in this work. The lignan (+)-syringaresinol 9 was the most active of the tested compounds against both parasites. Recently a study conducted by de Sousa et al. (2014) showed that syringaresinol is able to inhibit arginase from *L. amazonensis* a key enzyme involved in the polyamine synthesis and in the production of nitric oxide, and its ability to inhibit *T. b. brucei* growth has been described (Alamzeb et al., 2013). Inhibition of *L. amazonensis* arginase by *Cecropia pachystachya* flavonoids present antiparasitic activity associated to mitochondrial and kinetoplast DNA disorganization (Cruz et al., 2013). Previous studies have shown that lignans isolated from *Z. naranjillo* showed high trypanocidal effect against the trypanastigote forms of *T. cruzi* (Bastos et al., 1999). De Souza et al. (2005) demonstrated the activity of lignan derivatives against free amastigote forms of *Trypanosoma cruzi*. No report was found on the activity of (+)-syringaresinol 9 against *T. cruzi* or *L. amazonensis*. It is noteworthy that, under the conditions employed here, this bioagent was more trypanocidal than benznidazole, used as positive control.

The comparison of the IC50 values obtained for both parasites show that γ-fagarine had about the same potency against *L. amazonensis* and *T. cruzi* (IC50 31.34 and 33.35 μM, respectively), while the other tested compounds were more active against *T. cruzi*. In a study performed by Ferreira et al. (2010) the alkaloid γ-fagarine (12) demonstrated antiparasitic activity against promastigot forms of *L. amazonensis* (IC50 17.3 μM), *L. infantum* and *L. braziliensis* (26.5 μM and 22.2 μM, respectively). A significant in vitro inhibitory activity against the promastigotes of *L. tropica* (IC50 0.37 μM) was also shown by γ-fagarine (Östman et al., 2007).

**Conclusion**

The phytochemical study of the roots from *Zanthoxylum tingoasuiba* allowed the identification of six coumarins, six furoquinoline alkaloids and a furofuranic lignan. Eleven of these compounds were isolated for the first time in this species (isopimpeneline and braylin have been previously described). The results obtained in the in vitro test against *T. cruzi* and *L. amazonensis* showed that some of the compounds found in the species display antiparasitic activity. These findings contribute to a better understanding of the chemical composition of this species of occurrence in the Brazilian semi-arid region and to the knowledge of the Brazilian biodiversity as a source for drug discovery.

**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.

Authors' contributions

RSC contributed in collecting plant sample, running the laboratory work, data analysis and drafted the paper. OPSP contributed in collecting plant sample, running the laboratory work. OCSD and JJS contributed to biological studies. MLH contributed to data analysis and drafted the paper. MAVS and EV designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgments

We thank Prof. Norberto Peperino Lopes (Faculty of Pharmaceutical Sciences of Ribeirão Preto, University of São Paulo) for the availability of laboratory and supplies. The authors thank CAPES and CNPq for the grants.

References


