Zanthoxylum capense constituents with antimycobacterial activity against Mycobacterium tuberculosis in vitro and ex vivo within human macrophages

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ABSTRACT

Ethnopharmacological relevance: Zanthoxylum capense Thunb. (Rutaceae) is a medicinal plant traditionally used in Mozambique to treat tuberculosis.

Aims of the study: The main aim of the study was to find antimycobacterial lead compounds from Zanthoxylum capense. Another goal was to provide scientific validation for the use of this plant in traditional medicine.

Methods and materials: By bioassay-guided fractionation, 16 compounds were isolated and screened for their in vitro antimycobacterial activity against two different strains of Mycobacterium tuberculosis. Their in vitro cytotoxicity to human THP-1 macrophages was also assessed. The compounds with favourable selectivity index values (SI > 10) were further investigated for their ability to inhibit the growth of Mycobacterium tuberculosis H37Rv in an intracellular macrophage model of infection.

Results: The best results were obtained for a benzophenanthridine alkaloid, decarine (1), and an N-isobutylamide, N-isobutyl-(2E,4E)-2,4-tetradecadienamide (15), which showed high activity against Mycobacterium tuberculosis H37Rv (MIC of 1.6 µg/ml) and a low macrophage cytotoxicity (IC 50 > 60 µg/ml), indicating considerable selective activity. The benzophenanthridine alkaloid 6-acetonilidihydrinditine (6) revealed cytotoxicity (IC 50 1.7 µg/ml), despite the determined MIC of 6.2–12.5 µg/ml. In infected macrophages, decarine (1) was able to reduce bacterial survival by almost two log units at a concentration of 6.2 µg/ml 5 days post-drug exposure. Compound 15 exhibited an intermediate activity at drug concentrations ranging from 6.2 to 25 µg/ml.

Conclusions: The high antimycobacterial activity of decarine found, both in vitro and ex vivo against mycobacteria, and the low cytotoxicity towards human macrophages indicate that it may be valuable as a lead scaffold for the development of anti-TB drugs.

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1. Introduction

As one of the top public health concerns, tuberculosis (TB) was responsible for 1.4 million deaths of the world’s population in 2011, with an estimated 8.7 million new cases (Dye and Williams, 2010; WHO, 2012). The number of drugs currently in use against Mycobacterium tuberculosis is very limited and most of them were introduced more than 40 years ago (Pilcher, 2005; Lienhardt et al., 2012). Diverse factors, including limited drug efficacy, inadequate drug prescription or poor patient compliance, have resulted in the increasing rise in the number of TB cases worldwide, many of them being multidrug resistant (MDR) and extensively drug resistant (XDR). Drug resistant strains have acquired mutations in drug targets or enzymes activating pro-drugs (De Rossi et al., 2006).

Current anti-TB chemotherapy regimens usually last for at least 6 months. They are characterized by an initial phase of rapid clearance of bacilli from the sputum (early bactericidal activity), followed by a slower elimination of the remaining organisms (sterilizing activity) (Hartkoorn et al., 2007). Since Mycobacterium tuberculosis is a facultative intracellular pathogen that can survive and grow within macrophages, it is imperative that a new potential drug is capable of penetrating into macrophages and inhibiting the growth of intracellular bacteria.
Altogether, the above reasons point at the urgency to find new therapeutic agents acting on novel targets, presenting early bactERICidal activity as well as fast intracellular sterilizing activity that could shorten current therapeutic regimens. in spite of growing efforts to discover effective anti-tubercular molecules, the search for new agents still represents a great challenge (Lienhardt et al., 2012).

In our search for antimycobacterial compounds from medicinal plants, we have screened several plant species traditionally used in Mozambique for the treatment of TB (Luo et al., 2011). The promising growth inhibitory activity of Zanthoxylum capense Thumb. (Rutaceae) extracts on a range of mycobacterial strains prompted us to perform the phytochemical investigation of this species from which seven benzophenanthidine-type alkaloids (1–7) and two neoignlans (13, 14) had been previously isolated (Luo et al., 2012). In this study, we report the isolation and identification of seven additional compounds (8–12, 15, 16) from the same plant. Compounds 1–16 were evaluated for their in vitro antimycobacterial activity along with their cytotoxicity. Furthermore, the most active compounds with considerable selectivity index (SI) values were assessed for their intracellular activity against Mycobacterium tuberculosis H37Rv within human THP-1 macrophages.

2. Materials and methods

2.1. Plant material

The roots of Zanthoxylum capense were collected in southern Mozambique (Machava and Massingir) in March 2009, and were identified by botanist Dr. Silva Mulhovo. A voucher specimen (No. 45/SIM) was deposited at the herbarium (LMA) of the Instituto de Investigacao Agraria de Mozambique (IIAM), Maputo, Mozambique.

2.2. Tested compounds

Sixteen compounds (1–16) presenting different structural scaffolds were evaluated for their antimycobacterial activity. The chemical structures for these compounds are presented in Fig. 1. These included decarine (1), norchelerythrine (2), dihydrochelerythrine (3), 6-acetonyldihydrochelerythrine (4), tridecanonchelerythrine (5), 6-acetylindolindoline (6), zanthocapensol (7), rutacarpine (8), skimmianine (9), (−)-sesamin (10), (−)-episesamin (11), (−)-savinin (12), zanthocapensol (13), zanthocapensate (14), N-isobuty1-(2E,4E)-2,4-tetradecadienamide (15) and lupeol (16). The isolation and structural elucidation of compounds 1–7, 13 and 14 were previously reported (Luo et al., 2012). The isolation of compounds 8–12, 15, 16 is described below. The purity of all the compounds was more than 95% based on the $^3$H NMR integrations and HPLC.

2.3. Extraction and methods

The air-dried powdered roots of Zanthoxylum capense were exhaustively extracted with methanol and sequentially partitioned into n-hexane, dichloromethane, ethyl acetate and n-butanol fractions as described (Luo et al., 2012). The n-hexane and dichloromethane extracts, which showed a promising antimycobacterial activity, were further chromatographed yielding fractions FH1–11 and FD1–16. Fraction FH4 was subjected to a silica gel column, eluting with n-hexane/ethyl acetate gradient to obtain compound 16 (894 mg). Fraction FH6 was similarly rechromatographed to give compound 11 (1.9 g). Compound 10 (160 mg) was obtained by recrystallization of fraction FH7 from ethyl acetate/n-hexane. Re-chromatography of fraction FH7 yielded compound 15 (20 mg). Column chromatography of fraction FH9 and further purification by recrystallization from ethyl acetate/n-hexane gave 85 mg of compound 12. Compound 8 (3 mg) was obtained by semi-preparative RP-HPLC (254 nm, MeOH–H$_2$O, 17:3; 3 mL/min) from fraction FD3. Fraction FD7 was recrystallized from ethyl acetate/n-hexane yielding compound 9 (500 mg).

2.4. Mycobacterial strains and growth conditions

Mycobacterium smegmatis mc² 155 (ATCC 700084), rapidly growing mycobacteria, was used in bioassay-guided fractionation for evaluating the antimycobacterial activity of methanol, n-hexane, dichloromethane, ethyl acetate and n-butanol extracts. Two reference strains of Mycobacterium tuberculosis, H37Ra ATCC 25177 (avirulent) and H37Rv ATCC 27294 (virulent), slow growing mycobacteria, were used for testing the isolated compounds.

Mycobacterium smegmatis was grown as previously described (Anes et al., 2006). The Mycobacterium tuberculosis strains were cultured in Middlebrook 7H9 broth supplemented with 10% (v/v) OADC (Beckton Dickinson), 0.05% (v/v) Tween 80 (Sigma-Aldrich) and 0.2% (v/v) glycerol (Sigma-Aldrich). Strains were grown at 37°C in agitation until an OD$_{600}$ of 0.6–0.8 (approximately 3 x 10$^7$ CFU/ml) was reached, which was typically 1 day for Mycobacterium smegmatis mc² 155 and 7–10 days for Mycobacterium tuberculosis.

2.5. Minimum inhibitory concentration (MIC) determination

Screening assays were performed by using the broth microdilution method (Sethi et al., 2007). Solutions of each extract and compound were prepared by first dissolving in dimethyl sulfoxide (DMSO; Sigma-Aldrich) to a concentration of 20 mg/ml and 4 mg/ml, respectively, and then further diluting in the respective culture media for each bacteria (referred above). Serial two-fold dilutions were made in 96-well microtitre plates with concentrations ranging from 250 to 4 µg/ml for each extract, and from 50 to 0.8 µg/ml for each compound. Each well was inoculated with bacterial suspension at a concentration of 10$^6$ CFU/ml. No inhibitory effects were observed in the presence of DMSO at the concentrations used (< 1.25%). Isoniazid (Sigma-Aldrich) was used as a reference drug.

Mycobacterium smegmatis mc² 155 was incubated in microtitre plates for 2–3 days and Mycobacterium tuberculosis H37Ra ATCC 25177 and H37Rv ATCC 27294 for 7–8 days. Following this period they were observed under a light microscope to determine the lowest concentration with no visible mycobacterial growth. The results were confirmed by measuring the optical density at 600 nm in a Tecan M200 plate spectrophotometer.

All the MIC results were confirmed by at least three independent experiments.

2.6. THP-1 cell line

Human acute monocytic leukaemia cell line THP-1 (ATCC TIB-202) was maintained in RPMI-1640 medium containing 10% (v/v) foetal calf serum (FCS), 1% (v/v) l-glutamine, 1 mM sodium pyruvate, 10 mM HEPES at pH 7.4, 1 × MEM non-essential amino acids, 100 IU/ml penicillin and 100 µg/ml streptomycin (all reagents from Gibco) and incubated at 37°C in 5% CO$_2$ atmosphere. Prior to the experiments, 1 × 10$^6$ cells were seeded in 96-well plates and incubated overnight in cell culture medium (described above) supplemented with 20 nM phorbol 12-myristate 13-acetate (PMA) (Sigma-Aldrich) to induce differentiation of THP-1 monocytes into macrophages. The following day the PMA containing medium was replaced with fresh
PMA-free medium and maintained for 24 h to ensure that the cells reverted to a resting macrophage phenotype.

2.7. Cytotoxicity assay

Cells were incubated with the compounds at concentrations ranging from 50 to 0.8 μg/ml for 7 days. Culture medium with the compounds was replenished every two days during the course of the experiment. DMSO, at the same proportions as in the tested compounds, was used as a control. The highest concentration of DMSO used was 1.25%. Puromycin (Sigma-Aldrich) was used as a positive control for cell death. Cell viability was determined after 7 days of treatment using alamarBlue® (Molecular Probes) and following the manufacturer's indications. Briefly, 10% (v/v) of alamarBlue reagent was added to each well and incubated for 4 h at 37 °C in 5% CO₂. Fluorescence was measured at an excitation of 570 nm and emission of 595 nm in a Tecan M200 plate spectrophotometer. Viability was calculated as percentage fluorescence intensity relative to the untreated cells. The IC₅₀ values were calculated by using a 5 parameter logistic nonlinear regression model equation from the set of concentrations tested.

Selectivity index (SI) value of each compound was calculated by dividing the IC₅₀ value by the MIC value (Orme, 2001).

The antimycobacterial activity was considered to be specific when SI > 10 (Hartkoorn et al., 2007).

2.8. Intracellular antimycobacterial activity in THP-1 macrophages

Prior to infection, Mycobacterium tuberculosis H37Rv ATCC 27294 was prepared in order to achieve a single cell suspension. For this, a bacterial culture on exponential growth phase was centrifuged at 3000 × g for 10 min, washed with PBS and centrifuged again at the same conditions. Bacteria were then resuspended in the THP-1 culture medium without antibiotics. In order to dismantle bacterial clumps, the bacterial suspension was passed through a 21 G needle followed by sonication. Residual clumps were removed by 1 min centrifugation at 500 × g. Single-cell suspension was verified by light microscopy.

THP-1 cells were infected with a multiplicity of infection (MOI) of 10. Cells were allowed to uptake the bacteria for 3 h. To remove non-internalized extracellular bacteria, cells were washed three times with PBS. Following this step, selected compounds were added to the infected cells at the desired concentrations. DMSO, at the same proportions as in the compound solutions, was used as untreated control. The highest concentration of DMSO used was 1.25%. Isoniazid (1 μg/ml) and pyrazinamide (200 μg/ml) were used as reference drugs.
At 3 h, and 1, 3 and 5 days post-infection, cells were lysed with 0.5% Igceal (Sigma-Aldrich) solution. Serial dilutions of the lysate were performed in water and plated on Middlebrook 7H10 medium supplemented with 10% (v/v) OADC. Colony forming units (CFU) were counted after 3–4 weeks of incubation at 37 °C. A compound was considered bactericidal if it significantly reduced the colony forming units in the test samples compared with the control. The results were confirmed by two independent experiments performed in triplicate.

2.9. Statistical analysis

Statistical analysis was performed using SigmaPlot 11.0 software. Multiple group comparisons were made using ANOVA one-parameter tests followed by pairwise comparisons of the groups using the Holm–Sidak test. The considered nominal alpha criterion level was 0.001.

3. Results

3.1. In vitro antymycobacterial activity evaluation

During bioassay-guided fractionation, the methanolic extract from the roots of the medicinal plant Zanthoxylum capense showed antymycobacterial activity (MIC 62.5 μg/ml) against a fast growing strain of Mycobacterium smegmatis mc² 155. Further evaluation of the fractions obtained by sequential partition of methanol extract revealed that the non-polar n-hexane and dichloromethane soluble parts were the most active, with MIC values of 31.2 and 15.6 μg/ml, respectively. In contrast, the polar ethyl acetate and n-butanol fractions showed only weak activity with a MIC value of 250 μg/ml. Therefore, further phytochemical study was carried out with the non-polar fractions.

Sixteen compounds (1–16) (Fig. 1), isolated from the n-hexane and dichloromethane soluble fractions of the roots of Zanthoxylum capense, were evaluated for their in vitro antymycobacterial activity. The isolation of the benzophenanthridine alkaloids 1–7, and the neolignans 13 and 14 was previously reported (Luo et al., 2012). Further phytochemical studies led to the isolation of quinazoline- (8) and quinoline-type alkaloids (9) along with lignans (10–12), an N-isobutylamide (15) and a pentacyclic triterpene (16). The structural identification of these compounds was carried out by comparison of their spectroscopic data with those reported in the literature (Aynilian et al., 1972; Moura et al., 1997; Abarbri et al., 1998; Takaku et al., 2001; Mhaske and Argade, 2004; Miyake et al., 2005).

All of the compounds (1–16) were screened for growth inhibitory activity against two drug-susceptible strains including avirulent Mycobacterium tuberculosis H37Ra ATCC 25177 and virulent Mycobacterium tuberculosis H37Rv ATCC 27294. Most of the compounds showed similar antimicrobial profiles towards these two mycobacterial strains (Table 1). Among the benzophenanthridine-type alkaloids, decaric (1) presented the most potent inhibitory activity with MIC values of 1.6 and 3.1 μg/ml against H37Rv and H37Ra, respectively (Table 1). The alkaloid 6-acetylhydronitidine (6) also showed pronounced inhibitory activity with MIC values of 12.5 and 6.2 μg/ml against the referred strains. On the other hand, the benzophenanthridine analogues 2–5 and 7 exhibited reduced inhibition with MICs higher than 50 μg/ml. Besides, the N-isobutylamide (15) demonstrated strong antymycobacterial activity with MIC values of 1.6 μg/ml and 6.2 μg/ml for H37Rv and H37Ra, respectively. The quinoline-type alkaloid, skimmianine (9), and the lignans, (−)-sesamin (10) and (−)-episesamin (11), showed moderate activity (MIC values between 25 and 50 μg/ml) against the tested strains. Lupeol (16) only showed activity towards H37Rv (MIC 25 μg/ml). Furthermore, quinazoline alkaloid (8) and the lignans 12–14 did not display any antymycobacterial activity at the concentrations tested (MIC ≥ 50 μg/ml).

3.2. Cytotoxicity on human THP-1 macrophages

In order to evaluate the cytotoxicity profiles of all the isolated compounds, THP-1 macrophages were exposed to the compounds

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antimycobacterial activity (MIC (μg/ml))</th>
<th>Cytotoxicity (μg/ml)</th>
<th>SIa</th>
<th>Selectivity index (SI) = cytotoxicity (IC₅₀) / MIC</th>
<th>Selectivity index (SI) = antiycobacterial activity (MIC)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mycobacterium tuberculosis H37Ra ATCC 25177</td>
<td>Mycobacterium tuberculosis H37Rv ATCC 27294</td>
<td>C₅₀</td>
<td>μg/ml</td>
<td>SIb</td>
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<tr>
<td>1</td>
<td>3.1</td>
<td>1.6</td>
<td>66.0 ± 4.5</td>
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<td>41.2</td>
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<tr>
<td>2</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>75.4 ± 33.7</td>
<td>&lt; 1.5</td>
<td>&lt; 1.5</td>
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<tr>
<td>3</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>68.7 ± 0.4</td>
<td>&lt; 1.4</td>
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<tr>
<td>4</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>57.1 ± 5.8</td>
<td>&lt; 1.1</td>
<td>&lt; 1.1</td>
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<tr>
<td>5</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>24.5 ± 5.8</td>
<td>&lt; 1.9</td>
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<tr>
<td>6</td>
<td>6.2</td>
<td>12.5</td>
<td>1.7 ± 0</td>
<td>0.3</td>
<td>0.1</td>
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<tr>
<td>7</td>
<td>&gt; 50</td>
<td>50</td>
<td>40.3 ± 13.8</td>
<td>&lt; 0.8</td>
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<tr>
<td>8</td>
<td>&gt; 50</td>
<td>&gt; 50</td>
<td>58.2 ± 3.8</td>
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<td>9</td>
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<td>25</td>
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<td>11</td>
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<td>48.9 ± 12.8</td>
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<td>&gt; 50</td>
<td>3.7 ± ND</td>
<td>&lt; 0.07</td>
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<tr>
<td>13</td>
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<tr>
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<td>52.1 ± 3.4</td>
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<tr>
<td>15</td>
<td>6.2</td>
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<td>61.2 ± 11.6</td>
<td>9.9</td>
<td>38.2</td>
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<td>0.03</td>
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<td>&gt; 33333.3c</td>
<td>&gt; 33333.3c</td>
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<tr>
<td>Pyronycin</td>
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<td></td>
<td>&lt; 0.3</td>
<td></td>
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</tbody>
</table>

ND, not determined.

a Values are expressed as the mean ± SD.
b Selectivity index (SI) = cytotoxicity (IC₅₀) / antymycobacterial activity (MIC)

c Data from reference (Hearn and Cynamon, 2004), and the SI values of isoniazid were calculated according to the reported IC₅₀ towards Vero cells.
at a concentration range of 50–0.8 μg/ml. The calculated 50% growth-inhibitory concentration (IC_{50}) values of the compounds are shown in Table 1. The alkaloid 6-acetonyldihydronitidine (6) was observed to be the most cytotoxic compound exhibiting an IC_{50} value of 1.7 μg/ml. The lignan (−)-savinin (12) was found to possess lethal effect on THP-1 cells (IC_{50} 3.7 μg/ml) but lacked antimycobacterial activity (MIC > 50 μg/ml). Low cytotoxicity (IC_{50} 40.3–94.5 μg/ml) was observed for compounds 1–5, 7–11 and 13–16.

To assess the antimycobacterial specificity of these compounds, the selectivity index values (SI = IC_{50}/MIC) were calculated (Table 1). The antimycobacterial activity was considered to be specific when SI > 10 (Hartkoorn et al., 2007). Decarine (1) demonstrated the highest selectivity among all of the isolated compounds, showing SI = 21.3 for H37Ra and SI = 41.2 for H37Rv. The N-isobutylamide (15) was also found to be specifically active towards H37Ra and H37Rv with SI values of 9.9 and 38.2, respectively. The generally low SI values obtained for compounds 2–14 and 16 indicated either their antimycobacterial activity is poor or presented high cytotoxic effect.

3.3. Antimicrobial activity against intracellular Mycobacterium tuberculosis H37Rv in THP-1 macrophages

Considering the above results in which decarine (1) and N-isobutylamide (15) presented favourable selective activity against Mycobacterium tuberculosis strains, these compounds were chosen for further intracellular efficacy evaluation. Human THP-1 macrophages were infected with Mycobacterium tuberculosis H37Rv ATCC 27294, and then exposed to decarine (1) and N-isobutylamide (15) at concentrations of 1.6, 3.1, 6.2, 12.5 and 25 μg/ml (from MIC to 16-fold MIC). At 1, 3 and 5 days post-infection, macrophages were lysed and the viable bacteria enumerated by colony counts (Fig. 2). Pyrazinamide (200 μg/ml) and isoniazid (1 μg/ml) were included as control compounds of bacteriostatic and bactericidal activity, respectively. DMSO, at the same proportions as in the compound solutions, was used as untreated control. Although, neither decarine (1) nor N-isobutylamide (15) was capable of completely inhibiting growth of the bacteria comparable to isoniazid, yet, compound 1 showed some bactericidal activity in a dose-dependent manner. As observed in Fig. 2a, on day 5 post-infection, the intracellular antimicrobial activity of decarine at concentrations of 6.2–25 μg/ml was superior to that of pyrazinamide at 200 μg/ml, showing more than 90% reduction in colony forming units. A slightly higher activity than that of pyrazinamide was still observed for decarine at 3.1 μg/ml.

In contrast, the N-isobutylamide (15) showed only moderate inhibitory efficacy at all the tested concentrations, inhibiting less than 50% growth of intracellular bacteria during 5 days of drug exposure. As can be observed in Fig. 2b, this activity was neither dose- nor time-dependent.

4. Discussion

Plant-derived drugs have long been used worldwide in traditional medicine for the treatment of various infectious diseases including TB (Jachak and Jain, 2006). We initially screened 15 medicinal plants used in Mozambique for the treatment of TB and other respiratory diseases and highlighted the potential antimycobacterial activity of four plant species (Luo et al., 2011), namely Maerua edulis, Securidaca longepedunculata, Tabernae montana elegans and Zanthoxylum capense. Of these, Zanthoxylum capense appeared to be the most promising species, showing MIC values of 31.2–125 μg/ml for a range of mycobacterial strains (Luo et al., 2011). In this work, we undertook further phytochemical investigation of its antimycobacterial activity.

The phytochemical study of the n-hexane and dichloromethane soluble fractions of Zanthoxylum capense led to the isolation of compounds with different scaffolds (Fig. 1), most of them alkaloids (1–9). When analyzing the activity of the isolated benzopenanthridine analogues (1–7), decarine (1) and 6-acetylnitidine (6) displayed strong inhibitory effect against Mycobacterium tuberculosis. In addition to the promising in vitro antimycobacterial activity as well as low cytotoxicity on human THP-1 macrophages, decarine (1) also demonstrated bactericidal activity in an intracellular model of infection. Although this inhibitory activity on intracellular mycobacteria required a higher concentration (> 4-fold MIC), the IC_{50} of decarine on the host cells was still about 10 times higher than this value. Therefore, it is concluded that the host cells would not be adversely affected at concentrations that are effective against intracellular Mycobacterium tuberculosis. In contrast, 6-acetylnitidine (6) also exhibited prominent cytotoxicity on THP-1 macrophages, leading to a poor selective activity (SI 0.1–0.3). The other benzopenanthridine-type alkaloids 2–5 and
7, showed only marginal antimycobacterial activity as well as low cytotoxicity.

Previously, decarine (1) has shown potent antiplasmodial (Ross et al., 2004), anti-HIV (Cheng et al., 2005), and anti-inflammatory (Chen et al., 2011) activities. Moreover, our recent antibacterial evaluation indicated that decarine was also an inhibitor of Staphylococcus aureus ATCC 6538 (Luo et al., 2012). Decarine (1) has a free hydroxyl group at C-8, whereas norcheler-ythrine (2), which presented poor antimycobacterial activity, has a methoxyl group at that position, suggesting that this structural feature might account for the potent antimycobacterial activity of decarine. Similar results have been observed in recent reports, where decarine (1) exhibited significantly higher anti-HIV and anti-inflammatory activities than its C-8 methoxyl analogues (Cheng et al., 2005; Chen et al., 2011). To the best of our knowledge, this is the first report on the antimycobacterial activity of decarine.

When comparing the activity of 6-acetonyldihydronitidine (6) with that of its analogues 3–5 and 7, it seems that the oxygena-
tion patterns of ring A influences the cytotoxicity of this class of compounds. Compound 6, having two methoxyl groups at C-8 and C-9, exhibited low MIC values but the highest cytotoxicity of all compounds tested in this work. On the other hand, compounds 3–5 and 7, with methoxyl groups at C-7 and C-8, displayed poor antimycobacterial activity as well as low cytotoxicity. These results are in accordance with a structure-activity relationship study on benzophenanthidine alkaloids that highlighted the effect of ring A alkoxyl substituents on the antimycobacterial activity (Ishikawa, 2001). An earlier study (Stormitz et al., 1973), related to the antitumoral activity of this type of alkaloids, also emphasized the effect of ring A substituents. Several types of alkaloids, namely isoquinoline alkaloids, have also been reported as potential antimycobacterial leads (Copp, 2003; Kishore et al., 2009).

Among the remaining isolated compounds, N-isobutyl-(2E,4E)-2,4-tetradecadienamide (15) displayed pronounced activity against both strains of Mycobacterium tuberculosis H37Ra ATCC 25177 and H37Rv ATCC 27294 together with low cytotoxicity. N-alkylamides, such as compound 15, are a heterogeneous class of molecules that are found in several plant families. Various biological activities have been attributed to N-alkylamides, namely antibacterial, anti-
fungal, antiparasit, and insecticidal activities, which make them very promising in the development of novel drugs (Boonen et al., 2012). A moderate antimycobacterial activity was also found for N-isobutylamides similar to compound 15, namely pellitorine isolated from Piper sarmentosum (García et al., 2012).

5. Conclusions

In conclusion, our results demonstrate that the medicinal plant Zanthoxylum capense possesses antimycobacterial compounds and therefore support the traditional use of this plant for treat-
ment of TB. The promising activity of decarine both in vitro and intracellularly within human macrophages suggests its potential for use as an anti-TB drug scaffold. Nevertheless, further studies are necessary to determine its mechanism of action.

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