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Antimycobacterial evaluation and preliminary phytochemical investigation of selected medicinal plants traditionally used in Mozambique

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ABSTRACT

Ethnopharmacological relevance: Several medicinal plants are traditionally used in Mozambique to treat tuberculosis and related symptoms.

Aims of the study: It was aimed to assess the *in vitro* antimycobacterial activity of crude extracts from fifteen medicinal plants and to reveal main classes of compounds which may account for the activity of extracts.

Methods and materials: The plant materials were sequentially extracted by *n*-hexane, dichloromethane, ethyl acetate, and 70% ethanol. Decoction of each plant material was also prepared according to traditional use. Broth microdilution method was employed to screen extracts against two mycobacterial species: *Mycobacterium smegmatis* ATCC 607 and *Mycobacterium tuberculosis* H37Rv. The extracts with minimum inhibitory concentration(s) (MIC) below 125 µg/mL were considered active and further tested against different mycobacterial species and strains, namely *Mycobacterium tuberculosis* H37Ra, *Mycobacterium bovis* BCG ATCC 35734, *Mycobacterium smegmatis* mc² 155, *Mycobacterium avium* DSM 44156 and DSM 44157. Cytotoxic effect was evaluated against human macrophages from the monocytic THP-1 cells. Main classes of compounds in these active extracts were proposed from their ¹H NMR spectroscopic characterizations.

Results: *n*-Hexane extracts of *Maerua edulis* and *Securidaca longepedunculata*, ethyl acetate extract of *Tabernaemontana elegans* and dichloromethane extract of *Zanthoxylum capense* were found to possess considerable activity against *Mycobacterium bovis* BCG and *Mycobacterium tuberculosis* H37Ra with MIC 15.6–62.5 µg/mL. *Tabernaemontana elegans* ethyl acetate extract displayed strong activity against *Mycobacterium tuberculosis* H37Rv (MIC 15.6 µg/mL). Except for *Tabernaemontana elegans* ethyl acetate extract which presented potent cytotoxic effects in THP-1 cells (IC₅₀ < 4 µg/mL), the other three plant extracts showed moderate to none toxicity. Based on ¹H NMR spectroscopic analysis, major components in both *Maerua edulis* and *Securidaca longepedunculata* *n*-hexane extracts were linear chain unsaturated fatty acids. *Zanthoxylum capense* dichloromethane extract contained more complex constituents (mostly phenolic compounds). In the most potent extract, *Tabernaemontana elegans* ethyl acetate extract, the prominent compounds were identified as indole alkaloids.

Conclusions: The pronounced antimycobacterial activity of the medicinal plants *Maerua edulis*, *Securidaca longepedunculata*, *Zanthoxylum capense*, and *Tabernaemontana elegans* suggested that they might provide compounds which could be potential anti-TB drug leads.

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1. Introduction

Mycobacterium tuberculosis, a deadly bacterial pathogen and causative agent of tuberculosis (TB), infects about one third of the world's population. In 2009, there were 9.4 million new cases and 1.7 million people died from TB (WHO, 2010). Despite more than

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40 years of anti-TB chemotherapy, TB remains one of the leading infectious killers worldwide. The association with HIV epidemic, the increasing emergence of multi-drug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB) have worsened the situation and posed a serious health threat. Therefore, potent new anti-tubercular drugs with novel modes of action and low toxicity are urgently needed to combat the threat of TB.

Natural sources provide numerous examples of interesting secondary metabolites with antimycobacterial activity, indicating that natural products could be rewarding field for the discovery of new anti-TB leads (Newton et al., 2000; Copp, 2003; Pauli et al., 2005; Copp and Pearce, 2007). In Mozambique, where around 500 plant species are used as traditional medicine, people mainly rely on medicinal plants as their primary source for medication. However, scientific validation and efficacy of many of those plants have not been well documented so far (Bandeira et al., 2001).

The aim of the present study was to evaluate plant species used in Mozambique for treatment of TB and other respiratory diseases for their antimycobacterial activity. Fifteen traditional medicinal plants were selected according to ethnobotanical information. Preliminary screening was carried out using two mycobacterial species *Mycobacterium smegmatis* ATCC 607 (fast growing) and *Mycobacterium tuberculosis* H37Rv (slow growing). *Mycobacterium tuberculosis* H37Ra, *Mycobacterium bovis* BCG ATCC 35734, *Mycobacterium smegmatis* mc² 155, *Mycobacterium avium* DSM 44156 and DSM 44157 were used at a later stage to test active plant extracts in order to obtain wide spectrum antimycobacterial profiles. Cytotoxicity of active plant extracts towards human monocytic THP-1 cells was also performed. The main classes of compounds in the active extracts were proposed by ¹H NMR spectroscopic characterization.

2. Materials and methods

2.1. Collection of plant materials

A literature survey was carried out on the medicinal plants for curing TB in Mozambique. Fifteen plants were selected according to their traditional use in treatment of various ailments including cough, bronchitis, chest complaints, pneumonia, and TB. The information of plant materials is summarized in Table 1. All species were obtained from south of Mozambique (Machava and Massingir) and authenticated by one of the authors, Dr. Silva Mulhovo. The voucher specimens (Table 1) have been deposited at the herbarium (LMA) of the Instituto de Investigação Agrária de Moçambique (IIAM), Maputo, Mozambique.

2.2. Preparation of extracts

The collected plants were air-dried in the shade at room temperature for at least two weeks, and then ground into fine powder. Seventy-five crude extracts of the medicinal plants were obtained by both sequential extraction and decoction, according to traditional use.

Crude plant extracts were prepared by submitting 50 g of air-dried powdered plant material to a sequential extraction procedure with 500 mL of *n*-hexane, dichloromethane, ethyl acetate, and 70% ethanol (v/v) for 48 h, at room temperature. Then the extracts were filtrated through Whatman filter paper.

For *Opuntia* spp., fresh plant was chopped into small pieces and then was made into juice using a blender with addition of minimum amount of distilled water. The juice was filtered and the filtrate was divided into two portions. One portion was concentrated into dryness at reduced pressure as total extract, and the other portion was successively extracted with *n*-hexane, CH₂Cl₂, and EtOAc.

The decoctions were obtained by keeping 5 g of dried plant material each in 150 mL of distilled water to boil for 20 min, and then cooled down for 2 h at room temperature. The extracts were filtered to obtain the filtrates.

All seventy-five extracts were separately evaporated to dryness under vacuum below 40 °C by rotary evaporation and stored in the freezer at –22 °C until use.

2.3. Antimycobacterial assays

2.3.1. Microorganisms

Two species of mycobacteria: *Mycobacterium smegmatis* ATCC 607 and *Mycobacterium tuberculosis* H37Rv ATCC 25618 were used in the preliminary screening of the 75 extracts. *Mycobacterium smegmatis* ATCC 607 was cultured on Mueller Hinton agar plate at 37 °C for 2 days. Then the colonies were transferred to sterile saline (0.9%, w/v NaCl) to obtain a suspension comparable to 0.5 McFarland turbidity standard. *Mycobacterium tuberculosis* H37Rv was cultured in Middlebrook 7H9 broth supplemented with 10% (v/v) ADC (Becton Dickinson) enrichment, 0.05% (v/v) Tween 80 (Sigma–Aldrich) and 0.2% (v/v) glycerol (Sigma–Aldrich) at 37 °C for 7–10 days until a OD₆₀₀ of 0.6–0.8 was reached, indicative of 3 × 10⁷ cfu/mL.

According to the literature (Molina-Salinas et al., 2007), those extracts having a MIC of 125 µg/mL or below are considered active against mycobacteria. Therefore, the extracts with MIC values below 125 µg/mL were further tested against the strains: *Mycobacterium tuberculosis* H37Ra (Institute Pasteur collection), *Mycobacterium bovis* BCG ATCC 35734, *Mycobacterium smegmatis* ATCC 607 variant mc² 155, *Mycobacterium avium* DSM 44156 and DSM 44157. These bacteria were cultured in Middlebrook 7H9 broth supplemented with 10% OADC, 0.05% Tween 80 and 0.2% glycerol at 37 °C until a OD₆₀₀ of 0.6–0.8 was reached, indicative of 3 × 10⁷ cfu/mL; this typically was 1 day for *Mycobacterium smegmatis* ATCC 607 variant mc² 155, and 7–10 days for the rest of the strains.

For each assay, two replicates of each concentration were tested, and the experiment was repeated three times.

2.3.2. Preliminary screening against *Mycobacterium smegmatis* ATCC 607 and *Mycobacterium tuberculosis* H37Rv

Screening assays were performed using the broth microdilution method (Ramón-García et al., 2009), following the recommendations of the Clinical and Laboratory Standards Institute (CLSI, 2008). Solutions of each extract were prepared by first dissolving in dimethyl sulfoxide (DMSO; Sigma–Aldrich) to a concentration of 20 mg/mL and then further diluting in the respective culture media for each bacteria (referred above). Serial two-fold dilutions of each extract were made in 96-well microtitre plates with concentrations ranging from 1 mg/mL to 0.9 µg/mL for *Mycobacterium smegmatis* ATCC 607 and 500–15.6 µg/mL for *Mycobacterium tuberculosis* H37Rv. Each well was inoculated with bacterial suspension at concentration of 10⁵ cfu/mL. No inhibitory effects were observed in the presence of DMSO at the concentrations used. Rifampicin and isoniazid were used as reference drugs.

For the screening of antimycobacterial activity against *Mycobacterium smegmatis*, the microtitre plates were incubated for 2 days at 37 °C, and then measured by absorbance microplate reader (ELx808™, BioTek) at 630 nm. When the colours of extracts interfered with the absorbance reading, the results were confirmed by replicating the samples onto Mueller Hinton agar plate and incubating at 37 °C for 1 day to assess the growth of mycobacteria.

For the screening of antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv, the microtitre plates were incubated for 8 days at 37 °C, then the redox indicator resazurin (0.1 mg/mL; Sigma–Aldrich) was added to each well at 15% (v/v) and the plates

Table 1

Traditional uses of plant species collected from Mozambique for treating TB/related diseases.

Species	Family	Plant part	Voucher specimen	Traditional use	Reference
<i>Adansonia digitata</i> L.	Bombacaceae	Barks	34/SM	Tuberculosis, persistent cough, bronchitis, pneumonia, chest pain.	Jansen and Mendes (1990)
<i>Anacardium occidentale</i> L.	Anacardiaceae	Roots	28/SM	Cough	Jansen and Mendes (1982)
<i>Ansellia africana</i> Lindl.	Orchidaceae	Whole plant	35/SM	Asthma, respiratory problems	Bandeira et al. (2001)
<i>Artabotrys brachypetalus</i> Benth.	Annonaceae	Roots	36/SM	Cough, asthma	Jansen and Mendes (1982)
<i>Capparis tomentosa</i> Lam.	Capparaceae	Roots	37/SM	Cough, chest pain, tuberculosis	Jansen and Mendes (1990); Bandeira et al. (2001)
<i>Clerodendrum glabrum</i> E. Mey. Var. <i>glabrum</i>	Verbenaceae	Leaves	38/SM	Cough, fever, tuberculosis, bronchitis,	Iwalewa et al. (2007)
<i>Combretum zeyheri</i> Sond.	Combretaceae	Barks	39/SM	Cough	Jansen and Mendes (1991)
<i>Maerua edulis</i> (Gilg & Gilg-Ben.) DeWolf	Capparaceae	Roots	40/SM	Cough, tuberculosis	Jansen and Mendes (1990)
<i>Maerua juncea</i> Pax	Capparaceae	Roots	41/SM	Respiratory problems	Personal communication
<i>Opuntia</i> spp.	Cactaceae	Whole plant	42/SM	Cough, bronchitis	Jansen and Mendes (1990); Bandeira et al. (2001)
<i>Sarcostemma viminalis</i> (L.) R. Br.	Apocynaceae	Roots	43/SM	Tuberculosis	Jansen and Mendes (1983)
<i>Securidaca longepedunculata</i> Fresen.	Polygalaceae	Roots	44/SM	Cough, chest complaints, tuberculosis	Bandeira et al. (2001)
<i>Tabernaemontana elegans</i> Stapf	Apocynaceae	Roots	23/SM	Chest complaints, tuberculosis	Jansen and Mendes (1983); Bandeira et al. (2001)
<i>Zanthoxylum capense</i> (Thunb.) Harv.	Rutaceae	Roots	45/SM	Violent chronic coughing, tuberculosis, bronchitis	Steyn et al. (1998)
<i>Vernonia colorata</i> (Willd.) Drake subsp. <i>colorata</i>	Asteraceae	Leaves	27/SM	Cough, pneumonia	Bandeira et al. (2001)

were incubated for two additional days. A change in colour from blue to pink indicated the growth of mycobacteria, and the MIC was defined as the lowest concentration of drug preventing this colour change. When fungal and/or bacterial contamination of extracts was detected, assay was carried out in the presence of amphotericin B (9 µg/mL; Sigma–Aldrich) and vancomycin (0.25 µg/mL; Sigma–Aldrich), which did not affect the growth of *Mycobacterium tuberculosis* H37Rv. When the colours of extracts interfered with the resazurin assay, the BacTiter-Glo™ Microbial Cell Viability Assay (Promega) was used following manufacturer's recommendations.

2.3.3. Screening against *Mycobacterium tuberculosis* H37Ra, *Mycobacterium bovis* BCG ATCC 35734, *Mycobacterium smegmatis* mc² 155, *Mycobacterium avium* DSM 44156 and DSM 44157

Using the broth microdilution method, the selected plant extracts were dissolved in DMSO and serial two-fold dilutions were performed in Middlebrook 7H9 broth supplemented with 10% OADC, 0.05% Tween 80 and 0.2% glycerol in a range of concentrations from 250 to 4 µg/mL. Approximately 10⁵ cells were incubated in the diluted extracts. Kanamycin was used as a reference drug. The microtitre plates were observed under a light microscope and the optical density at 600 nm was measured in a Tecan's M200 plate spectrophotometer on day 5 and 10 to assess the bacterial growth and to determine the MIC.

2.4. Cytotoxicity assay

2.4.1. Cell culture

Human acute monocytic leukemia cell line, THP-1 (TIB-202, ATCC), was maintained in RPMI-1640 medium containing 10% fetal calf serum (FCS), 1% 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% CO₂ atmosphere. Prior to infection, 1 × 10⁵ cells were seeded in 96-well plates and incubated over night 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 insure that the cells reverted to a resting macrophage phenotype.

2.4.2. Assay

Cells were incubated with the plant extracts at concentrations ranging from 125 µg/mL to 4 µg/mL for 7 days. Culture medium was replenished with the extracts in every two days during the course of the experiment. DMSO at the same proportions as in the tested extracts was used as a control. Puromycin 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. This reagent becomes fluorescent when metabolized by the cells, allowing quantification of cell viability by measuring their metabolic function. Briefly, 10% (v/v) of alamarBlue reagent was added to each well and incubated for 4 h at 37 °C and 5% CO₂. Fluorescence was measured at an excitation of 570 nm and emission of 595 nm in a Tecan's M200 plate spectrophotometer. Viability was calculated as percentage fluorescence intensity relative to the untreated cells. The IC₅₀ values were calculated by linear regression from the set of concentrations tested.

2.5. ¹H NMR spectroscopic analysis

¹H NMR spectra of the active extracts were recorded on a Bruker ARX-400 nuclear magnetic resonance (NMR) spectrometer (¹H NMR, 400 MHz). Deuterated chloroform (CDCl₃) and methanol (CD₃OD) were used as solvents. Chemical shifts are reported with reference to the respective residual solvents or deuterated solvent peaks (δ_H 7.26 for CDCl₃; δ_H 3.30 for CD₃OD).

3. Results and discussion

3.1. Preliminary screening

Based on ethnobotanical information about medicinal plants used by local people in Mozambique for treatment of TB-related symptoms (Table 1), a total of fifteen plant species were selected

Table 2
Antimycobacterial activity (MIC, $\mu\text{g}/\text{mL}$) and cytotoxicity towards human monocytic THP-1 cells (IC_{50}) of the active extracts.

Species	Extract	Antimycobacterial activity MIC ($\mu\text{g}/\text{mL}$)					Cytotoxicity			
		<i>Mycobacterium smegmatis</i> ATCC 607	<i>Mycobacterium tuberculosis</i> H37Rv	<i>Mycobacterium tuberculosis</i> H37Ra	<i>Mycobacterium bovis</i> BCG	<i>Mycobacterium avium</i> DSM 44156	<i>Mycobacterium avium</i> DSM 44157	<i>Mycobacterium smegmatis</i> mc ² 155	$\text{IC}_{50} \pm \text{SD}$ ($\mu\text{g}/\text{mL}$)	THP-1
<i>Maerua edulis</i>	n-Hexane	62.5	250	62.5	31.2	125	250	125	>125	>125
<i>Securidaca longepedunculata</i>	n-Hexane	500	125	62.5	31.2	250	>250	62.5	>125	>125
<i>Tabernaemontana elegans</i>	EtOAc	500	15.6	31.2	15.6	>250	250	125	<4	<4
<i>Zanthoxylum capense</i>	CH_2Cl_2	125	62.5	31.2	31.2	125	>250	62.5		45.7 ± 4.1
Rifampicin		125	<0.08							
Isoniazid			0.5							
Kanamycin				0.8	0.8	6.2	12.5	0.8		<0.3
Puromycin										

in the present investigation. Seventy-five crude extracts were obtained by both successive extraction with four solvents of varying polarity and decoction. *Mycobacterium smegmatis* ATCC 607 and *Mycobacterium tuberculosis* H37Rv ATCC 25618 were used as target organisms in the preliminary assays. *Mycobacterium smegmatis* ATCC 607, which is a fast growing, avirulent, saprophytic mycobacterium, has been used extensively in current research for primary screening of antimycobacterial activity. However, *Mycobacterium smegmatis* ATCC 607 possesses a limited degree of similarity to *Mycobacterium tuberculosis* with respect to drug susceptibility (Pauli et al., 2005). During our screening, only *Maerua edulis* n-hexane and *Zanthoxylum capense* CH_2Cl_2 extracts showed considerable activity against *Mycobacterium smegmatis* ATCC 607 with MIC of 62 $\mu\text{g}/\text{mL}$ and 125 $\mu\text{g}/\text{mL}$, respectively. Although *Mycobacterium tuberculosis* H37Rv is a virulent, slow growing strain, it is an ideal organism for anti-TB discovery effort, and has a drug susceptibility profile which is fairly representative of the majority of drug susceptible clinical isolates. A total of eight extracts from four plant species exhibited moderate to significant activity towards *Mycobacterium tuberculosis* H37Rv, namely, *Capparis tomentosa* 70% EtOH extract (MIC 125 $\mu\text{g}/\text{mL}$), *Securidaca longepedunculata* n-hexane extract (MIC 125 $\mu\text{g}/\text{mL}$), *Tabernaemontana elegans* EtOAc extract (MIC 15 $\mu\text{g}/\text{mL}$), 70% EtOH extract (MIC 125 $\mu\text{g}/\text{mL}$) and H_2O extract (MIC 125 $\mu\text{g}/\text{mL}$) and *Zanthoxylum capense* n-hexane extract (MIC 125 $\mu\text{g}/\text{mL}$), CH_2Cl_2 extract (MIC 62 $\mu\text{g}/\text{mL}$), and EtOAc extract (MIC 125 $\mu\text{g}/\text{mL}$).

The remaining experimental plant species, in spite of being reported to be used in the treatment of TB and related diseases, failed to display any activity against the screened strains in our assays. The possible explanations could be that the anti-TB effect of those plants is mediated through immuno-stimulation or immuno-modulation rather than direct inhibition on mycobacterial growth; or that the potential active compounds need to be metabolically activated *in vivo* by specific enzymes or may have a pH dependant biological activity (Ríos and Recio, 2005). Therefore, the negative results obtained from our work could not preclude the potential anti-TB effect of those medicinal plants.

3.2. Extensive antimycobacterial activity study and cytotoxicity of active extracts

The above initial screening results led us to select the aforementioned five plant species for further antimycobacterial study. The tested organisms comprised two slow growing strains *Mycobacterium tuberculosis* H37Ra and *Mycobacterium bovis* BCG ATCC 35734, closely related to *Mycobacterium tuberculosis* H37Rv, two slow growing strains of the *Mycobacterium avium* complex, *Mycobacterium avium* DSM 44156 and DSM 44157, which are more resistant to a number of antibiotics than *Mycobacterium tuberculosis* (Inderlied et al., 1993; Heifets, 1996), and one fast growing strain *Mycobacterium smegmatis* mc² 155. The antimycobacterial results are presented in Table 2. As no more activity was observed during further tests, the data of *Capparis tomentosa* 70% EtOH extract are not included.

As shown in Table 2, the n-hexane extracts of both *Maerua edulis* and *Securidaca longepedunculata*, the EtOAc extract of *Tabernaemontana elegans*, and the CH_2Cl_2 extract of *Zanthoxylum capense* demonstrated promising activity against *Mycobacterium tuberculosis* H37Ra, and *Mycobacterium bovis* (MIC 15.6–62.5 $\mu\text{g}/\text{mL}$). However, only slight activity was observed for these four plant extracts towards the two antibiotic resistant strains of *Mycobacterium avium*. The n-hexane extract of *Securidaca longepedunculata* and the CH_2Cl_2 extract of *Zanthoxylum capense* displayed considerable activity against *Mycobacterium smegmatis* mc² 155 (MIC 62.5 $\mu\text{g}/\text{mL}$). These results indicated that these four plant extracts do have potent antimycobacterial activity against slow growing

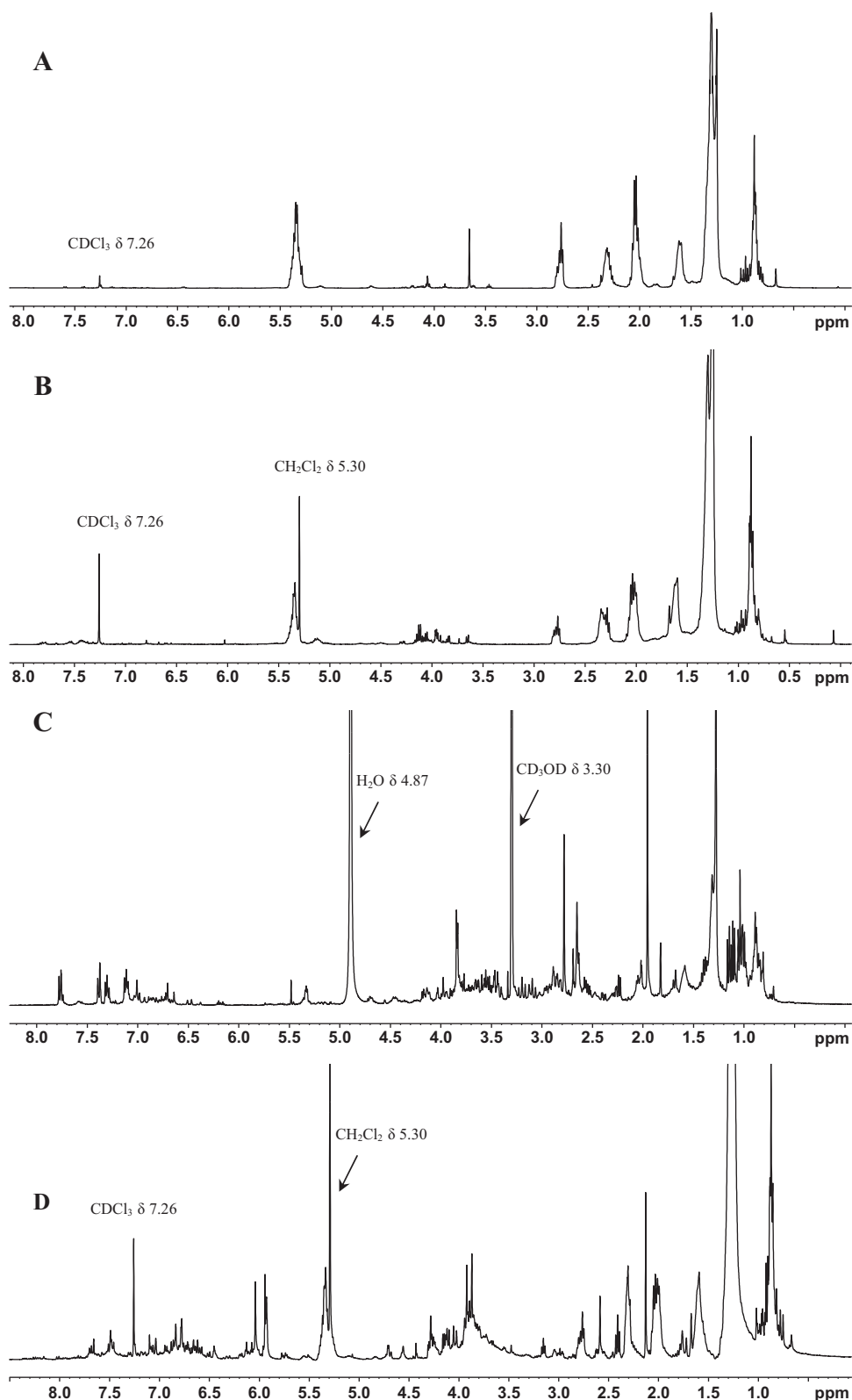


Fig. 1. ^1H NMR spectra (400 MHz) of active crude extracts. (A) *Maerua edulis* *n*-hexane extract; (B) *Securidaca longepedunculata* *n*-hexane extract; (C) *Tabernaemontana elegans* EtOAc extract; and (D) *Zanthoxylum capense* CH_2Cl_2 extract.

mycobacterial strains, but less direct inhibitory activity towards antibiotic resistant strains at this unpurified stage (crude extracts of compounds mixture). Hence, further purification and identification of the compounds responsible for antimycobacterial activity are required for these active plants.

Following the promising antimycobacterial activity, the cytotoxicity of these active extracts was evaluated using human monocytic THP-1 cells and the results are reported in Table 2. The *n*-hexane extracts of *Maerua edulis* and *Securidaca longepedunculata* had $\text{IC}_{50} > 125 \mu\text{g/mL}$, showing no toxicity

towards THP-1 cells at the highest dose tested, while the CH₂Cl₂ extract of *Zanthoxylum capense* exhibited low toxicity with IC₅₀ = 45.7 µg/mL. The highest toxicity observed for the EtOAc extract of *Tabernaemontana elegans* (IC₅₀ < 4 µg/mL) may be associated with its medicinal usage in treating cancer.

3.3. Preliminary phytochemical investigation

In order to reveal the components, which may account for the pronounced antimycobacterial activity observed for the four plant extracts, preliminary phytochemical investigation was carried out by direct ¹H NMR analysis. ¹H NMR spectroscopy has proved to be a particularly successful probe as it provides a rapid and non-destructive detector for a wide range of compounds, which are still in crude extract forms (Politi et al., 2008). And as a result, direct indications could be established whether a biologically active extract contains compounds of interest or only well known metabolites.

Interestingly, the ¹H NMR spectra of the *n*-hexane extracts of both *Maerua edulis* and *Securidaca longepedunculata* showed quite similar signal patterns except for minor differences in the range of 3.5–4.4 ppm (Fig. 1). The signals at δ 5.3–5.4, δ 2.7–2.8 and δ 2.0–2.1 are characteristic for unsaturated compounds with olefinic methines, and bis-allylic and allylic methylenes, respectively. Methylene signals at δ 2.3 are due to their adjacency to carbonyl functions. Signals at δ 1.2–1.4 and δ 0.8–0.9 are assignable to alkyl chains and terminal methyl groups, respectively. These features are in agreement with linear-chain unsaturated fatty acids which have been isolated from both *Maerua* (Abdel-Mogib, 1999) and *Securidaca* (Smith et al., 1979) genera.

The antimycobacterial properties of fatty acids have been known for decades. Unsaturated fatty acids have been reported to possess activity against mycobacteria which was dependent on the degree of unsaturation, chain length, and the bacterial species tested (Carballeira, 2008).

To the best of our knowledge, few phytochemical investigations have been carried out on the genus *Maerua* (Abdel-Mogib, 1999), and there has been no report on the chemical composition of *Maerua edulis* so far. Previous study of a related species *Maerua oblongifolia* reported the isolation of six known fatty acid derivatives, including palmitic acid and its methyl ester, stearic acid, palmitoleic acid, oleic acid methyl ester and linoleic acid methyl ester (the esters were likely formed due to methanolysis of fatty acids during extraction) (Abdel-Mogib, 1999). The ¹H NMR analysis suggested that *Maerua edulis* *n*-hexane extract may also contain the above fatty acids in both free and ester forms (singlet at δ 3.65 are corresponding to methoxyl groups). Palmitleic acid, oleic acid and linoleic acid, which are very common unsaturated fatty acids, were reported to possess potent antimycobacterial activity against a panel of fast growing mycobacteria (MIC 2–32 µg/mL) (Seidel and Taylor, 2004). In addition, oleic acid and linoleic acid were also found to be lethal for *Mycobacterium bovis* and *Mycobacterium tuberculosis* H37Ra when assayed in mildly acidic buffer (Kondo and Kanai, 1976, 1977). Thus, these findings of significant antimycobacterial activity of unsaturated fatty acids are consistent with our results that the *n*-hexane extract of *Maerua edulis* was active towards *Mycobacterium smegmatis* ATCC 607, *Mycobacterium bovis* and *Mycobacterium tuberculosis* H37Ra (MIC 31.2–62.5 µg/mL).

The phytochemistry of *Securidaca longepedunculata* has been extensively studied, and a number of references reported the main components as xanthenes, saponines, and alkaloids (Meyer et al., 2008; Mitaine-Offer et al., 2010), whereas the isolation of hydroxydienonic fatty acids with unique characters was also reported (Smith et al., 1979). The ¹H NMR signals ranging from

3.5 to 4.4 ppm (Fig. 1B) are attributed to the hydroxylation of unsaturated fatty acids, suggesting the presence of hydroxylated unsaturated fatty acids in the *n*-hexane extract of *Securidaca longepedunculata*. A series of hydroxylated unsaturated fatty acids have been reported as potent acetyl CoA carboxylase inhibitors (Watanabe et al., 1999) further indicating that these compounds exert antimycobacterial effect by inhibiting fatty acid synthesis. Hence, the observation of antimycobacterial activity of *Securidaca longepedunculata* *n*-hexane extract may closely be associated with its main composition of hydroxylated unsaturated fatty acids.

Analysis of the ¹H NMR spectrum of *Tabernaemontana elegans* EtOAc extract revealed the presence of vobasine-type indole alkaloids by comparison with our group data (Mansoor et al., 2009a). The characteristic signals at δ 7.75 (dd, H-9), δ 7.4 (dd, H-12), δ 7.3 (td, H-11), δ 7.1 (td, H-10), δ 5.3 (H-19), and δ 2.65 (OMe-22) are comparable to those found for vobasine, dregamine, and tabernaemontanine, which are also the main components of aerial parts of *Tabernaemontana elegans* (Schmelzer and Gurib-Fakim, 2008). Among these three compounds, dregamine was reported to possess convulsant and respiration-stimulant activities. It has been used in treatment of muscular and nervous asthma, respiratory depression and type III poliovirus (HPV-3). Consistently, another two indole alkaloids, ibogaine and voacangine, isolated from another member of *Tabernaemontana* genus (*T. citrifolia*) have been reported for their antimycobacterial activity against *Mycobacterium tuberculosis* H37Rv (MIC 50 µg/mL) (Rastogi et al., 1998). In addition, the aqueous extract of the roots of *Tabernaemontana elegans* was also found to be active against *Mycobacterium smegmatis* ATCC 14468 at MIC 1 mg/mL, and the presence of alkaloids was detected by a preliminary phytochemical study (Pallant and Steenkamp, 2008). Our group has extensively studied the leaves of *Tabernaemontana elegans*, and reported the isolation of novel monoterpene indole alkaloid along with three new β-carbolines in addition of other known compounds. We found that the alkaloids tabernaemontanine and vobasine showed a promising apoptosis induction activity in human hepatoma HuH-7 cells (Mansoor et al., 2009a, 2009b). Therefore, the high toxicity observed from *Tabernaemontana elegans* EtOAc extract may be due to its content in vobasine and tabernaemontanine, and consequently such apoptosis inducing effect may contribute to the significant antimycobacterial activity of the extract.

Zanthoxylum is a genus containing about 250 species with reported phytochemically important compounds mainly belonging to benzophenanthridine alkaloids. Additionally, lignans, coumarins, terpenoids, and flavonoids have also been isolated from this genus (Chen et al., 2009). However, thus far very limited studies have been carried out on the phytochemical composition of *Zanthoxylum capense* (Steyn et al., 1998). The ¹H NMR spectrum (Fig. 1) of *Zanthoxylum capense* CH₂Cl₂ extract showed the signals for aromatic protons ranging from 6.5 to 7.8 ppm, methylenedioxy groups at δ 5.9 and 6.05, olefinic protons at δ 5.3–5.4, methoxyl and oxymethene groups at δ 3.5–4.5, allylic and bis-allylic methylenes at δ 2.0 and 2.8, singlets at δ 2.6 attributed to *N*-Me and methylenes adjacent to carbonyl groups at δ 2.3. Additionally, signals at δ 1.6 and 1.2 are alkyl chain groups, and δ 0.8–0.9 are terminal methyls. Although as complex as its spectrum appeared, we could recognize the presence of benzophenanthridine alkaloids, as well as lignans and linear-chain fatty acids in *Zanthoxylum capense* CH₂Cl₂ extract. Since all these three classes of compounds have been well reported to possess antimycobacterial activity (Copp, 2003; Copp and Pearce, 2007), it seems interesting to clarify whether the potent antimycobacterial activity displayed by *Zanthoxylum capense* CH₂Cl₂ extract is due to one of these compounds, or whether this activity is due to synergistic effects of some or all of these compounds.

4. Conclusions

In the present investigation, four plant species, *Maerua edulis*, *Securidaca longepedunculata*, *Tabernaemontana elegans*, and *Zanthoxylum capense* displayed potent activity towards a panel of mycobacteria indicating their potential as sources of anti-TB drug leads. Direct analysis of the ^1H NMR spectra of the most active extracts revealed their main composition, which linked closely with the observed antimycobacterial activity. Consequently, the obtained association between the supposed main classes of compounds in the extracts and promising activity may guide future isolation and antimycobacterial evaluation of the active principles. Further phytochemical and pharmacological studies of these plants are evidently worthwhile and our group has already focused on this effort.

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