Inhibitory properties of selected South African medicinal plants against Mycobacterium tuberculosis Ezekiel Green a,b, Amidou Samie a, Chikwelu L. Obic, Pascal O. Bessonga, Roland N. Ndip b,d,∗

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A B S T R A C T

Ethnopharmacological relevance: Tuberculosis (TB), caused by Mycobacterium tuberculosis (MTB) is the most commonly notified disease and the fifth largest cause of mortality. One in 10 cases is resistant to treatment in some areas. Several plants are used locally to treat TB-related disease. Aims of the study: The aim was to screen selected South African medicinal plants used to treat TB and related symptoms by traditional healers for antimycobacterial activity. Materials and methods: Ethnobotanical information on these plants was obtained. Crude acetone, methanol, hexane and ethanol extracts of 21 selected medicinal plants obtained in Venda, South Africa were screened for their ability to inhibit MTB H37Ra and a clinical strain resistant to first-line drugs and one second-line drug using tetrazolium microplate assay to determine the minimum inhibitory concentration (MIC). Results were analyzed using Microsoft Excel 2007 and One way ANOVA; p<0.05 was considered for statistical significance. Results: Few acetone extracts were active against MTB with MIC under 100?g/mL. Four plants showed lower MIC values; Berchemia discolor Klotzsch Hemsl 12,5?g/mL on H37Ra and 10.5?g/mL on the clinical isolate, Bridelia micrantha Hochst. Baill (25?g/mL), Warbugia salutaris Bertol. F Chiov (25?g/mL), and Terminalia sericea Burch ex D. F (25?g/mL) on both H37Ra and clinical isolate. However, the roots of Ximenia caffra Sond. Var. caffra, barks of Sclerocarya birrea (A Rich) Hochst, Asclepias fruticosa L, tubers of Allium sativum L, leaves of Carica papaya L, Solanum panduriforme E. Mey C, and roots of Securidaca longepedunculata Fresen gave MIC greater than 100?g/mL. Conclusion: The acetone extracts of Berchemia discolor, Bridelia micrantha, Terminalia sericea and Warbugia salutaris could be important sources of mycobactericidal compounds against multidrug-resistant MTB. © 2010 Elsevier Ireland Ltd. All rights reserved.

Introduction

MTB is the aetiological agent of tuberculosis (TB), one of the most prevalent infections in the world causing high mortality in developing countries. The disease spreads more easily in over-crowded settings and in conditions of malnutrition and poverty (Pereira et al.,

New TB cases have been estimated at 9.2 million during 2006, with 1.7 million MTB-related deaths. This is an increase over the 9.1 million new cases reported in 2005 (World Health Organization, 2008). One-third of the world’s population is estimated to have latent MTB (World Health Organization, 2008). Although the increase in new TB cases is attributed to population growth, new drug-resistant strains that threaten to increase this number, undermine efforts to eradicate this disease, and exacerbate the healthcare burden. Incidences of multidrug-resistant TB and extensively drug-resistant TB are on the rise, requiring the development of new treatment regimens, drugs, and drug targets (World Health Organization, 2008; Green et al., 2010). Few alternative drugs are available in cases where drug resistance is a problem. Despite highly effective first-line drugs, morbidity and mortality due to MTB are still increasing (Ballell et al., 2005). It is estimated that between the years 2000 and 2020 nearly one billion people will be newly infected, 200 million will develop TB and 35 million will die from the disease (World Health Organization, 2000). In South Africa, multidrug-resistant tuberculosis (MDR-TB) has also been identified (Green et al., 2008, 2010) and these emerging MDR strains complicate treatment of TB (Victor et al., 2007). In 2005, large numbers of patients with MDR-TB and XDR-TB were identified at a rural hospital in Tugela Ferry, KwaZulu-Natal (Gandhi et al., 2006). Coinfection of MTB with the human immunodeficiency virus (HIV) and the emergence of strains resistant to available therapies (Chungetal., 1995; Ballelletal., 2005) have also been linked to morbidity and mortality due to MTB. South Africa is home to one of the largest populations of HIV infected individuals in the world and has more patients receiving antiretroviral therapy (ART) than does any other country (World Health Organization, 2007b). The increase in TB drug resistance in this context undermines the strides that the national ART rollouthas made and may potentiallylimit its successful expansion. Fifty-eight percent of patients with TB were coinfected with HIV. Of significant concern, the TB cure rate (64%) was the lowest among the 10 countries with the highest TB burden (Andrews et al., 2007). The emergence of XDR-TB is a signpost for the necessity to find new drugs for the management of TB. An alternative source of new drugs is in natural products isolated from medicinal plants. Natural products isolated from plants have played an important role in discovery of drugs against infectious diseases. Almost 75% of the approved anti-infective drugs are derived from medicinal plants (Cragg et al., 1997). According to World Health Organization, more than 65% of the global population uses medicinal plants as a primary healthcare modality (Farnsworthetal., 1985). South Africa is a rich source of medicinal plants containing approximately 10% of the world’s terrestrial plants, some being medicinal (Arnold et al., 2002); and members of different indigenous communities in South Africa consult traditional herbalists for the treatment of infections. Over 350 plant species used in traditional medicine have been assessed for their antituberculosis activities (Eldeen and van Staden, 2007). Apart from the antituberculosis activities, plant extracts have also...
demonstrated immunomodulatory effects on different cell cultures and in experimental animals (Eloff, 2001). However, there is paucity of information and scientific validation on plants used in Venda region to cure TB and its related symptoms. The aim of the present study was to evaluate plant species for antimycobacterial activities. Plants were chosen based on ethnobotanical information, that is, they have been used in traditional medicine for treatment of TB or symptoms of this disease in our environment.

2. Materials and methods
2.1. Mycobacterium tuberculosis isolates
We used MTB H37Ra (American Type Culture Collection 25177) because it is sensitive to all first-line drugs, while our isolates (Green et al., 2010) are resistant to all first-line drugs.

2.2. Plant material and extraction
Two traditional healers in the Limpopo province who receive TB patients were interviewed on the type of plants they employ in treating these individuals. These individuals have either disclosed their condition confirmed by a medical report or the healers suspected TB when the patients presented with a combination of two or more of the following conditions: chronic diarrhoea, sweating at night, persistent cough, progressive weight loss, and skin infections. Based on responses, 24 plants were collected and identified at the Botany Unit, Department of Biological Sciences, University of Venda, South Africa, where voucher specimens have been deposited. Plants were at different times collected from their natural habitats between August 2002 and April 2003. All plants were collected in Venda. Table 1 presents ethnobotanical information on the selected plants.

2.3. Preparation of aqueous, ethanol, methanol and acetone extracts
Plant barks, roots or leaves were washed with distilled water and dried at room temperature for 2–3 weeks. Dried plant material was chopped and ground into powder. Two hundred grams of powdered material were soaked in 2 L of either aqueous, acetone, ethanol or methanol overnight on a rotatory platform. The resulting mixture was subsequently strained through a cheese cloth and then vacuum-aided filtered through Whatman filter paper No. 3 (W&R, England, UK). The residue was further extracted twice with 250 mL of the extractant. Filtrates were evaporated to dryness on a rotatory evaporator (Rotavapor R-144 Buchi, Switzerland) at 40°C to obtain the acetone, ethanol and methanol extracts, respectively. The aqueous extracts were concentrated by freeze-drying. Extracts were stored in the dark at –20°C until used.

2.4. Antituberculosis activity
The activity of all plant extracts against the aforementioned Mycobacterium tuberculosis strains was tested using the resazurin microplate assay (REMA) according to the method of Jadaun et al. (2007). Briefly, each of the above strains were cultured at 37°C in Middlebrook 7H9 broth (Becton Dickinson, Sparks, MD) supplemented with 0.2% glycerol (Sigma Chemical Co., St. Louis, MO) and 10% oleic acid–albumin–dextrose–catalase (OADC; Becton Dickinson) until logarithmic growth was reached. Each culture was mixed with a sufficient volume of sterile supplemented Middlebrook 7H9 broth to achieve a turbidity equivalent to that of McFarland’s No. 1 standard. To obtain the test inoculum, this suspension was further diluted 1:20 with the same culture medium to approximately 6 × 10^6 colony-forming units (CFU)/mL immediately before use. The extracts were dissolved in 100% dimethyl sulfoxide (DMSO, Sigma) and maintained at room temperature for 1 h to assure their sterilization (Molina-Salinas et al., 2006). These extracts were further diluted to their final concentrations with fresh culture broth. The final concentration of DMSO in all assays was 2% or less, which is nontoxic for mycobacteria (Molina-Salinas et al., 2006). The organic and aqueous extracts from each plant were assayed in duplicate. All tests were carried out in sterile flat-bottomed 96-well microplates with low-evaporation polystyrene lids (Costar Corning, New York, NY). Perimeter wells were filled with sterile double-distilled water to prevent dehydration of the medium during incubation. A 100 μL of Middlebrook 7H9 broth supplemented with OADC

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was added to the remaining (test) wells. Each microplate was divided into six-well rows to establish a twofold dilution series in each row from each plant extract. The working plant extracts (100?L) were poured into the first well of each row, from which twofold dilution series were made with Middlebrook 7H9 broth. The final concentrations of these preparations ranged from 3.125 to 100?g/mL. Each MIC was determined three times and standard deviation calculated from the mean. The antituberculosis drugs streptomycin, isoniazide, and ethambutol (Sigma) were dissolved in double-distilled water and rifampin (Sigma) in 100% DMSO, respectively (Molina-Salinas et al., 2006). All the above antitubercular agents were diluted to a final concentration of 1mg/mL, divided into 0.5mL aliquots, and stored at −70°C until use. The final concentrations of these E. Green et al. / Journal of Ethnopharmacology 130 (2010) 151–157

Table 1 Ethnobotanical and relevant information on the plants used in this study. Species (family name) Local name Plant part used Voucher specimen Traditional use of plant

<table>
<thead>
<tr>
<th>Species (family name)</th>
<th>Local name</th>
<th>Plant part used</th>
<th>Voucher specimen</th>
<th>Traditional use of plant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Allium sativum L (Alliceae)</td>
<td>Gallic (E)</td>
<td>Tuber</td>
<td>EG01</td>
<td>For treating arthritis, backache, fever, rheumatism, and worms, febrifuge, tuberculosis, stimulant, carminative, antiseptic, anthelmintic, diaphoretic, expectorant, diuretic, hypotensive, and whooping cough (Thring and Weitz, 2006) Roots, barks and leaf are used for diarrhoea, dysentery, stomach problems, fever, cough, malaria, tonic and diabetes (Iwalewa et al., 2007) – Sclerocarya birrea (A Rich) Hochst (Anacardardiaceae) Mufula (V) Marula tree bark</td>
</tr>
<tr>
<td>Terminalia sericea Burch ex D.C. (Combretaceae) Muvuvhu (V) River bush willow</td>
<td>ST07</td>
<td>Mususu (V) Silver Terminalia tree (E) Leaves Bark</td>
<td></td>
<td>Used to treat wounds bacterial infections, diarrhoea, hypertension and fevers (Msonthi and Magombo, 1983; Fyhrquist et al., 2002; Arnold and Gulumian, 1984) Colds, sinuses, influenza and other chest complaints, antiparasitic powder applied to sores (Iwalewa et al., 2007) Immuno-stimulant, post-testicular antifertility drug, wounds and burns, anthelmintic activity relieves, symptoms of asthma, gastric problem, fever and amoebic dysentery (Aruoma et al., 2006; Mehdipour et al., 2006) Dried leaves are used for sleeping sickness, malaria, headache and anthelmintic, dried barks are used for cough and leprosy (Kerharo, 1974; Etkin, 1997; Khan et al., 1980; Arnold and Gulumian, 1984) Stomach aches, tapeworms, diarrhoea, headaches, sore joints, sore eyes, venereal diseases, and fevers (Betti, 2004; Bessong et al., 2006) Tuberculosis, stomach complains and intestinal parasites (Iwalewa et al., 2007) A decoction of the roots together with Diospyros lycioides (Ebenaceae) and Euclea natalensis (Ebenaceae) are taken to treat epilepsy in Venda, South Africa (Arnold and Gulumian, 1984) The roots are used to treat dysentery and diarrhoea (Hutchings et al., 1996) Tuberculosis (Bashir et al., 1987) Diarrhoea and dysentery (Fabry et al., 1996), fever, cough, venereal diseases Used for cough, bronchial problems, leprosy and infertility Used by Vha-Venda people to cure erectile dysfunction Warbugia salutaris (Bertol. f.) Chiov (Canellaceae) Carica papaya L (Caricaceae) Mulanga (V) Pepper bark</td>
</tr>
<tr>
<td>Diospyros mespiliformis Hochst (Ebanaceae)</td>
<td>Mususa (V) Jackalberry Tree/African ebony</td>
<td>Leaves/barks</td>
<td>SA12</td>
<td>Bridelia micrantha (Hochst.) Baill (Euphorbiaceae) Munzere (V) coast gold leaf</td>
</tr>
</tbody>
</table>
Monkey pod (E) Bark BP01 BarkSA03 Schotia brachypetala Sond (Caesalpinaceae) Grewia villosa (Malvaceae) Ximenia caffra Sond. Var. caffra (Olacaceae) Piper capense L.f (Piperaceae) Securidaca longipedunculata Fresen. (Polygalaceae) Berchemia discolor (Klotzsch) Hemsl. (Rhamnaceae) Ziziphus mucronata Willd (Rhamnaceae) Mulubí (V) Weeping boer bean (E) Mupunzu (V) Mutswili (V) BarkSA14 Roots Leaves SA01 AS15 Mulilwe (V) Mpesu (V), Violet tree (E) Munie (V) Roots Roots TT02 Barks/leavesAS21 Infertility and Menorrhagia (Arnold and Gulumian, 1984) Mukhalu (V) Buffalo thorn (E) Bark, leaves, roots SA15 Boils, sores, glandular swellings, diarrhoea, dysentery, expectorant, emetic for coughs, chest problems, boils, sores, glandular swellings (Iwalewa et al., 2007) Pelvic pains, wounds, toothache (Hutchings et al., 1996) Solanum panduriforme E. Mey. (Solanaceae) Lippia javanica (Burm. f.) Spreng (Verbanaceae) Thuthula (V) Leaves EG04 Musudzungwane (V) Leaves AS19 Used for coughs or colds and also for skin infections or wounds. The leaves and stems are often used and in some cases the roots as well. Strong leaf infusions are made which are commonly used as inhalants. The leaf infusions are also used topically for scabies and lice. More commonly, leaf and stem infusions are used as a tea, and this is taken to treat coughs, colds, fever and bronchitis. The plant has also been used for bronchial. The Vha-Venda people use leaf infusions as anthelmintics, for respiratory and febrile ailments and as prophylactic against dysentery diarrhoea and malaria. Roots are used as an-tidotes suspected food poisoning and for bronchitis and sore eyes. Used for fever and influenza in combination with leaves of Artemisia afra (Iwalewa et al., 2007) To prevent miscarriages, diarrhoea (Steenkamp, 2003) Rhoicissus tridentate Wild and Drum (Vitaceae) Murumbulambidzana (V), Bitter grape (E) Leaves/tubers AS18 E, English; V, Venda.