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Inhibitory properties of selected South African medicinal plants against *Mycobacterium tuberculosis*

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ABSTRACT

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 $H_{37}Ra$ 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 μg/mL. Four plants showed lower MIC values; *Berchemia discolor* Klotzsch Hemsl 12, 5 μg/mL on $H_{37}Ra$ and 10.5 μg/mL on the clinical isolate, *Bridelia micrantha* Hochst. Baill (25 μg/mL), *Warbugia salutaris* Bertol. F Chiov (25 μg/mL), and *Terminalia sericea* Burch ex D. F (25 μg/mL) on both $H_{37}Ra$ 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 μg/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.

1. 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 overcrowded settings and in conditions of malnutrition and poverty (Pereira et al., 2005). 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 extensive 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

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2005, it was estimated that 285,000 cases of TB developed in South Africa, representing the seventh highest total number of cases in the world and the second highest total in Africa (World Health Organization, 2007a). Second-line drugs such as kanamycin, p-aminosalicylate, ethionamide and fluoroquinolones are either toxic or less effective (Blumberg et al., 2003). 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 (Chung et al., 1995; Ballell et al., 2005) have also been linked to morbidity and motality 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 rollout has made and may potentially limit its successful and continued 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 health care modality (Farnsworth et al., 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 the treatment of TB or symptoms of this disease in our environment.

2. Materials and methods

2.1. Mycobacterium tuberculosis isolates

We used MTB $H_{37}Ra$ (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 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 μL) 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 μg/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 antimycobacterial agents were diluted to a final concentration of 1 mg/mL, divided into 0.5 mL aliquots, and stored at −70 °C until use. The final concentrations of these

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
Allium sativum L (*Alliceae*)	Gallic (E)	Tuber	EG01	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)
Sclerocarya birrea (A Rich) Hochst (*Anacardardiaceae*)	Mufula (V) Marula tree	Bark	SA16	Roots, barks and leaf are used for diarrhoea, dysentery, stomach problems, fever, cough, malaria, tonic and diabetes (Iwalewa et al., 2007)
Rhus rogersii Schönland (*Anacardiaceae*)	Rogers Currant		TT01	–
Carisa edulis Forssk. Vahl (*Apocenaceae*)	Murungulu (V) Natal plum (E)	Leaves	JF07	Skin infection, ectoparasitic disease, abdominal problems, headaches and sexually transmitted disease (Venter and Venter, 1996)
Asclepias fruticosa L (*Asclepiadaceae*)	Mutshule/Mushulwa (V)	Roots	ST04	Used for diarrhoea and stomach pain, facilitate child-birth, asthma and diabetes (Watt and Breyer-Brandwyk, 1962; Iwalewa et al., 2007)
Combretum erythrophyllum (Burch) Sond. (*Combretaceae*)	Muvuvhu (V) River bush willow (E)	Leaves		Abdominal pains, venereal diseases (Hutchings et al., 1996)
Terminalia sericea Burch ex D.C. (*Combretaceae*)	Mususu (V) Silver Terminalia tree (E)	Bark	ST07	Used to treat wounds bacterial infections, diarrhoea, hypertension and fevers (Msonthi and Magombo, 1983; Fyhrquist et al., 2002; Arnold and Gulumian, 1984)
Warbugia salutaris (Bertol. f.) Chiov (*Canellaceae*)	Mulanga (V) Pepper bark (E)	Leaves	SA08	Colds, sinuses, influenza and other chest complaints, antiparasitic powder applied to sores (Iwalewa et al., 2007)
Carica papaya L (*Caricaceae*)	Papawe (V) Pawpaw (E)	Leaves	EG03	Immuno-stimulant, post-testicular antifertility drug, wounds and burns, antihelmintic activity relieves, symptoms of asthma, gastric problem, fever and amoebic dysentery (Aruoma et al., 2006; Mehdipour et al., 2006)
Diospyros mespiliformis Hochst (*Ebanaceae*)	Musuma (V) Jackalberry Tree/African ebony	Leaves/barks	SA12	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)
Bridelia micrantha (Hochst.) Baill (*Euphorbiaceae*)	Munzere (V) coast gold leaf (E)	Bark	BP03	Stomach aches, tapeworms, diarrhoea, headaches, sore joints, sore eyes, venereal diseases, and fevers (Betti, 2004; Bessong et al., 2006)
Peltophorum africanum Sond (*Fabaceae*)	Musese (V) African Wattle (E)	Bark	BP01	Tuberculosis, stomach complains and intestinal parasites (Iwalewa et al., 2007)
Cassia petersiana Bolle (*Fabaceae*)	Munumbenembe (V) Monkey pod (E)	Bark	SA03	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)
Schotia brachypetala Sond (*Caesalpinaceae*)	Mulubi (V) Weeping boer bean (E)	Bark	SA14	The roots are used to treat dysentery and diarrhoea (Hutchings et al., 1996)
Grewia villosa (*Malvaceae*)	Mupunzu (V)	Roots	SA01	Tuberculosis (Bashir et al., 1987)
Ximenia caffra Sond. Var. caffra (*Olacaceae*)	Mutswili (V)	Leaves	AS15	Diarrhoea and dysentery (Fabry et al., 1996), fever, cough, venereal diseases
Piper capense L.f (*Piperaceae*)	Mulilwe (V)	Roots	TT02	Used for cough, bronchial problems, leprosy and infertility
Securidaca longepedunculata Fresen. (Polygalaceae)	Mpesu (V), Violet tree (E)	Roots		Used by Vha-Venda people to cure erectile dysfunction
Berchemia discolor (Klotzsch) Hemsl. (*Rhamnaceae*)	Munie (V)	Barks/leaves	AS21	Infertility and Menorrhagia (Arnold and Gulumian, 1984)
Ziziphus mucronata Willd (*Rhamnaceae*)	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)
Solanum panduriforme E. Mey. (*Solaneceae*)	Thuthula (V)	Leaves	EG04	Pelvic pains, wounds, toothache (Hutchings et al., 1996)
Lippia javanica (Burm. f.) Spreng (*Verbaneceae*)	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)
Rhoicissus tridentate Wild and Drum (*Vitaceae*)	Murumbulambidzana (V), Bitter grape (E)	Leaves/tubers	AS18	To prevent miscarriages, diarrhoea (Steenkamp, 2003)

E, English; V, Venda.

Table 2
Antituberculous activities of selected medicinal plants from Venda, South Africa.

Plants	Plant part	Extract	MIC μg/mL microorganisms			
			Mtb $H_{37}R_a$	SD	*Mtb 2*	SD
Ziziphus mucronata	B	Acetone	50	0.6	50	1.5
Berchemia discolor	B	Acetone	12.5	2.1	10.5	2.8
	L	Acetone	>100	7.9	>100	8.9
Warbugia salutaris	L	Acetone	25	2.0	25	5
Cassia petersiana	B	Acetone	50	2.0	50	2.0
Rhoicissus tridentate	L	Acetone	50	4.0	50	1.0
Ximenia caffra	R	Acetone	>100	2.6	>100	7.2
Carisa edulis	R	Acetone	>100	6.5	>100	10.2
		Methanol	>100	6.5	>100	10.2
Terminalia sericea	B	Acetone	25	0	25	0
Combretum erythrophyllum	L	Water	>100	13	>100	13.1
		Methanol	>100	13	>100	13
Rhus rogersii	B	Acetone	50	0	50	0
Peltophorum africanum	B	Acetone	>100	2.6	>100	10.2
Grewia villosa	R	Acetone	>100	7.2	>100	2.6
Piper capense	R	Acetone	100	13.1	100	13
Bridelia micrantha	B	Acetone	25	0	25	1
Lippia javanica	L	Methanol	>100	3.5	>100	3.5
Dyospyros mespiliformis	L	Hexane	100	4.1	100	4.3
Scotia brakepetale	B	Acetone	50	0	50	0
Scherocarya birrea	B	Acetone	>100	10.8	>100	6.2
Asclepias fruticosa	R	Acetone	>100	32	>100	13.9
Allium sativum	T	Water	>100	0.57	>100	3.5
		Methanol	>100	2.5	>100	3
Carica papaya	L	Water	>100	1.73	>100	4.0
		Methanol	>100	4.3	>100	4.0
Solanum panduriforme	L	Water	>100	2.6	>100	6.4
		Methanol	>100	0.6	>100	1.2
Securidaca longepedunculata	R	Acetone	>100	1.2	>100	1.5
Reference drugs						
Rifampicin			0.06	0.01	100	0
Isoniazid			0.06	0.01	4.6	6.11
Ethambutol			2.00	0	12	0
Streptomycin			0.50	0.2	120	2

B, bark; L, leaves; MTB 2, clinical strain resistant to INH, RIF, EMB and STR; SD, standard deviation.

drugs were 0.25–8 μg/mL (streptomycin), 0.03–1 μg/mL (isoniazide), 0.06–2 μg/mL (rifampin), and 0.50–16 μg/mL (ethambutol) in the assays performed with *Mycobacterium tuberculosis* H_{37}Ra. Those used in the clinical *Mycobacterium tuberculosis* strain (resistant to INH, RIF, EMB, and SM) assays were 0.20–100 μg/mL. The test inoculum (100 μL) was added to all test wells. Each microplate was incubated for 5 days at 37 °C in a 5% CO_2 atmosphere in a sealed plastic CO_2-permeable bag (Ziploc; Johnson & Son, Racine, WI). After 5 days of incubation, 32 μL of freshly prepared resazurin solution (Trek Diagnostic, Westlake, OH) was added to one growth-control well. The microplates were incubated again at 37 °C for 24 h. If a color shift from blue to pink was observed in the growth-control sample, 32 μL of resazurin solution was added to each of the remaining wells, and the microplate was further incubated for 24 h. A well-defined pink color was interpreted as positive bacterial growth, whereas a blue color indicated an absence of growth. The MIC corresponded to the greatest dilution of plant extract in which the color shift from blue to pink was not observed. Those extracts with MIC ≤100 μg/mL were considered active (Molina-Salinas et al., 2006).

2.5. Statistical analysis

Analysis was performed using Microsoft Excel 2007. The One way ANOVA test was used to determine if there was any statistically significant difference in the MIC of the extracts and the antibiotics. *p*-Values <0.05 were considered significant.

3. Results and discussion

The increase of resistance to conventional antibiotics by microorganisms has necessitated the search for new, efficient and cost effective ways for the control of infectious diseases (Samie et al., 2005; Ndip et al., 2007). A number of South African plants have been shown to contain antituberculosis activities (Mativandlela et al., 2008; Thring et al., 2007; Lall and Meyer, 1999). However, such studies were based on a very limited number of plant species with the probability of losing species with untaped antimicrobial potentials. In our study, a total of 21 plant extracts were tested for antituberculosis activities. Table 1 presents the botanical names, vernacular names, voucher specimen numbers and their uses in traditional medicine according to the information obtained from traditional healers in Limpopo province, South Africa and published literature. The MIC value of ≤100 μg/mL was chosen as a representative of MTB and MDR-TB susceptibility as this has previously been established (Molina-Salinas et al., 2006). The results of the screening of crude plants extracts for their antimycobacterial activity are presented in Table 2.

Various plants are used in the treatment of TB-related diseases or their symptoms in Limpopo Province, among which *Terminalia*

sericea, *Bridelia micrantha*, *Ziziphus mucronatha*, *Berchemia discolor*, *Scotia brakepetale*, *Rhus rogersii*, *Piper capense*, *Cassia petersiana*, *Rhoicissus tridentate* and *Warbugia salutaris* showed no difference in activity at concentrations $\leq$100 μg/mL against *Mycobacterium tuberculosis* $H_{37}Ra$ and our clinical strain of MTB resistant to isoniazid, rifampicin, ethambutol and streptomycin.

Antimycobacterial compounds from plants may be extracted using different solvents and the type of solvent used determines the success of the isolation process (Masoko et al., 2007). Several studies have shown acetone to be an efficient extractant of diverse bioactive compounds (Eloff, 1998; Kotze and Eloff, 2002; Madamombe and Afolayan, 2003; Asres et al., 2006) that may act in synergy and produce a greater antimycobacterial activity, resulting in high susceptibility patterns with low MIC values observed in this study. Flavonoids and steroids have been extracted using acetone (Eloff, 1998). Plants produce flavonoids in response to microbial infection (Hernandez et al., 2000; Schinor et al., 2007), which may account for their *in vitro* activity against a wide array of microorganisms.

The highest level of antimycobacterial activity was demonstrated by the acetone bark extract of *Berchemia discolor*. There was no difference in MIC values for the clinical isolate that showed resistance to first-line drugs and that observed with the $H_{37}Ra$ (12.5 μg/mL) strain susceptible to first-line drugs. This shows that compounds found in *Berchemia discolor* acted potently and different from the first-line drugs. Prenylated flavonoids have been isolated from the root bark of *Berchemia discolor* (Chin et al., 2006). These flavonoids were shown to be active against hormone-dependant human prostate cancer cells (LNCaP). However, flavonoids have also been shown to be active against *Mycobacterium tuberculosis* (Okunade et al., 2004). They serve as health promoting compound as a results of their ability to scavenge for hydroxyl radicals, and superoxide anion radicals (Ferguson, 2001). These observations support the usefulness of *Berchemia discolor* in folklore remedies in the treatment of infections caused by MTB.

Acetone extracts of *Bridelia micrantha*, *Terminalia sericea*, and *Warbugia salutaris* showed a MIC of 25 μg/mL against the two tested MTB strains. Compounds including friedelin, epi-friedelin and phenolic derivatives such as gallic acid, ellagic acids, anthocyanidin, taraxerol, taraxerone and caffeic acid from the leaves and stem bark of *Bridelia micrantha* have been reported (Pegel and Rogers, 1968). Onunkwo et al. (1996) indicated the presence of flavonoids from *Bridelia ferruginia*. Generally, flavonoids are thought to have antituberculosis properties (Okunade et al., 2004), and are seen to be of potential therapeutic value (Asres and Bucar, 2005). One or more of these fractions may be responsible for MTB inhibition observed in this study.

Compounds from *Terminalia sericea* isolated so far include termilignan B and arjunic acid from the roots of the plant (Eldeen et al., 2008). Ethanol, ethyl acetate and dichloromethane root and bark extracts have been reported to be active against *Mycobacterium aurum* with MIC ranging from 1.56 to 3.12 mg/mL (Eldeen and van Staden, 2007). Our results therefore corroborate the established antimycobacterial properties of this plant. It was reported that muzigadial (Rabe and van Staden, 2000), warburganal (Appleton et al., 1992; Nakanishi and Kubo, 1977), polygodial, isopolygodial, mukaadial, and salutarisolide (Mashimbye et al., 1999) were isolated from *Warbugia salutaris*. These compounds, including muzigadial, all belong to the class of compounds known as drimane sesquiterpenoids. Partially purified compounds from *Warbugia salutaris* have been shown to have inhibitory activity on mycobacterial arylamine N-acetyltransferase (Madikane et al., 2007) thereby inhibiting the growth of MTB.

Four plants, *Ziziphus mucronatha*, *Scotia brakepetale*, *Rhus rogersii* and *Piper capense* presented lower but interesting activities (MIC between 50 and 100 μg/mL). However, the weak activity demonstrated by these extracts *in vitro* to the organism, does not necessarily imply that they would demonstrate weak activities *in vivo*. As with some drugs, some of these plant maybe more potent *in vivo* due to metabolic transformation of their components into highly active intermediates (Ngemenya et al., 2006). Such results provide evidence that these plants might be potential sources of new antibacterial agents even against some resistant strains. Although all the plants showed activity against the tested organisms, there were no major differences when their MICs were compared with each other ($p > 0.05$); but for the antimycobacterial drugs tested, there were major differences ($p < 0.05$).

It was also interesting to note that a number of plants reportedly used to treat TB in Venda, did not demonstrate any antituberculosis activity against the two MTB strains at the concentrations tested. It may be because the plants are used to treat symptoms of the disease rather than the cure of the disease itself. Bever (1986) demonstrated immuno-modulation of chemical compounds from medicinal plants, many of which had been proven inactive or weakly active *in vitro* against pathogens. It has also been reported that individual plants within a species may vary according to a number of factors, including where the plant is growing, temperature, rainfall, season in which the plant is collected, soil type, length of day, altitude and storage conditions (Evans, 1996; WHO, 1992). The extracts might also contain little of the active ingredient (Apak and Olila, 2006). The lack of *in vitro* activity by these plants could be as a result of the aforementioned facts.

Although some of these plant extracts were previously reported to have strong antibacterial and antiviral activities (More et al., 2008; McGaw et al., 2002; Arnold et al., 2002), they showed weak or no activity against MTB in this study. A key target for antimycobacterial chemotherapy is cell wall biosynthesis. Due to the complex lipoglycan calyx on the cell surface, which provide a significant physical barrier to intracellular acting, compounds (Ballell et al., 2005), many antibiotics do not work on MTB. This may explain the lack of activities showed by some of the plant extracts against MTB in this study.

In conclusion, this study highlights some plants which are worthy of further investigation for their antituberculosis activities including *Berchemia discolor*, *Warbugia salutaris*, *Terminalia sericea* and *Bridelia micrantha*. These plants may provide new lead compounds as potential antituberculosis agent; and provide preliminary scientific validation of the traditional medicinal claims of the use of the plants for relieving TB-related symptoms such as cough, chest pains and fever. Isolated compounds from these plants which are already receiving attention in our group may have pronounced inhibitory effects on the test strains.

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References

Andrews, J.R., Shah, N.S., Gandhi, N., Moll, T., Friedland, G., 2007. Multidrug-resistant and extensively drug-resistant tuberculosis: implications for the HIV epidemic and antiretroviral therapy rollout in South Africa. Journal of Infectious Diseases 196, S482–S490.

Apak, L., Olila, D., 2006. The *in-vitro* antibacterial activity of *Annona senegalensis*, *Securidata longepediculata* and *Steganotaenia araliacea*—Ugandan medicinal plants. African Health Sciences 6, 31–35.

Appleton, C.C., Drewes, S.E., Mashimbye, M.J., Cunningham, A.B., 1992. Observations on the molluscicidal properties of (−)-warburganal on the South African *Bulinus africanus* (Planorbidae). Journal of Medical and Applied Malacology 4, 37–40.

Arnold, H., Gulumian, M., 1984. Pharmacopoeia of traditional medicine in Venda. Journal of Ethnopharmacology 12, 35–74.

Arnold, T.H., Prentice, C.A., Hawker, L.C., Snyman, E.E., Tomalin, M., Crouch, N.R., Pottas-Bircher, C., 2002. Medicinal and Magical Plants of Southern Africa: An

Annotated Checklist. Strelitzia 13. National Botanical Institute, Pretoria, South Africa, pp. 1–203.
Aruoma, O.I., Colognato, R., Fontana, I., Gartlon, J., Migliore, L., Koike, K., Coecke, S., Lamy, E., Mersch-Sundermann, V., Laurenza, I., Benzi, L., Yoshino, F., Kobayashi, K., Lee, M.C., 2006. Molecular effects of fermented papaya preparation on oxidative damage, MAP kinase activation and modulation of the benzo[a]pyrene mediated genotoxicity. Biofactors 26, 147–159.
Asres, K., Bucar, F., 2005. Ant-HIV activity against immunodeficiency virus type 1 (HIV-1) and type II (HIV-II) of compounds isolated from the stem bark of *Combretum molle*. Ethiopian Medical Journal 43, 15–20.
Asres, K., Mazumder, A., Bucar, F., 2006. Antibacterial and antifungal activities of extracts of *Combretum molle*. Ethiopian Medical Journal 44, 269–277.
Ballell, L., Field, R.A., Duncan, K., Young, R.J., 2005. New small-molecule synthetic antimycobacterials. Antimicrobial Agents and Chemotherapy 49, 2153–2163.
Bashir, A.K., Ross, M.F., Turner, T.D., 1987. The alkaloids of *Grewia villosa* root. Fitoterapia 58, 141–142.
Bessong, P.O., Rojas, L.B., Obi, L.C., Tshisikawe, P.M., Igunbor, E.O., 2006. Further screening of Venda medicinal plants for activity against HIV type 1 reverse transcriptase and integrase. African Journal of Biotechnology 5, 526–528.
Betti, J.L., 2004. An ethnobotanical study of medicinal plants among the Baka pygmies in the Dja Biosphere reserve, Cameroon. African Study Monograms 25, 1–27.
Bever, B.O., 1986. Anti-infective activity of chemical components of higher plants. In: Medicinal Plants of Tropical West Africa. Cambridge University Press, Cambridge, pp. 68–70.
Blumberg, H.M., Burman, W.J., Chaisson, R.E., Daley, C.L., Etkind, S.C., Friedman, L.N., Fujiwara, P., Grzemska, M., Hopewell, P.C., Iseman, M.D., Jasmer, R.M., Koppaka, V., Menzies, R.I., O'Brien, R.J., Reves, R.R., Reichman, L.B., Simone, P.M., Starke, J.R., Vernon, A.A., 2003. American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America: treatment of tuberculosis. American Journal of Respiratory and Critical Care Medicine 167, 603–662.
Chin, Y.-W., Mdee, L.K., Mbwambo, Z.H., Mi, Q., Chai, H.-B., Cragg, G.M., Swanson, S.M., Kinghorn, A.D., 2006. Prenylated flavonoids from the root bark of *Berchemia discolor*, a Tanzanian Medicinal Plant. Journal of Natural Products 69, 1649–1652.
Chung, G.A.C., Aktar, Z., Jackson, S., Duncan, K., 1995. High-throughput screen for detecting antimycobacterial agents. Antimicrobial Agents and Chemotherapy 39, 2235–2238.
Cragg, G.M., Newman, D.J., Snader, K.M., 1997. Natural products in drug discovery and development. Journal of Natural Products 60, 52–60.
Eldeen, I.M.S., van Staden, J., 2007. Antimycobacterial activity of some trees used in South African traditional medicine. South African Journal of Botany 73, 248–251.
Eldeen, I.M.S., van Heerden, F.R., van Standen, J., 2008. Isolation and biological activities of termilignan B and arjunic acid from *Terminalia sericea* roots. Planta Medica 74, 411–413.
Eloff, J.N., 1998. Which extractant should be used for screening and isolation of antimicrobial components from plants? Journal of Ethnopharmacology 60, 1–8.
Eloff, J.N., 2001. Antibacterial activity of Marula (*Sclerocarya birrea* (A. rich) Hochst. Subsp. *Caffra* (Sond Kokwaro) (Anacardiaceae) bark and leaves. Journal of Ethnopharmacology 76, 305–308.
Etkin, N.L., 1997. Antimalarial plants used by Hausa in Northern Nigeria. Tropical Doctor 27, 12–16.
Evans, W.C., 1996. Trease and Evans' Pharmacognosy, 14th edition. WB Saunders Company Limited, London, pp. 176–177.
Fabry, W., Okemo, P., Ansorg, R., 1996. Activity of East African medicinal plants against *Helicobacter pylori*. Chemotherapy 42, 315–317.
Farnsworth, N.R., Akerele, O., Bingel, A.S., Soejarto, D.D., Guo, Z., 1985. Medicinal plants in therapy. Bulletin of the World Health Organization 63, 965–981.
Ferguson, L.R., 2001. Role of plant polyphenols in genomic stability. Mutation Research 475, 89–111.
Fyhrquist, P., Mwasumbi, L., Haeggstrom, C.A., Vuorela, H., Hiltunen, R., Vuorela, P., 2002. Ethnobotanical and antimicrobial investigation on some species of *Terminalia* and *Combretum* (Combretaceae) growing in Tanzania. Journal of Ethnopharmacology 79, 169–177.
Gandhi, N.R., Moll, A., Sturm, A.W., Pawinski, R., Govender, T., Lalloo, U., Zeller, K., Andrews, J., Friedland, G., 2006. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. Lancet 368, 1575–1580.
Green, E., Obi, C.L., Nchabeleng, M., de Villiers, B.E., Sein, P.P., Letsoalo, T., Hoosen, A.A., Bessong, P.O., Ndip, R.N., 2010. Drug-susceptibility pattern of *Mycobacterium tuberculosis* in Mpumalanga Province, South Africa: possible guiding of retreatment regimen. Journal of Health, Population and Nutrition 28, 7–13.
Green, E., Obi, L.C., Nchabeleng, M., de Villiers, B.E., Sein, P.P., Letsoalo, T., Hoosen, A.A., Bessong, P.O., 2008. Molecular characterization of resistant *Mycobacterium tuberculosis* isolates from Dr George Mukhari Hospital, Pretoria, South Africa. Southern African Journal of Epidemiology and Infection 23, 11–14.
Hernandez, N.E., Tereschuk, M.L., Abdala, L.R., 2000. Antimicrobial activity of flavonoids in medicinal plants from Tafi del Valle (Tucumán, Argentina). Journal of Ethnopharmacology 73, 317–322.
Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A.B., 1996. Zulu Medicinal Plants—An Inventory. University of Natal Press, Pietermaritzburg, South Africa.
Iwalewa, E.O., McGaw, L.J., Naidoo, V., Eloff, J.N., 2007. Inflammation: the foundation of diseases and disorders. A review of phytomedicines of South African origin used to treat pain and inflammatory conditions. African Journal of Biotechnology 6, 2868–2885.
Jadaun, G.P.S., Agarwal, C., Sharma, H., Ahmed, Z., Upadhyay, P., Faujdar, J., Gupta, A.K., Das, R., Gupta, P., Chauhan, D.S., Sharma, V.D., Katoch, V.M., 2007. Determination of ethambutol MICs for *Mycobacterium tuberculosis* and *Mycobacterium avium* isolates by resazurin microtiter assay. Journal of Antimicrobial Chemotherapy 60, 152–155.
Kerharo, J., 1974. Historic and ethnopharmacognostic review on the belief and traditional practices in the treatment of sleeping sickness in West Africa. Bulletin de la Societe de Medecine d'Afrique Noire de Langue Francaise 19, 400.
Khan, M.R., Ndaalio, G., Nkunya, M.H., Wevers, H., Sawhney, A.N., 1980. Studies on African medicinal plant. Part 1. Preliminary screening of medicinal plants for antibacterial activity. Planta Medica 40, 91S–97S.
Kotze, M., Eloff, J.N., 2002. Extraction of antibacterial compounds from *Combretum microphyllum* (Combretaceae). South African Journal of Botany 68, 62–67.
Lall, N., Meyer, J.J.M., 1999. In vitro inhibition of drug-resistant and drug sensitive strains of *Mycobacterium tuberculosis* by ethnobotanically selected South African plants. Journal of Ethnopharmacology 66, 347–354.
Madamombe, I.T., Afolayan, A.J., 2003. Evaluation of antimicrobial activity of extracts from South African *Usnea barbata*. Pharmaceutical Biology 41, 199–202.
Madikane, V.E., Bhakta, S., Russell, A.J., Campbell, W.E., Claridge, T.D.W., Elisha, B.G., Davies, S.G., Smith, P., Sim, E., 2007. Inhibition of mycobacterial arylamine N-acetyltransferase contributes to anti-mycobacterial activity of *Warburgia salutaris*. Bioorganic & Medicinal Chemistry 15, 3579–3586.
Masoko, P., Picard, J., Eloff, J.N., 2007. The antifungal activity of twenty-four Southern African *Combretum* species (Combretaceae). South African Journal of Botany 73, 173–183.
Mativandlela, S.P.N., Meyer, J.J.M., Hussein, A.A., Houghton, P.J., Hamilton, C.J., Lall, N., 2008. Activity against *Mycobacterium smegmatis* and *M. tuberculosis* by extracts of South African medicinal plants. Phytotherapy Research 22, 841–845.
Mashimbye, M.J., Maumela, M.C., Drewes, S.E., 1999. A drimane sesquiterpenoid lactone from *Warburgia salutaris*. Phytochemistry 51, 435–438.
McGaw, L.J., Jäger, A.K., van Staden, J., 2002. Isolation of antibacterial fatty acids from *Schotia brachypetala*. Fitoterapia 73, 431–433.
Mehdipour, S., Yasa, N., Dehghan, G., Khorasani, R., Mohammadirad, A., Rahimi, R., Abdollahi, M., 2006. Antioxidant potentials of Iranian *Carica papaya* juice in vitro and in vivo are comparable to a tocopherol. Phytotherapy Research 20, 591–594.
Molina-Salinas, G.M., Ramos-Guerra, M.C., Vargas-Villarreal, J., Mata-Cárdenas, B.D., Becerril-Montes, P., Said-Fernández, S., 2006. Bactericidal activity of organic extracts from *Flourensia cernua* DC against strains of *Mycobacterium tuberculosis*. Archives of Medical Research 37, 45–49.
More, G., Tshikalangea, T.E., Lall, N., Botha, F., Meyer, J.J.M., 2008. Antimicrobial activity of medicinal plants against oral microorganisms. Journal of Ethnopharmacology 119, 473–477.
Msonthi, J.D., Magombo, D., 1983. Medicinal herbs in Malawi and their uses. Hamdard 26, 94–100.
Nakanishi, K., Kubo, I., 1977. Studies on warburganal, muzigadial and related compounds. Israel Journal of Chemistry 16, 28–31.
Ndip, R.N., Tarkang, A.E.M., Mbullah, S.M., Luma, H.N., Malongue, A., Ndip, L.M., Nyongbela, K., Wirmum, C., Efange, S.M.N., 2007. *In vitro* anti-*Helicobacter pylori* activity of selected medicinal plants from North West Cameroon. Journal of Ethnopharmacology 114, 452–457.
Ngemenya, M.N., Mbah, J.A., Tane, P., Titanji, V.P.K., 2006. Antibacterial effects of some Cameroonian medicinal plants against common pathogenic bacteria. African Journal of Traditional Complimentary and Alternative Medicine 3, 84–93.
Okunade, A.L., Elvin-Lewis, M.P.F., Lewis, W.H., 2004. Natural antimycobacterial metabolites: current status. Phytochemistry 65, 1017–1032.
Onunkwo, G.C., Akah, P.A., Udeala, O.K., 1996. Studies on *Bridelia ferruginea* leaves. Stability and hypoglycemic actions of the leaf extract tablets. Phytotherapy Research 10, 418–420.
Pegel, K.H., Rogers, C.B., 1968. Constituents of *Bridelia micrantha*. Phytochemistry 7, 655–656.
Pereira, M., Tripathy, S., Indamdar, V., Ramesh, K., Bhavsar, M., Date, A., Iyyer, R., Acchammachary, A., Mehendale, S., Risbud, A., 2005. Drug resistance pattern of *Mycobacterium tuberculosis* in seropositive and seronegative HIV-TB patients in Pune, India. Indian Journal for Medical Research 121, 235–239.
Rabe, T., van Staden, J., 2000. Isolation of an antibacterial sesquiterpenoid from *Warburgia salutaris*. Journal of Ethnopharmacology 73, 171–174.
Samie, A., Obi, C.L., Bessong, P.O., Namrita, L., 2005. Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species. African Journal of Biotechnology 4, 1443–1451.
Schinor, E.C., Salvador, M.J., Ito, I.Y., Dias, D.A., 2007. Evaluation of the antimicrobial activity of crude extracts and isolated constituents from *Chresta scapigera*. Brazilian Journal of Microbiology 38, 145–149.
Steenkamp, V., 2003. Traditional herbal remedies used by South African women for gynaecological complaints. Journal of Ethnopharmacology 86, 97–108.
Thring, T.S.A., Weitz, F.M., 2006. Medicinal plant use in the Bredasdorp/Elim region of the southern Overberg in the Western Cape Province of South Africa. Journal of Ethnopharmacology 103, 261–275.
Thring, T.S.A., Springfield, E.P., Weitz, F.M., 2007. Antimicrobial activities of four plant species from the Southern Overberg region of South Africa. African Journal of Biotechnology 6, 1779–1784.
Venter, F., Venter, J.A., 1996. Making the Most of Indigenous Trees. Briza Publications, Pretoria.
Victor, T.C., Streicher, E.M., Kewley, C., et al., 2007. Spread of an emerging *Mycobacterium tuberculosis* drug-resistant strain in the Western Cape of South

Africa. International Journal of Tuberculosis and Lung Diseases 11, 195–201.
Watt, C., Breyer-Brandwyk, M.G., 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. E&S. Livingstone Ltd., Edinburgh and London, p. 449.
World Health Organization, 2000. Global tuberculosis control. WHO report 2000, Geneva, WHO, 2000 (document WHO/CDS/TB/2000.275, http://www.who.int/gtb/publications/globrep00/index.html) (accessed 4 February 2010).
World Health Organization Expect committee on specification for Pharmaceutical preparation, 1992. Quality Assurance of Pharmaceuticals. Thirty-second report. World Health Organization, Geneva. pp. 44–76.
World Health Organization, 2007a. Global Tuberculosis Control: Surveillance, Planning, Financing: WHO Report. WHO/HTM/TB/2007.376. WHO, Geneva.
World Health Organization WHO, 2007b. Towards Universal Access: Scaling Up Priority HIV/AIDS Interventions in the Health Sector; Progress Report, April 2007. WHO, Geneva.
World Health Organization, 2008. Global Tuberculosis Control: Surveillance, Planning and Financing. WHO, Geneva, www.sahealthinfo.org/noveldrug/novelpamphlet.htm.