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ANTIMYCOBACTERIAL EVALUATION OF FIFTEEN MEDICINAL PLANTS IN SOUTH AFRICA**T.J. Mmushi, P. Masoko*, L.K. Mdee, M.P. Mokgotho, L.J. Mampuru, R.L. Howard**

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Abstract

Fifteen plant species were collected from the Nelspruit Botanical Garden based on a list of plants provided by Phytomedicine Programme at the University of Pretoria and their ethnopharmacological information. Hexane, dichloromethane (DCM), acetone and methanolic extracts were screened for antimycobacterial activity against *Mycobacterium smegmatis*. The acetone extract of *Milletia stulhimannii* was the most active, showing activity against *Mycobacterium smegmatis* with minimum inhibitory concentration (MIC) value of 0.13 mg/ml. Acetone extracts for all plants had lower MIC values ranging between 0.11-1.25 mg/ml against *M. smegmatis*. *Milletia stulhimannii*, *Albizia gummifera*, *Xanthocercis zambesiaca* and *Barringtonia racemosa* have shown great potential as anti-tuberculosis agents. They were active against *M. smegmatis* with average MIC values of acetone extracts of 0.13 mg/ml.

Keywords: Tuberculosis, Antimycobacterial activity, *Milletia stulhimannii*, Minimum inhibitory concentration

Introduction

Tuberculosis (TB) is a serious public health challenge of the 21st century and is still one of the most devastating diseases of mankind. It is estimated that there are 1.7 million deaths and up to 9.2 million new clinical cases of TB each year (World Health Organisation, 2008). The recent increase of TB is associated with the increasing infection with the human immunodeficiency virus (HIV) and the rapid spread of multidrug resistant TB strains.

Natural products from plants are proven templates for new drug development (Okunade *et al.*, 2004), and have many interesting biological activities. A wide structural diversity of antimycobacterial compounds has been discovered from plants and other organisms including fungi and marine organisms. Several recent reviews have highlighted the underutilized potential of plant species and natural products as sources of antimycobacterial extracts and chemicals (Gibbons, 2004). Plant-derived antimycobacterial compounds belong to an exceptionally wide diversity of classes, among them alkaloids, terpenoids, coumarins, peptides and phenolics. Thus, medicinal plants remain an important resource to find original active drugs or new therapeutic agents. Over 350 natural products have been evaluated for their antimycobacterial activities (Newton *et al.*, 2002).

Herbal remedies play an essential role in traditional medicine in rural areas of South Africa, where these are often the therapeutic treatment of choice. The preparation of herbal medicine which depends on a cultural context may be obtained from healers as already prepared mixtures, or as unprepared raw materials. Although South Africa possesses a rich tradition in the use of medicinal plants and an outstanding floral diversity estimated at 251 220 species of vascular plants (Cracraft and Grifo, 1999), little research has been done on these plants for antimycobacterial leads which could be used as therapeutic agents.

Since there has been no anti-TB drug introduced in the past 30 years, there is an urgent need to search for and develop new, effective and affordable anti-TB drugs (Gautam *et al.*, 2007). Therefore, in this study we screened 15 plants traditionally used for treatment of TB-related symptoms (frequent coughs, chest ailments, bloody sputum, fever, etc) for bioactivity against tuberculosis.

Materials and Methods

Plant collection and Storage

Fifteen medicinal plants (Table 1) were selected based from a list of plants that were crudely screened by Phytomedicine Programme at the University of Pretoria. Leaves were collected at the Lowveld National Botanical Garden, Mpumalanga, South Africa, transported in sterile sealed, labelled containers to the laboratory where they were separately allowed to dry completely at room temperature. The dried leaves were ground into a fine powder using an electric grinder and stored in airtight containers in a dark place to prevent oxidation until the extraction stage.

Table1: Indigenous medicinal plants selected for antimycobacterial screening.

Selected Medicinal Plants	
Scientific names	Family names
<i>Albizia gummifera</i> (J.F. Gmel.) C.A. Sm.	Mimosaceae
<i>Annona senegalensis</i> Pers.	Annonaceae
<i>Apodytes dimidiata</i> subsp <i>dimidiata</i> E.Mey. ex Arn.	Icacinaceae
<i>Antidesma venosum</i> E Mey. ex Tul.	Euphorbiaceae
<i>Barringtonia racemosa</i> (L.) Roxb.	Lecythidaceae
<i>Kigelia africana</i> (Lam.) Benth.	Bignoniaceae
<i>Kirkia acuminata</i> Oliv.	Simaroubaceae
<i>Macaranga capensis</i> Baill. Benth. ex Sim	Euphorbiaceae
<i>Maytenus senegalensis</i> (Lam.) Excell	Celastraceae
<i>Maytenus udanta</i> (Thunb.) Blakelock	Celastraceae
<i>Millettia stuhlmannii</i> Taub.	Fabaceae
<i>Sclerocarya birrea</i> (A.Rich.) Hochst.	Anacardiaceae
<i>Vangueria infausta</i> subsp <i>infausta</i> Burch.	Rubiaceae
<i>Warburgia salutaris</i> (Bertol. f.) Chiov.	Canellaceae
<i>Xanthocercis zambesiaca</i> (Baker) Dumaz-le Grand.	Fabaceae

Extraction procedure

The crude extracts were prepared according to the method of Kotze and Eloff (2002). Plant materials from each species were individually extracted by weighing 1 g of each finely ground samples and extracted with 10 ml of a different solvent: hexane, dichloromethane (DCM), acetone and methanol in 50 ml Erlenmeyer flasks, respectively. The mixtures were vigorously shaken for 10 mins at high speed. After centrifuging at 959 xg for 10 mins, the supernatants were decanted into pre-weighed 50 ml Erlenmeyer flasks. The extraction process was repeated three times to exhaustively extract the plant material. The solvents were evaporated by air in a fume cupboard at room temperature and the amount of extracts obtained was quantified.

Antibacterial activity assays

Test organisms

The *Mycobacterium smegmatis* was obtained from the School of Molecular and Cell Biology, University of Witwatersrand. The *M. smegmatis* was maintained on Middlebrook 7H9 broth containing 0.05% Tween 80 and 10% (v/v) ADC supplement (Albumin Fraction V, Dextrose and Catalase). The purity of the culture was checked by Ziehl-Neelsen staining before used in the antimicrobial assays.

In vitro broth microdilution screening assay

The antimycobacterial activities of the extracts were performed in 96-well microtiter plates as described by Eloff (1998). Fresh solutions of each crude extract and rifampicin (positive control) were prepared by first dissolving these in acetone to 10 mg/ml; all tests were carried out in triplicate. Two-fold serial dilutions of each extract and rifampicin were made with 100 µl of each; sterile distilled water and 7H9 Middlebrook medium for *M. smegmatis* in 96-well microplates, to yield final concentrations ranging from 2.5 mg/ml to 0.02 mg/ml for the crude extracts and the antibiotic. Rifampicin was included together with untreated controls. The plates were incubated at 37°C overnight. Forty microlitres of 0.2 mg/ml iodinitrotetrazolium chloride (INT) (Sigma-Aldrich) was added to each well and plates were further incubated for 30 mins at 37°C. Bacterial growth in the wells was indicated by a change in colour, whereas clear wells indicated inhibition by the extracts or positive control. Minimum Inhibitory Concentration values were recorded as the lowest concentrations of extracts showing no growth. The assay was repeated twice in triplicates each time.

Total activity of the extracts

The total activity in ml/g was calculated by dividing the quantity extracted from 1 g of plant material (in mg) with the MIC value. The resultant value indicates the volume to which the extract can be diluted and still inhibit the growth of a microorganism (Eloff, 2004).

Results and Discussion

Mass extracted from the leaves of 15 medicinal plants using different solvents of varying polarity (hexane, dichloromethane, acetone and methanol) are shown in Figure 1. Methanol was the best solvent, extracting a greater quantity of plant material from leaves than the other solvents used. Hexane extracted the least amount of material. The extractability of these solvents is consistent with observations made by Masoko et al. (2008).

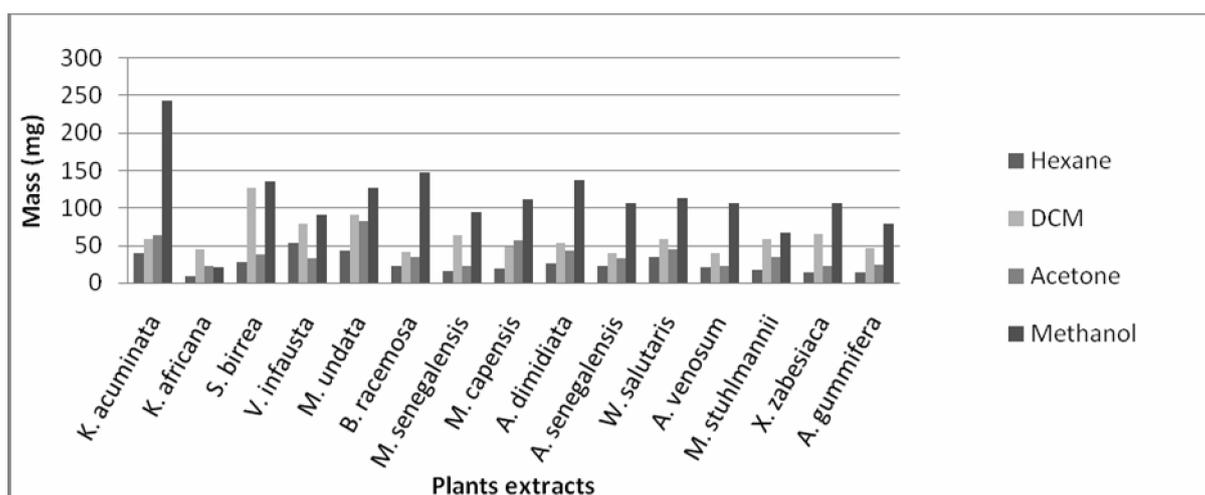


Figure 1: Mass of samples extracted by different solvents with varying polarity from 1 g of starting material.

Table 2: Average MIC (mg/ml) and total activity (ml/g) of selected plant species after 24 hrs incubation at 37°C.

Minimal inhibitory concentration (mg/ml)													
	<i>Albizia gummifera</i>	<i>Kirkia acuminata</i>	<i>Xanthocercis zambesiaca</i>	<i>Milletia stuhlmannii</i>	<i>Barringtonia racemosa</i>	<i>Sclerocarya birrea</i>	<i>Maytenus udanta</i>	<i>Vangueria infausta</i> subsp <i>infausta</i>	<i>Macaranga capensis</i>	<i>Maytenus senegalensis</i>	<i>Apodytes dimidiata</i> subsp <i>dimidiata</i>	<i>Kigelia Africana</i>	<i>Antidesma venosum</i>
Hexane	1.25	0.63	1.667	0.31	0.63	0.523	1.25	1.25	0.63	0.104	1.25	0.63	1.25
DCM	0.31	0.31	1.25	0.31	0.63	0.63	1.25	1.25	0.523	na	1.043	1.667	1.25
Acetone	0.16	0.31	0.106	0.133	0.107	0.260	1.25	0.523	0.260	0.523	0.63	0.63	0.260
Methanol	0.31	0.63	0.63	0.260	0.837	0.417	1.25	1.043	0.417	1.25	na	0.63	0.837
Average	0.51	0.47	0.91	0.25	0.55	0.46	1.25	1.02	0.46	0.63	0.97	0.89	0.90
Total activity (ml/g)													
Hexane	11.2	63.5	8.40	54.8	34.9	53.5	34.4.	43.2	30.2	144.2	20	14.3	16
DCM	151.6	190.3	52	190.3	65.1	200	72.8	62.4	91.8	na	51.8	26.4	31.2
Acetone	150	203.2	207.6	263.2	327.1	146.2	66.4	61.2	219.2	44	68.3	36.5	88.5
Methanol	254.8	387.3	168.3	257.7	176.8	326.1	101.6	87.3	266..2	75.2	na	33.3	126.6
Average	141.9	211.1	109.1	191.5	151.0	181.5	80.3	63.5	113.7	87.8	46.7	27.6	65.6

na = No activity at 2.5 mg/ml

Rifampicin= 125 µg/ml

Table 3: Average minimum inhibitory concentrations values of 4 medicinal plant acetone extracts showing the highest antimicrobial activity against *Mycobacterium smegmatis* after 24 hrs incubation at 37°C.

Medicinal plants	Minimum inhibitory concentration (mg/ml)
<i>Albizia gummifera</i>	0.16
<i>Xanthocercis zambeziaca</i>	0.106
<i>Barringtonia racemosa</i>	0.107
<i>Milletia stuhlmannii</i>	0.133
Average	0.13

The results of the antimicrobial activity are shown in Table 2. *Milletia stuhlmannii* demonstrated the greatest antimicrobial effect as compared to the other plant extracts with MIC values as low as 0.13 mg/ml against *Mycobacterium smegmatis*. The MIC of the positive control was 125 µg/ml against *M. smegmatis*.

Acetone extracts of all plants had lower MIC values ranging between 0.11- 1.25 mg/ml for *M. smegmatis* after 24 hrs (Table 2). Acetone extracts of *Milletia stuhlmannii*, *Albizia gummifera*, *Xanthocercis zambeziaca* and *Barringtonia racemosa* showed promising antimycobacterial activities against *M. smegmatis* with an average MIC value of 0.13 mg/ml (Table 3). The observation that some crude extracts had higher MIC (low potency) may be due to two possibilities. Firstly, positive anti-TB compounds may be partially inhibited by other agents present in the crude extracts (antagonistic effect). Secondly, it could be that the active compounds are present in very low concentrations (Amoo et al., 2009).

Extracts with higher total activity values were considered the best for isolation of potential bioactive compounds. The extracts of *Kirkia acuminata* exhibited the highest total activity, followed by *M. stuhlmannii* when compared to the others (Table 2). Thus, 1 g of *K. acuminata* and *M. stuhlmannii* acetone extract can be diluted to 211.1 ml and 191.5 ml, respectively, with water and still inhibit the growth of *M. smegmatis*.

In conclusion, four plants showed low MIC values (Table 3) and these may have potential anti-TB compounds. Efforts are underway to identify and characterise the active constituents from these plants.

Acknowledgments

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