Ethnobotanical study of some selected medicinal plants used by traditional healers in Limpopo Province (South Africa)

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Abstract

Ten plant species were collected from the Nelspruit Botanical Garden based on their uses by traditional healers in Limpopo Province of South Africa. Hexane, dichloromethane (DCM), acetone and methanolic extracts of this plants were screened for antibacterial activity against *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Escherichiacoli* ATCC 25922. Ampicillin was used as the positive control. Methanol extract of *Kirkia acuminata,* acetone extracts *of Maytenus senegalensis* and *Millettia stuhlmannii* were the most active with average minimum inhibitory concentration (MIC) values of 0.32 to 0.33 mg/ml. *E. faecalis* was the most sensitive microorganism with the average MIC value of 0.25 mg/ml and *S. aureus* was resistant with average MIC value of 1.18 mg/ml. Acetone extracts for all plants had lower MIC values ranging between 0.02 – 0.63 mg/ml. Average total activity, a measure of potency, was highest for acetone and methanol extract of *Maytenus udanta* 1352 and 2022 ml/g respectively and followed by acetone extract of *Maytenus udanta* (1640 ml/g). Most of the selected plants have shown great potential as antibacterial agents.

Keywords: Antibacterial activity, Medicinal plants, Minimum inhibitory concentration, Total activity

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Introduction

Most of the population in urban South Africa, as well as smaller rural communities, relies on herbal medicines for their health care needs. Apart from their cultural significance, this is because herbal medicines are generally more accessible and affordable. Currently there is a problem of microbial drug resistance and there is an increase of opportunistic infections especially with AIDS patients and individuals on immunosuppressive chemotherapy. Many antibacterial, antifungal and antiviral drugs are of limited use due to toxicity, while other viral diseases have not yet found a cure. These problems pose a need of searching more new substances (Mareggesi *et al*., 2008). It is known that many plants especially those used by traditional healers produce pharmaceutically active compounds that have antimicrobial, antihelminthic, antifungal, antiviral, anti-inflammatory and antioxidant activity (McGaw *et al*., 2000). The prescription and use of traditional medicine in South Africa is currently not regulated, with the result that there is always the danger of misadministration, especially of toxic plants. The potential genotoxic effects that follow prolonged use of some of the more popular herbal remedies, are also cause for alarm. (Fennell *et al*., 2004)

The uses of traditional medicine; during last decade, have expanded globally and are gaining popularity. The world health organization (WHO) reported in 2001 that the herbal medicine serve the health need of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries.

Albizia gummifera (Mimosaceae) is geographically distributed from the northern parts of the eastern parts of South Africa and northern Limpopo throughout the tropical countries up into Senegal in the west and Ethiopia in the east of Africa. The stem bark is employed in the preparation of medicines for the treatment of coughs, gonorrhea, and

fever. The plant has also been indicated for use for the treatment of skin diseases, malaria,and stomach pains (Kokwaro, 1976). *Barringtonia racemosa* (Lecythidaceae) is commonly known as powderpuff tree. *Barringtonia racemosa* is the only indigenous species of this genus occurring in South Africa and the plant is found in very humid, moist conditions. It is common along tropical and subtropical coasts in the Indian Ocean, starting at the east coast of South Africa. The seeds, bark, wood and roots contain the poison saponin and is used to stun fish. The bark is also high in tannin content and is frequently used in powdered form for this purpose. Extracts from the plant are effective insectides and are also used medicinally in South Africa; the Zulus use the fruit to treat malaria. The fruits are effective in cough, asthma and diarrhea; the seeds are aromatic and useful in colics and ophthalmia (Nadkarni, 1982).

Kirkia acuminata (Simaroubaceae) with a vernacular name white syringe is distributed throughout Southern Africa and extends from Gauteng, Botswana, Namibia, and to the north in Tanzania. In South Africa the wood is made into furniture and floor blocks. An infusion of the bark is taken against vomiting and abdominal pain. An infusion of the root is taken to treat cough. The fruit sap is applied on wounds and as an antidote on snake bites. Pulverized roots are a remedy for toothache (Heywood *et al*., 2007). *Macaranga capensis* (Euphorbiaceae) is widely distributed throughout Africa. The associated species *Macaranga peltata* and *Macaranga indica* are used for treatment of venereal sores (Kirtikar and Basu, 1984); cuts, wounds, stomach-ache (Jain *et al.,* 2004).

Maytenus senegalensis and *Maytenus udanta* belongs in the spike-thorn family, Celastraceae, a large, cosmopolitan and diverse family of trees, shrubs and woody climbers. The leaves of *M. senegalensis* are used for treatment of diarrhoea and intestinal worms in calf, dog bites (Koné and Kamanzi, 2008); respiratory ailments, tuberculosis (Lall and Meyer, 1999). *Millettia stuhlmannii* (Fabaceae) is found throughout the Congo and southern regions of Africa, Tanzania and Mozambique. The bark from the tree is used for its toxins to stun fish for harvest. It is also used in parts of Africa for mask carving. The plant is often used for treatment of toothache, spleen disorders (Jain, 1991).

Sclerocarya birrea (Anacardiaceae) is commonly known as marula tree. The marula tree is widespread in Africa from Ethiopia in the north to KwaZulu-Natal in the south and is abundant in the Limpopo Province (South Africa). A decoction of the bark treats dysentery, diarrhoea, rheumatism and has a prophylactic effect against malaria. The bark is an excellent remedy for haemorrhoids. Roots and bark are also used as laxatives. A drink made from marula leaves is used for the treatment of gonorrhoea (Van Wyk and Wink (2004). *Vangueria infausta* subsp *infausta* (Rubiaceae) and commonly known as wild medlar. The plant is distributed from the Eastern Cape to Limpopo and North-West (South Africa) and it is common in open, exposed grassland. It is fed to cattle suffering from East Coast Fever, and people take it as a cure for parasitic worm infections in a form of a decoction (De Boer *et al.,* 2005); anthelmintic action (Teichler, 1935); antiplasmodial activity (Nundkumar and Ojewole, 2002).

Xanthocercis zambesiaca (Fabaceae) is commonly known as Nyala tree. The plant is commonly found in Malawi, Mozambique, South Africa, Zambia and Zimbabwe. It is used for diabetic people (Nojima *et al*., 1998).

This study was designed to investigate medicinal plants used by traditional healers in Limpopo (South Africa) for potential antibacterial activity by preliminary bioassay screening. The traditional healers used the selected plants for treatment of symptoms such as wounds, boils, purulent sores and diarrhea among other things. Forty plant extracts were tested for antibacterial activity using the microdilution method (Eloff, 1998) .

Materials and Methods

Plant collection and Storage

Ten medicinal plants (Table 1) were selected based on their use by traditional healers in Limpopo to treat different diseases. 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.

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 different solvents: 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.

Microorganisms used

Staphylococcus aureus ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212 and *Escherichia coli* ATCC 25922 species are the major cause of nosocomial infections in hospitals (Sacho and Schoub, 1993) and are mainly the strains recommended for use by the National Committee for Clinical Laboratory Standards (NCCLS, 1992).

Phytochemical analysis

Chemical constituents of the extracts were analyzed by thin layer chromatography (TLC) using aluminium-backed TLC plates (Merck, silica gel 60 F234). The TLC plates were developed with one of the three eluent systems, i.e., ethyl acetate/methanol/water (40:5.4:5): [EMW] (polar/neutral); chloroform/ethyl acetate/formic acid (5:4:1): [CEF] (intermediate polarity/acidic); benzene/-ethanol/ammonium hydroxide (90:10:1): [BEA] (non-polar/basic) (Kotze and Eloff, 2002). Development of the chromatograms was

done in a closed tank in which the atmosphere had been saturated with the eluent vapour by lining the tank with filter paper wetted with the eluent.

TLC analysis of the extracts

Visible bands were marked under daylight and ultraviolet light (254 and 360 nm, Camac Universal UV lamp TL-600) before spraying with freshly prepared *p*-anisaldehyde (1 ml *p*-anisaldehyde, 18 ml ethanol, 1 ml sulphuric acid) or vanillin (0.1 g vanillin, 28 ml methanol,

1 ml sulphuric acid) spray reagents (Stahl, 1969). The plates were carefully heated at 105ºC for optimal colour development.

Qualitative 2,2 -diphenyl-1-picrylhydrazyl (DPPH) assay on TLC

TLCs were used to separate extracts as described earlier. The plates were dried in the fumehood. To detect antioxidant activity, chromatograms were sprayed with 0.2% 2,2-diphenyl-2-picrylhydrazyl (Sigma®) (DPPH) in methanol, as an indicator (Derby and Margotteaux, 1970). The presence of antioxidant compounds were detected by yellow spots against a purple background on TLC plates sprayed with 0.2% DPPH in methanol.

Quantitative antibacterial activity assay by minimum inhibitory concentration (MIC)

The microplate serial dilution method (Eloff, 1998) was used to determine the minimum inhibitory concentration (MIC) of extracts against *S. aureus, P. aeruginosa*, *E. faecalis* and *E. coli*. Extracts (10 mg/ml) were dissolved in acetone and serially diluted with sterile water in microplates in a laminar flow cabinet. The same volume of an actively growing culture of the test bacteria was added to the different wells and cultures were grown overnight in 100% relative humidity at 37^{o}C. As an indicator of growth, 40 µl of 0.2 mg/ml of *p*-iodonitrotetrazolium violet (Sigma®) (INT) dissolved in water was added to each of the microplate wells. Growth was indicated by a violet colour of the culture. The lowest concentration of the test solution that led to an inhibition of growth was taken

as the MIC. The negative control acetone had no influence on the growth at the highest concentration used (25%). Ampicillin was used as positive controls.

Densities of bacterial cultures for use in the screening procedures were as follows: *S. aureus*, 2.6×10^{12} cfu/ml; *E. faecalis*, 1.5×10^{10} cfu/ml; *P. aeruginosa*, 5.2×10^{13} cfu/ml; *E. coli*, 3.0×10^{11} cfu/ml.

Total activity of the extracts

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

Results and discussion

Leaves samples of 10 plants used by Limpopo Province people for various ailments that are normally caused by bacterial infection were collected Table 1 from Lowveld National Botanical Garden. Hexane, dichloromethane, acetone and methanol extracts were obtained. A total of 40 extracts were tested against four bacterial strains, two Gram-positive and two Gram-negative. Methanol was the best extractant giving the highest mass of extracts while n-hexane yielded the lowest mass of extracts. The masses of acetone extracts were second highest to methanol for all the plant species extracted (results not shown). These results are comparable with data reported by Eloff (1999) in a study involving the biological activity of 27 different members of the Combretaceae family.

The majority of traditional healers use water to extract active compounds from these plants, because water is not harmful to domestic animals and humans and is generally the only extractant available, consequently the prepared drug would not contain all the pharmacologically active compounds. Successful isolation of compounds from plant material is largely dependent on the type of solvent used in the extraction procedure. Use of water alone leads to difficulties in extracting non-polar active compounds.

After evaporation of extracting solvents (hexane, DCM, acetone and ethanol) extracts were redissolved in acetone because this solvent was found not to be harmful towards bacteria and fungi (Eloff *et al.,* 2007).

Table1. Indigenous medicinal plants selected for antibacterial screening

Selected Medicinal Plants	
Scientific names	**Family names**
Albizia gummifera (J.F. Gmel.) C.A. Sm.	Mimosaceae
Barringtonia racemosa (L.) Roxb.	Lecythidaceae
Kirkia acuminata Oliv.	Simaroubaceae
Macaranga capensis Baill. Benth. ex Sim	Euphorbiaceae
Maytenus senegalensis (Lam.) Excell	Celastraceae
Maytenus udanta (Thunb.) Blakelock	Celastraceae
Millettia stuhlmannii Taub.	Fabaceae
Sclerocarya birrea (A.Rich.) Hochst.	Anacardiaceae
Vangueria infausta subsp *infausta* Burch.	Rubiaceae
Xanthocercis zambesiaca (Baker) Dumaz-le Grand.	Fabaceae

The results of the antimicrobial activity are shown in table 2. *Enterococcus faecalis* was observed to be the most sensitive microorganism with average MIC value of 0.25 mg/ml followed by *E. coli* (0.71 mg/ml) and *P. aeruginosa* (0.73 mg/ml) and *S. aureus* was resistant with the average MIC value of 1.18 mg/ml. All extract of *Maytenus senegalensis* and *Maytenus udanta* were active against *E. faecalis* with MIC values of 0.02 mg/ml and 0.04 mg/ml respectively. *M. senegalensis is used to* treat a variety of ailments like pneumonia, tuberculosis, sore throat, earache, eye infections, rheumatism and venereal diseases (Watt and Breyer-Brandwijk, 1962 and Van Wyk *et al*., 2002). The roots are mostly used in preparing traditional remedies. Leaves and stem-bark are also used, but less frequently. In this study leaves were used. The bioactivity in *Maytenus* species has been attributed to various groups of secondary metabolites such

as triterpenes and several types of alkaloids (Hutchings *et al.,* 1996). The bioactivity can be due to these secondary metabolites.

Low MIC values were also observed in all extracts of *Sclerocarya birrea* and *Vangueria infausta* subsp *infausta*, which were 0.02 mg/ml against *E. faecalis*. Eloff (2001) has also reported good bioactivity of bark and leaves of *S. birrea* extracted with acetone, using a microplate serial dilution technique with *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli* and *Enterococcus faecalis* as test organisms. All extracts were active with MIC values from 0.15 to 1.25 mg/ml.

Low MIC values of some of the plant extract on different bacterial strains explains their broad spectrum activity while most of the plant extracts found to have good activity on one organism may be due to their narrow spectrum of activity. Previous studies on these plants phytochemical analysis show that these plants have diverse compounds (Eloff, 2001; Van Wyk *et al*., 2002; Watt and Breyer-Brandwijk, 1962). There are number of factors which contribute to this, i.e. area where the plants are collected and climate changes but in this study we tried to exclude these factors by collecting around the same area with almost similar conditions. The preliminary results of the present study, therefore, not only confirms the justifiable use of some of the plants against these micro-organisms in the traditional health care system but also reflects the hope for development of effective chemotherapeutic agents in the future from same or similar plants.

Table 2. Minimum inhibitory concentration of selected plant species after 24 h incubation at 37°C

Plant species	Extractants	MIC values (mg/ml)				
		E. coli	***E. faecalis***	***S. aureus***	***P. aeruginosa***	**Average**
Albizia gummifera (J.F. Gmel.) C.A. Sm.	Hexane	n/a	1.25	2.5	n/a	**1.88**
	DCM	0.63	1.25	2.5	0.31	**1.17**
	Acetone	0.63	0.16	0.63	0.31	**0.43**
	Methanol	0.84	0.16	0.84	n/a	**0.61**
Barringtonia racemosa (L.) Roxb.	Hexane	1.25	0.63	1.25	0.52	**0.91**
	DCM	0.84	0.63	1.13	0.31	**0.73**
	Acetone	0.63	0.16	1.67	0.05	**0.63**

	Methanol	1.04	0.31	2.5	0.31	**1.04**
Kirkia acuminata Oliv.	Hexane	n/a	0.21	n/a	n/a	**0.21**
	DCM	0.31	0.05	1.04	n/a	**0.47**
	Acetone	n/a	0.11	1.25	1.25	**0.87**
	Methanol	0.31	0.02	0.63	n/a	**0.32**
Macaranga capensis Baill. Benth. ex Sim	Hexane	1.04	0.04	n/a	n/a	**0.54**
	DCM	0.63	0.26	0.31	n/a	**0.40**
	Acetone	0.26	0.31	0.31	1.25	**0.53**
	Methanol	1.25	0.31	0.31	n/a	**0.62**
Maytenus senegalensis (Lam.) Excell	Hexane	n/a	0.06	n/a	n/a	**0.06**
	DCM	1.25	0.02	n/a	n/a	**0.64**
	Acetone	0.31	0.02	0.63	0.31	**0.32**
	Methanol	1.25	0.02	n/a	n/a	**0.64**
Maytenus udanta (Thunb.) Blakelock	Hexane	n/a	0.04	0.63	0.63	**0.43**
	DCM	n/a	0.04	0.63	0.63	**0.43**
	Acetone	0.63	0.04	0.26	1.04	**0.49**
	Methanol	1.67	0.04	0.52	1.04	**0.82**
Millettia stuhlmannii Taub.	Hexane	0.63	0.63	1.67	0.31	**0.81**
	DCM	0.21	0.63	2.08	0.31	**0.81**
	Acetone	0.16	0.26	0.63	0.26	**0.33**
	Methanol	0.21	0.13	0.63	0.31	**0.32**
Sclerocarya birrea (A.Rich.) Hochst.	Hexane	n/a	0.02	n/a	n/a	**0.02**
	DCM	1.04	0.02	n/a	0.16	**0.41**
	Acetone	n/a	0.02	1.25	1.25	**0.84**
	Methanol	n/a	0.02	1.67	1.25	**0.98**
Vangueria infausta subsp *infausta* Burch.	Hexane	n/a	0.02	n/a	n/a	**0.02**
	DCM	0.42	0.02	n/a	n/a	**0.22**
	Acetone	0.16	0.02	n/a	0.63	**0.27**
	Methanol	1.25	0.02	n/a	1.25	**0.84**
Xanthocercis zambesiaca (Baker) Dumaz-le Grand.	Hexane	0.84	n/a	1.67	1.25	**1.25**
	DCM	0.63	1.04	2.5	1.67	**1.46**
	Acetone	n/a	0.63	1.04	n/a	**0.84**
	Methanol	0.31	0.31	1.67	1.67	**0.99**
Average		**0.71**	**0.25**	**1.18**	**0.73**	
Ampicillin (µg/ml)		0.16	0.16	0.08	0.13	

n/a -not available

The quantity of antibacterial compounds present was also determined as shown in Table 3. To determine which extract can be used for further testing and isolation, not only the MIC value is important, but also the total activity. Because the MIC value is inversely related to the quantity of antibacterial compounds present, an arbitrary measure of the quantity of antibacterial compounds present was calculated by dividing the quantity extracted in milligrams from 1 g leaf by the MIC value in mg/ml. This value indicates the volume to which the biologically active compound present in 1 g of the dried plant material can be diluted and still kill the bacteria (Eloff, 2004). Extracts with higher values were considered the best to work with. From Table 3, substantial total activity was observed against *E. faecalis* followed by *P. aeruginosa* after 24 h; *S. aureus* was relatively resistant. Average total activity, a measure of potency, was highest for methanol and dichloromethane extracts of *Maytenus udanta,* 2022 and 1640 ml/g respectively and followed by methanol extract of *Kirkia acuminata* (967 ml/g) and lowest was hexane extract of *Xanthocercis zambesiaca* (47 ml/g).

Table 3. Total activity in ml/g of selected plants extracts after 24 h incubation at 37^{o}C

Plant species	Extractants	Total activity (ml/g)				
		E. coli	*E. faecalis*	*S. aureus*	*P. aeruginosa*	Average
Albizia gummifera (J.F. Gmel.) C.A. Sm.	Hexane		58	29		**44**
	DCM	218	110	55	444	**203**
	Acetone	120	473	120	244	**279**
	Methanol	201	1057	201		**629**
Barringtonia racemosa (L.) Roxb.	Hexane	64	128	64	158	**117**
	DCM	342	342	191	696	**410**
	Acetone	147	580	55	1858	**831**
	Methanol	229	770	95	770	**545**
Kirkia acuminata Oliv.	Hexane		405			**405**
	DCM	235	1377	70		**724**
	Acetone		1323	113	113	**516**
	Methanol	1965		967		**967**
Macaranga capensis Baill. Benth. ex Sim	Hexane	24	625			**625**
	DCM	141	342	287		**315**
	Acetone	419	352	352	87	**264**
	Methanol	157	632	632		**632**

Maytenus senegalensis (Lam.) Excell	Hexane		394			**394**
	DCM	53				
	Acetone	155		76	155	**116**
	Methanol	146				
Maytenus udanta (Thunb.) Blakelock	Hexane		2472	156	156	**928**
	DCM		4365	277	277	**1640**
	Acetone	216	3402	523	130	**1352**
	Methanol	130	5440	418	209	**2022**
Millettia stuhlmannii Taub.	Hexane	113	113	42	230	**128**
	DCM	655	218	67	444	**243**
	Acetone	551	339	140	339	**273**
	Methanol	753	510	251	510	**424**
Sclerocarya birrea (A.Rich.) Hochst.	Hexane					
	DCM	93			606	**606**
	Acetone			65	648	**357**
	Methanol			197	262	**230**
Vangueria infausta subsp *infausta* Burch.	Hexane					
	DCM	444				
	Acetone	531			135	**135**
	Methanol	154			154	**154**
Xanthocercis zambesiaca (Baker) Dumaz-le Grand.	Hexane	80		40	54	**47**
	DCM	309	189	77	116	**127**
	Acetone		146	89		**118**
	Methanol	669	669	124	124	**306**
Average		**321**	**994**	**199**	**357**	

The main problem with traditional healers is preparation of treatment using these plants. All of them use more than one plant to prepare treatment and there is no systematic approach in doing that. Some use spoon while some just add until they feel it's enough, which affect the quality of the remedy. During interaction with traditional healers it was noted that some of the plants they add are very old and seasonal variations is not taken care of and lastly handling is not the issue. In this study we are trying to also assist healers on effect of factors mentioned as that affect the quality. The low quality of herbal products may be due to several factors. These include factors that are difficult to control such as the time and methods of collection of raw materials, processing methods and complex heterogeneity of plant secondary metabolites, and unpredicted consequences when herbs are mixed with western medicines as well as a general lack of scientific validation (McIntyre, 1998).

High demand of traditional medicine is a concern. Researchers and clinicians are usually concerned about safety, effectiveness and consistency of herbal mixtures and simple preparations. Results obtained need further investigations. Bioassay guided fractionation of the constituents of the most promising plants as well as acute toxicity studies are needed in order to conclude on their activity. We are currently working on the promising plants for further analysis.

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