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Full Length Research Paper

Activity profiles of fourteen selected medicinal plants from Rural Venda communities in South Africa against fifteen clinical bacterial species

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Fourteen plants used in traditional medicine in the Venda region of South Africa were screened for activity against fifteen bacterial species. Methanol, acetone and hexane extracts and in some cases essential oils were tested using the disc diffusion and the microdilution methods. Most of the extracts were active against at least one bacterial species. Methanol and acetone extracts were the most active while Gram positive bacteria were the most sensitive as compared to Gram negative bacteria. This study has revealed the strong *in vitro* activity of *Syzigium cordatum*, *Peltophorum africanum*, *Rhoicissus tridentata, Bridelia micrantha* and *Ximenia caffra* against Gram positive and Gram negative bacteria. Essential oils of *Lippia javanica* was also effective against most of the bacterial species studied. However, *Pouzolzia mixta* and *Mucuna coriaceae* showed less activity. Some plants were more active than commercial antibiotics. This study is the first to test the activity of the selected plants from the Venda region against such number of bacterial isolates and justifies their use by local traditional healers. The identification of the active components of the plants and the determination of the effect of these plants on the immune system will give more information on their activity. Finally, these results may be of importance in identifying candidate plants and essential oils for eventual drug design and other therapeutic purposes, respectively.

Key words: Bacteria, Disc diffusion, Medicinal plants, Microdilution, South Africa, Venda.

INTRODUCTION

During the last decade, infectious diseases and particularly infectious diarrhoea have threatened the life of millions of people around the world (Ashbolt, 2004). It is estimated that one in five children die before his fifth birth day due to diarrhea in developing countries (Haque et al., 2003). This situation has been compounded by the increase in the development of antimicrobial resistance by different microorganisms including bacteria and parasites, and the high cost of current antimicrobials. The development of novel, efficient and inexpensive drugs is thus of great importance.

For centuries, medicinal plants have been used all over the world for the treatment and prevention of various ailments, particularly in developing countries where infectious diseases are endemic and modern health facilities and services are inadequate. Elsewhere, many potent drugs have been purified from medicinal plants including anti-malarial, anti-cancer, anti-diabetic and antibacterial compounds. In Africa, traditional medicine is of great value and more than 70% of the population refers to traditional healers concerning health issues (Kamanzy et al., 2002). In South Africa, traditional medicine is well recognized and different communities use a wide variety of plants to treat gastrointestinal disorders such as diarrhea and infection by intestinal parasites, which are particularly prevalent in rural areas (McGaw et al., 2000). The Venda region of South Africa, situated in the far North of the country, has a very strong tradition

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Table 1. Ethnobotanical information of selected Venda medicinal plants used in the study.

Scientific names (Family)	Common names	Plant part used	Traditionally used to treat:	Voucher specimen #
Annona senegalensis Pers. (Annonaceae)	Muembe (V) Wild Custard Apple (E)	Essential oils from leaves	Venereal disease, Diarrhoea, dysentery (McGaw et al, 2000)	AS 11
Peltophorum africanum Sond (Fabaceae)	African Wattlle (E) Musese (V)	Bark and root	Tuberculosis, stomach complains, intestinal parasites (Mabogo, 1990)	BP01
Elaeodendron transvaalensis Burtt (Celastraceae)	Mulumanama (V) Bushveld saffron (E)	Root	Fungal infections, Stomach disorders, Stomach ulcers, Fungal infections, Venereal diseases.	BP05
Androstachys johnsonii (Euphorbiaceae)	Musimbiri (V) Ironwood, (E)	Root, bark, leaves	Stomach problems	AS 21
Bridelia micrantha Baill (Euphorbiaceae)	Munzere (V) Mitzeerie (E)	Root, bark seed	Stomach aches, tapeworms, diarrhoea, headaches, sore joints, sore eyes, venereal diseases, and fevers	BP 03
Sida alba Forrsk (Malvacea)		Leaves	Diarrhoea and dysentery	AS 12
Cissampelos torulosa (Menispermaceae)	Lukandulula (V)	Leaves plant	Diarrhoea and dysentery, sore throat (Mabogo, 1990)	AS 13
Syzygium cordatum (Myrtaceae)	Mutu (V) Water berry (E)	Bark and leaves	Stomach troubles, cold and fever, babies food, diarrhoea wounds (Mabogo, 1990)	AS 14
Ximenia caffra Sond (Olacaceae)	Mutshili (V) Sourplum (E)	Leaves and root	Diarrhoea and dysentery, fever, cough, venereal diseases (Mabogo, 1990)	AS 15
Mucuna coriacea baker (Fabaceae)	Mulada (V)	Root	Tooth ache	BP 02
Zornia milneana (Papilionoideae)	Lukandulula (V)	Whole plant	Dysentery and diarrhoea.	AS 16
Pouzolzia mixta (Urticaceae)	Soap Nettle (E) Muthanzwa (V)	Root, stem and Leaves	Diarrhoea, dysentery, general body health (Mabogo, 1990)	AS 17
Lippia javanica (Verbenaceae)	Musudzungwane (V) fever tea (E)	Leaves	Asthma, malaria, diarrhoea	AS 19
Rhoicissus tridentata Wild & Drum (Vitaceae)	Murumbulashedo(V) Bitter grape (E)	Root, tubers and fruits	To prevent miscarriages, Diarrhea (Mabogo, 1990)	AS18

E, English; V, Venda (South African language).

of medicinal plants which are very commonly used by the population, particularly those in rural areas. Dosage and the method of preparation and administration are very important. The Vhavendas in the Venda region most often prepare a decoction of the plant part in soft porridge (Arnold and Gulumian, 1984). For example, babies are generally given a soft porridge made from maize flour mixed with a number of medicinal plants called “tshiunza” (Mabogo, 1990). This preparation is believed to eliminate enteropathogens in case of diarrhoea. The immune system of the child is also expected to be strengthened by this preparation. The part of the plant used varies among species and traditional healers and also depends on the nature and state of the disease (Mabogo, 1990).

In this study, the antibacterial activity of 50 extracts and essential oils of 14 medicinal plants used by the Vhavendas of South Africa to cure diarrhoea and fever, amongst other diseases, was determined and compared to that of commonly used antibiotics.

MATERIALS AND METHODS

Plant collection

Plants used by local population to cure different ailments such as diarrhoea, stomach ache, malaria or as prophylaxis against diarrhea and malaria were collected with the help of botanists and two traditional healers. Appropriate plant parts were collected during summer between November 2003 and February 2004 from Mbaye, Makwarela, Thohoyandou and Mulima, in the Venda region, South Africa. The plants were identified by Mr. P. Tshisikhawe of the Department of Biological sciences, University of Venda. Voucher specimens have been deposited at the herbarium of the

Thohoyandou Botanical garden. The fourteen plants and parts used as well as other ethno medicinal information is shown in Table 1.

Preparation of extracts and essential oils

Plant material was washed with distilled water and air dried in the laboratory for two weeks and ground in a Wiley grinder with a 2 mm wire mesh. 50 g of each ground material was soaked in 500 ml of methanol, acetone or hexane for at least 72 h with frequent shakings. The samples were then suction filtered through Whattman No.1 filter paper. The filtrate was evaporated to dryness under reduced pressure at 40^{o}C. A stock solution of 0.2 g/ml in dimethyl sulfoxide (DMSO) was made for each extract. Essential oils were prepared from fresh leaves of *Lippia javanica* and *Annona senegalensis* by hydrodistillation for 3 h using a Cleveland type apparatus. All the extracts and essential oils were kept at 4^{o}C in the dark until used.

Microorganisms

The plants extracts were tested against fifteen clinical bacterial species obtained from the Department of Microbiology and the Department of Biological Sciences, University of Venda. These included five Gram positive bacteria: *Bacillus cereus, Bacillus pumilus, Bacillus subtilis, Staphylococcus aureus, Enterococcus fecalis*; and ten Gram negative bacteria: *Enterobacter cloacae, Escherichia coli, Pantoea agglomerans, Pseudomonas aeruginosa, Shigella flexneri, Aeromonas hydrophila, Proteus mirabilis, Klebsiella pneumoniae, Salmonella cholerae-suis* and *Serratia marcescens*. The bacterial strains were maintained on nutrient agar and subcultured every three days. An inoculum of each bacterial strain was suspended in 5 ml of Mueller Hinton broth and incubated overnight at 37^{o}C. The overnight cultures were diluted with Mueller Hinton Broth and adjusted to give a concentration of bacterial cells equivalent to a McFarland No. 0.5 standard prior to the antibacterial testing.

Cytotoxicity assays

A solution of dimethyl sulfoxide (DMSO, Merck, Germany) was used for the dilution o the plants extracts. Thus the effect of DMSO on the microorganisms was determined using the disc diffusion and the micro dilution method. Different concentrations of the DMSO ranging from 0.1 to 25% were tested against all the bacterial species in the microdilution method using the Mueller Hinton broth.

Antimicrobial evaluation of medicinal plants by the disc diffusion method

The disc diffusion method was used as described previously (Nostro et al., 2000). Briefly, Mueller Hinton Agar (MHA) was supplemented with 0.01% Tween 80 to enhance the solubilisation of oils and extract. 100 µl of an 18 h old culture of each test organism was spread on the agar using a multipoint applicator and the plate was left for 30 min in order to dry. Whatman paper was used to prepare discs of 6 mm diameter and sterilized by autoclaving. The blank sterile discs were deposited on top of the seeded MHA and 15 µl (3 mg) of each extract or essential oil was added on top of the disc. The plate was incubated at 37^{o}C for 24 h. All experiments were run in quadruplets using 10 µl of a 50 mg/ml gentamycin as a positive control and 15 µl (6%) of DMSO as negative control. We had determined that DMSO had no effect on bacterial growth below 15%. Each test was repeated four times and the antibacterial activity was expressed as the mean of diameter of the inhibition zones (mm) produced by the plant extracts.

Determination of minimum inhibitory concentration by the microdilution method

The microdilution method was used to determine the MIC of the crude plant extracts and essential oils. Serial dilutions of the extracts and essential oils were made in microtiter wells with Mueller–Hinton broth (0.01% Tween 80) to obtain at the end of experiment a concentration ranging from 0.08 to 12 mg/ml and a final concentration of DMSO of 2.5%. A McFarland No.1 standard suspension of test bacteria was made in Mueller–Hinton broth, from which 100 µl of the final inoculum containing approximately 1.5x10^{6} colony forming units (cfu) was added to the appropriate wells to a final volume of 200 µl. Inoculated plates were incubated at 37°C for 24 h. One hour before the end of incubation 40 µl of a 0.2% solution of Iodo-Nitro Tetrazolium (INT) (Merck, Germany) was added to the wells and the plate was incubated for another hour. Since the colourless tetrazolium salt is reduced to a red coloured product by biologically active organisms, the inhibition of growth can be detected when the solution in the well remains clear after incubation with INT. The assay was run three times. The lowest concentration of each extract showing no visible growth was recorded as the minimum inhibitory concentration (MIC). Gentamicin was used as the positive control while the negative control comprised the test bacteria with DMSO.

Sensitivity to standard antibiotic discs

Nine commercial antibiotics were also tested against all the organisms for comparison using the Kirby Bauer method. Standard antibiotic discs (Oxoid, Hampshire, England) were used as indicated by the manufacturer and included: erythromycin (30 µg), ampicilin (25 µg), ofloxacin (5 µg), meropenem (10 µg), cloramphenicol (30 µg), nalidixic acid (30 µg), kanamycin (30 µg), tetracyclin (30 µg) and gentamycin (30 µg) using the Mueller Hinton Agar and the diameter of the inhibition zones for each disc was recorded (mm).

RESULTS

The disk diffusion and the micro dilution methods were used to test the activity of thirty-nine extracts from fourteen plants and nine commercial antibiotics against fifteen bacterial species and the minimum inhibitory concentration, respectively.

Disc diffusion assay

The acetone extract of the leaves of *Pouzolzia mixta* was the most active with a diameter of zone of inhibition of 39 mm against *Proteus mirabilis*. Little or no activity was observed for al the other extracts of this plant. The acetone extract of the bark of *Syzygium cordatum* gave a diameter of zone of inhibition of 22 mm against *Staphylococcus aureus*, 19 mm against *Bacillus subtilis* and 18 mm against each of *Enterococcus fecalis*, *Enterobacter cloacae* and *Proteus mirabilis*. The methanol extract of the whole plant of *Zornia milneana*

Table 2. Antibacterial activity of fourteen medicinal plants against fifteen bacterial species of medical importance determined by the disc diffusion method. Activity indicated by the diameter of the zone of inhibition of growth in mm.

Plant	Part used and solvent	Zones of inhibition of growth (mm, diameter)														
		Bc	***Bp***	***Bs***	***Sa***	***Ef***	***Et***	***Ec***	***Pa***	***Ps***	***Sf***	***Ah***	***Pm***	***Kp***	***Sc***	***Sm***
X. caffra	Leaves, acetone	9	8	10	12	10	8	10	10	8	10	0	0	0	0	8
	Roots, acetone	12	10	10	10	14	8	10	10	8	8	8	10	0	0	8
B. micrantha	Roots, methanol	9	8	10	12	8	10	9	8	9	10	nd	10	0	0	8
	Bark, methanol	8	10	14	12	10	10	10	10	14	10	10	10	0	0	10
	Seeds, acetone	8	0	8	8	8	0	8	8	11	0	0	0	0	0	0
S. cordatum	Bark, acetone	10	18	19	22	18	18	14	12	14	14	10	18	8	14	12
	Bark, methanol	10	12	8	8	10	8	10	10	10	14	18	14	0	8	8
	Leaves, acetone	nd	nd	nd	nd	nd	17	11	nd	12	nd	nd	12	nd	nd	nd
	Leaves methanol	10	14	10	8	12	10	10	14	10	14	18	10	0	8	10
	Leaves, hexane	8	0	0	8	0	0	0	0	0	0	0	0	0	8	0
P. mixta	Roots, methanol	0	0	8	0	8	0	8	0	0	8	8	8	0	0	0
	Stem, acetone	8	0	8	0	8	8	9	0	8	8	0	8	0	0	0
	Stem methanol	0	8	0	0	8	0	0	0	0	0	0	0	0	0	0
	Leaves, acetone	0	0	0	8	0	0	0	8	0	0	0	39	0	0	0
	Leaves, methanol	8	0	0	0	0	0	0	8	0	0	0	0	0	8	0
C. torulosa	Leaves, methanol	8	10	0	10	8	8	8	8	0	10	18	10	0	0	0
	Leaves, acetone						0	0		0			0			
	Leaves, hexane	9	0	8	0	0	0	0	0	0	0	0	8	0	0	0
Z. milneana	Whole plant, acetone	8	8	10	10	14	10	10	8	0	8	0	0	0	8	0
	methanol	18	8	11	18	14	8	10	8	0	8	0	8	0	8	0
	hexane	8	0	8	0	8	0	0	0	8	0	0	0	0	nd	0
L. javanica	Leaves, methanol	8	10	8	8	10	8	10	8	0	10	10	10	0	8	0
	Leaves, acetone	14	10	10	14	10	8	8	10	8	10	10	10	12	8	8
	Essential oil	14	8	8	14	8	8	8	10	0	10	0	8	8	10	0
	Leaves, hexane	8	8	9	8	8	0	0	10	0	0	0	0	0	0	0
A. senegalense	Leaves, oil	22	8	8	10	8	8	8	0	0	0	0	0	0	0	0
P. africanum	Roots. methanol	8	12	14	12	8	10	12	8	16	8	10	10	0	0	8
	Bark, methanol	10	14	18	14	14	10	10	8	8	10	10	9	0	0	8
M. coreaceae	Roots, methanol	8	0	0	8	8	8	0	0	0	0	0	0	0	8	0
S. alba	Leaves, acetone	8	0	0	8	8	0	0	8	0	10	10	10	0	0	0
	Leaves, methanol	8	8	8		8	8	10	10	0	14	18	0	0	0	8
R. tridentata	Fruits, methanol	8	8	8	8	10	8	8	0	0	0	0	0	0	0	0
	Roots, methanol	12	8	14	10	10	10	10	14	14	nd	10	10	0	8	10
	Tubers, methanol	10	10	12	10	12	10	10	12	10	8	0	10	14	0	8
E.transvaalensis	Roots, methanol	8	8	10	8	10	8	8	10	8	0	8	8	0	8	0
A. johnsonii	Roots, acetone	nd	8	8	8	8	0	0	nd	0	nd	nd	0	nd	nd	nd
	Leaves, acetone	nd	10	10	10	14	10	8	nd	8	nd	nd	9	nd	nd	nd
	Bark, acetone	nd	8	8	8	8	0	0	nd	0	nd	nd	0	nd	nd	nd
Gentamicin		29	30	30	30	30	29	29	28	19	30	30	30	18	28	18

Bacillus cereus (Bc), Bacillus pumilus (Bp), Bacillus subtilis(Bs), Staphylococcus aureus (Sa), Enterococcus fecalis (Ef), Enterobacter cloacae (Et), Escherichia coli (Ec), Pantoea agglomerans (Pa), Pseudomonas aeruginosa(Ps), Shigella flexneri (Sf), Aeromonas hydrophila (Ah), Proteus mirabilis (Pm), Klebsiella pneumoniae(Kp), Salmonella cholerae-suis(Sc), Serratia marcescens (Sm).
nd= Not done.

Table 3. Minimum Inhibitory Concentrations of different plants extracts on bacterial isolates using the micro dilution method (mg/ml).

Plants	Part used and solvent	Minimum inhibitory concentration (mg/ml)														
		Bc	***Bp***	***Bs***	***Sa***	***Ef***	***Et***	***Ec***	***Pa***	***Ps***	***Sf***	***Ah***	***Pm***	***Kp***	***Sc***	***Sm***
X. caffra	Leaves, acetone	6	6	6	6	3	6	6	6	6	3	6	6	6	6	3
	Roots, acetone	1.5	6	3	3	1.5	6	3	1.5	12	1.5	3	6	3	6	3
B. micrantha	Roots, methanol	>12	6	6	3	3	12	6	>12	>12	1.5	12	>12	12	>12	12
	Barck, methanol	3	3	3	3	3	6	3	6	6	1.5	12	6	12	>12	6
	Seeds, acetone	3	12	>12	6	6	>12	3	12	>12	1.5	12	>12	6	>12	6
S. cordatum	Bark, acetone	0.75	3	3	0.35	0.17	6	3	0.35	0.75	0.35	3	3	1.5	3	0.35
	Bark, methanol	0.35	0.35	1.5	0.35	0.35	3	1.5	0.35	0.35	0.35	1.5	1.5	1.5	1.5	0.75
	Leaves, acetone	0.31	0.31	0.31	2.5	0.31	5	2.5	0.31	5	0.62	1.25	1.25	5	2.5	2.5
	Leaves, methanol	0.75	0.35	1.5	0.75	0.75	1.5	1.5	0.35	0.75	0.35	0.35	1.5	1.5	1.5	0.75
	Leaves, hexane	>12	12	>12	>12	>12	12	12	>12	12	12	>12	>12	>12	>12	>12
P. mixta	Roots, methanol	6	12	12	6	6	>12	6	12	12	3	12	>12	12	12	6
	Stem, acetone	12	12	12	12	12	12	3	12	12	12	12	>12	12	12	12
	Stem, methanol	6	>12	12	6	6	>12	6	12	12	3	3	3	12	12	12
	Leaves, acetone	12	12	12	12	12	6	12	12	12	6	12	12	12	6	12
	Leaves, methanol	12	12	12	12	12	12	12	12	12	>12	6	6	6	6	6
C. torulosa	Leaves, methanol	>12	>12	>12	>12	>12	>12	12	>12	>12	3	>12	>12	>12	12	>12
	Leaves, acetone	>12			>12			12		12				12	12	
	Leaves, hexane	>12	>12	>12	>12	>12	>12	12	>12	12	12	12	12	12	12	12
Z. milneana	Whole plant (WP), acetone	12	12	6	3	1.5	6	1.5	3	6	1.5	6	6	3	3	12
	WP, Methanol	6	3	12	6	1.5	6	6	6	12	3	12	12	6	12	>12
	WP, hexane	12	12	12	12	6	12	12	12	12	3	12	12	12	12	12
L. javanica	Leaves, methanol	1.5	6	>12	12	3	6	6	12	12	1.5	6	>12	12	6	12
	Leaves, acetone	3	6	>12	12	6	6	6	12	12	1.5	12	>12	12	6	>12
	Essential oils	6	6	3	1.5	3	12	6	6	12	3	3	>12	3	1.5	>12
	Leaves, hexane	12	12	12	6	12	12	6	12	12	6	12	12	12	6	12
A.senegalensis	Essential oils	12	>12	>12	>12	12	>12	>12	>12	>12	12	>12	>12	>12	>12	>12

Table 3. Contd.

P. africanum	Roots, methanol	3	6	6	3	1.5	>12	6	1.5	6	3	6	6	6	6	3
	Bark, methanol	1.5	3	6	3	3	6	3	3	6	1.5	3	6	6	3	1.5
M. coriaceae	Roots	>12	12	>12	>12	>12	>12	>12	>12	>12	12	>12	>12	>12	>12	>12
S. alba	methanol															
	Leaves, acetone	12	6	6	6	12	6	6	12	12	3	12	12	12	12	12
	Leaves, methanol	3	6	6	1.5	3	6	6	3	1.5	1.5	3	6	6	3	1.5
	Leaves, hexane	12	6	12	12	12	6	12	12	12	6	12	>12	12	12	12
R. tridentate	Fruits, methanol	6	12	>12	6	3	12	1.5	12	6	1.5	12	12	6	12	12
	Roots, acetone	1.5	3	6	3	1.5	6	3	3	6	1.5	3	3	3	3	1.5
	Tubers, acetone	0.75	1.5	6	3	3	6	3	3	6	1.5	3	3	3	1.5	1.5
E. transvaalensis	Roots, methanol		12	12	6		12	12				>12	>12	>12	6	>12
A. johnsonii	Roots, acetone	5	5	0.62	>12	2.5	5	12	0.62	>12	10	>12	>12	>12	12	>12
	Leaves, acetone	5	2.5	0.62	3	0.62	10	6	0.62	3	1.25	10	>12	3	6	>12
	Bark, acetone	0.62	2.5	0.62	6	0.62	0.62	6	0.62	>12	10	>12	>12	>12	12	>12
Gentamicin		0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.017	0.008	0.008	0.008	0.008	0.008	0.017

Bacillus cereus (Bc), Bacillus pumilus (Bp), Bacillus subtilis(Bs), Staphylococcus aureus (Sa), Enterococcus fecalis (Ef), Enterobacter cloacae (Et), Escherichia coli (Ec), Pantoea agglomerans (Pa), Pseudomonas aeruginosa(Ps), Shigella flexneri (Sf), Aeromonas hydrophila (Ah), Proteus mirabilis (Pm), Klebsiella pneumoniae(Kp), Salmonella cholerae-suis(Sc), Serratia marcescens (Sm).

inhibited *S. aureus* by 18 mm. The sensitivity of all the test bacteria to the different plant extracts are shown in Table 2.

Micro dilution assay

The extracts of Syzygium cordatum gave the lowest MICs. The least were observed for the acetone extract of the bark with 0.17 mg/ml against *Enterococcus fecalis*, 0.3 mg/ml against S*. aureus*, *Pseudomonas aeruginosa* and *Shigella flexneri*, each. The methanol extract of the bark and the acetone extract of the leaves gave similar MICs of 0.3 mg/ml against *Bacillus cereus*, *B subtilis* and *B. pumilus*. The other plants extracts gave higher MICs. Details are shown in Table 3. Gentamicin was used as a positive control and gave MICs of 0.008 mg/ml. Most of the extracts were active against at least one bacterial species and the activities were dose dependant. There were some differences in the activity of some extracts according to the two methods used. Some extracts that did not give any inhibition with the disc diffusion method were active when tested by the microdilution method and some extracts showing inhibition in the disc diffusion method did not show any antibacterial activity in the microdilution method. However, most of the extracts showing activity in the microdilution method were also active in the disc diffusion method.

The concentrations of the plants extracts tested by microdilution method varied between 0.08 and 12 mg/ml. Other active plants included *Ximenia caffra*, *Peltophorum africanum*, *Bridelia micrantha*, *Rhoicicus tridentata*, *Lippia javanica* and S*ida alba*. *Mucuna coriacea* was the least active followed by the essential oil of *Annona senegalensis* with MIC values of more than 12 mg/ml against almost all the bacteria. *Pouzolzia mixta* and *Cissampelos torulosa* were also not very active with most MIC values of more than 12 mg/ml. The Gram-positive bacteria (*Bacillus spp*. and *Staphylococcus aureus*) were the most sensitive whereas the Gram-negatives were more resistant.

Sensitivity to standard antibiotics

All the bacterial organisms were empirically resistant to ampicilin followed by nalidixic acid and tetracyclin. However, gentamycin was the most active against all the species tested with diameter of zones of inhibition for all the organisms ranging from 16 to 30 mm. *P. aeruginosa* was the least sensitive to all the antibiotics followed by *Serratia marcescens, Klebsiella pneumoniae* and *Proteus mirabilis.*

DISCUSSION

The increase of antibiotic resistance of microorganisms to conventional drugs has necessitated the search for new, efficient and cost effective ways for the control of infectious diseases. Many studies have showed that medicinal plants constitute a great source for the isolation of active drugs. For instance emetine, which has been used for a long time for the treatment of amebiasis and other diseases, was isolated from plant as well as quinine used for the treatment of malaria (Cowan, 1999). The use of medicinal plants is part of the African tradition and the Venda region of South Africa has a great variety of vegetation used by the local population to treat and prevent diseases. Other Venda medicinal plants have been shown to be inhibitory to different bacterial species (Obi et al., 2003). In this study different extracts from 14 plants were tested for antibacterial activities and has confirmed some results obtained in earlier studies on Venda medicinal plants (Obi et al., 2003). For example, *Mucuna coriacea* was not very active using the agar dilution method whereas *Peltophorum africanum* was active against *E. coli, S. aureus*, *A. hydrophila*, *S. sonei* and *C. jejuni*. However, the MICs were not determined. In the present study, the MIC values for *Peltophorum africanum* ranged between 1.5 and 12 mg/ml and the extracts were active against all the organisms tested apart from *Enterobacter cloacae*. Compounds isolated from *Peltophorum africanum* comprised flavonol glycosides and flavonol glucoside gallates (El Sherbeiny et al., 1977). A protein inhibitor has also been isolated from the seed of this plant (Joubert et al., 1981) . Other compounds have been isolated from this plant. These include 11-(*E*)-*p*-coumaric acid ester of bergenin (Mebe and Makuhunga, 1992), cyanomaclurin, bergenin, catechin and gallic acid (Bam et al., 1988).

Lippia javanica has been used as a mosquito repellent by the population in Southern Africa for a long time and previous studies in Zimbabwe have shown that essential oils from *L. javanica* have very strong and lasting repellent activity against starved female *Anopheles arabiensis* (Lukwa, 1994). Other studies in Durban showed that extracts of *L. javanica* were not active against *E. coli* and other bacteria (McGaw et al., 2000). However, we found in this study that acetone and methanol extract of this plant were active against most of the bacteria with MICs varying from 1.5 mg/ml against *S. aureus* to greater than 12 mg/ml for *Bacillus subtilis, Proteus mirabilis and Serratia marcescens*. This variation might be due to the impact of geographical and climatic difference or differences in the methods used and the concentrations tested. Traditionally, this plant is also used to treat or prevent diarrhea and fever (Mabogo, 1990). Its action might then be direct on the microorganisms or indirect affecting the immune system of the individuals. The antimicrobial activities of this plant might be due to some chemotypes identified elsewhere (Viljoen et al., 2005) including: geranial, limonene, germacrene-D, camphor, linalool, β-caryophyllene and myrcene). The components of this plant and consequently the activity have been found to be variable according to the geographical location. Thus plants collected at different times and from different regions might have different activities on microorganisms.

Extracts of *Bridelia micrantha* were active against most of the microorganisms tested and the MICs varied from 3 mg/ml to greater than 12 mg/ml. These results agree with previous studies (Lin et al., 2002) where extract of *B. micrantha* demonstrated inhibitory activities against different bacterial species including *S. flexneri* and *S. plesiomonas* and showed potent anti-diarrhoeic activities. In this study *B. micrantha* was mostly active against *S. flexneri* with the least MIC value of 1.5 mg/ml. However, it was not active against *Salmonella cholerae-suis* and *Klebsiella pneumoniae.* Compounds responsible for the antimicrobial activity might include: friedelin, taraxerone, epifriedelinol, taraxerol, gallic acid and ellagic acid isolated from this plant (Pegel and Rogers, 1968). Aqueous and methanolic extracts of *Bridelia crenulata*, a close relative of *B. micrantha* showed activities against *E. coli*, *K. pneumoniae* and *P.* aeruginosa (Ramesh et al., 2001) and compounds isolated included: friedelin, epi - friedelinol, n -octacosanol, a- amyrin, β-sitosterol, β-sitosterol-3-β-D-glucopyranoside and luteoforol. Similar compounds have been found in *Syzygium cordatum*.

Syzygium cordatum extracts were the most active against all the organisms tested with the least MIC of 0.31mg/ml. Previous studies (Candy et al., 1968) showed that wood and bark extracts of *Syzygium cordatum* contain friedelin, epi-friedelinol, β-sitosterol, arjunolic acid, gallic acid, ellagic acid (hexahydroxydiphenic acid), glucose and a gallic acid-ellagic acid complex. Leucodelphinidin and leucocyanidin were detected in bark and leaf. However, no antimicrobial study of crude extracts of this plant has been conducted. In this study, acetone and methanol extracts of the stem bark and leaves of this plant were very active against all the bacterial species tested with MICs varying between 0.31 and 6 mg/ml. Hexane extracts were very limited in amount and were not active against most of the organisms. Other members of the *Syzygium* family have been shown to be active against different microorganisms

(Nakashima et al., 1992; Fujioka et al., 1992; Vermani and Garg 2002).

Rhoicicus tridentata is used by populations in South Africa for gynaecological purposes and diarrhea. In other studies, *R. tridentata* had proved to possess direct uterotonic activity (Katsoulis et al., 2000). One of the interesting results was the seasonal effect on the potency of uterotonic activity of this plant. It was found that tubers harvested in the wet months of summer and autumn were more potent than those harvested in the drier months of winter and spring. This prompted us to collect the plants for our studies during the summer period. Roots and tubers of this plant showed very good activities against all the organisms tested with MICs varying from 0.75 mg/ml against *Bacillus cereus* to more than 12 mg/ml against *P. aeruginosa*. Fruit extracts were not as active as the underground parts and exhibited MICs of more that 12 mg/ml. Else where, this plant has been shown to be poisonous (Veale et al., 1992). It is then necessary to investigate its phytochemical profiles in order to identify the toxic chemicals and differentiate them from antimicrobial compounds or to determine whether the same compounds are responsible for the antimicrobial activities of this plant.

Some extracts had discordant results with the disc diffusion and microdilution methods. For instance, extracts of *X. caffra* gave no inhibition zone for some organisms with the disc diffusion whereas the microdilution method gave MICs of 6 mg/ml for the same organisms. Similar results were obtained previously (Silva et al., 1996; Rios et al., 1988). This might be due to the difference in solubility of possible active compounds. In the disc diffusion method, the limited diffusion of the less polar active compounds in solid media might explain the lack of inhibition zone around the disc, whereas in the microdilution method the compounds in solution get easily in contact with the organisms. Extracts of *X. caffra* were active against all the organisms when tested by the microdilution method and the root extracts were more active than the leaves extracts. *X. americana*, a close relative of *X. caffra* has been shown to be active against different bacteria. However, *X. caffra* has not been studied before for antimicrobial activities.

Annona senegalensis is a very common plant distributed throughout the African continent and is used by local populations from South Africa to Senegal for many purposes. Experimental studies have demonstrated anti-helminthic (Alawa et al., 2003), anti-plasmodial and cytotoxic activities (Kraft et al., 2003). However, documentation on the antibacterial activity of extracts of this plant is scarce. Compounds responsible for the above mentioned activities have been isolated and included mono-tetrahydrofuran acetogenins, diterpenoids and alkaloids. Studies on the essential oils from this plant are also rare. In this study, the essential oils of *A. senegalensis* showed very limited activity against most of the organisms tested.

To our knowledge, the activity of *Pouzolzia mixta, Zornia milneana, Syzygium cordatum, Sida alba* and *Cissampelos torulosa extracts* has not been tested against microorganisms. These plants demonstrated good antimicrobial activities against most of the bacteria tested. Some members of the Cissampelos family however have been studied. For example, hydrophilic extract of *Cissampelos mucronata* have shown good antiplasmodial activities (Tshibangu et al., 2002). *Androstachis johnsonii* is a gregarious species that occurs only in very selected areas in Mozambique, South Africa, Swaziland and Madagascar. It is mostly appreciated for its wood and is also used for construction. Although several studies have documented the antimicrobial properties of medicinal plants from different regions of South Africa (McGaw et al., 2000; Obi et al., 2003; Meyer et al., 1996) most plants studied in this investigation have not bee tested against all the bacterial species considered in this study.

This study has shown that many Venda medicinal plants have a wide range of antibacterial activity and confirms the traditional use of these plants as medicines by the local populations. Some plants were more active than commercial antibiotics particularly ampicilin, nalidixic acid and tetracyclin. *Syzigium cordatum*, *Peltophorum africanum*, *Rhoicissus tridentata, Bridelia micrantha* and *Ximenia caffra* were very active against Gram positive and Gram negative bacteria. Essential oils of *Lippia javanica* was also effective against most of the bacterial species studied. Studies of the activity of these plants on other organisms such as protozoan, viruses, and helminthes will be necessary as well as the determination of their phytochemistry. Studies of the activities of these plants on the immune system will also throw some lights on their possible effects on the immune system. *P. mixta and Mucuna coriacea* for example, has been claimed by many traditional healers to have very good healing effect on intestinal problems and diarrhoeal diseases although we could not find much activity with the disc diffusion and the micro dilution methods. This study has also shown the importance of the use of at least two different methods to test for the antimicrobial activity of medicinal plants.

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REFERENCES

Alawa CB, Adamu AM, Gefu JO, Ajanusi OJ, Abdu PA, Chiezey NP, Alawa JN, Bowman DD (2003). *In vitro* screening of two Nigerian medicinal plants (*Vernonia amygdalina* and *Annona senegalensis*) for anthelmintic activity. Vet. Parasitol. 113(1): 73-81.

Arnold HJ, Gulumian M (1984). Pharmacopoeia of traditional medicine in Venda. J. Ethnopharmacol. 12: 35–74.

Ashbolt JN (2004). Microbial contamination of drinking water and disease outcomes in developing regions. Toxicol. 198: 229–238.

Bam M, Ferreira D, Brandt EV (1988). Novel cyanomaclurin analogue from *Peltophorum africanum.* Phytochem. 27(11): 3704-3705.

Candy HA, McGarry EJ, Pegel KH (1968). Constituents of *Syzygium cordatum*. Phytochemistry 7(5): 889-890.

Cowan MM (1999). Plant products as antimicrobial agents. Clin. Microbiol. 12(4): 564-82.

El Sherbeiny AE, El Ansari MA, Nawwar MA, El Sayed NH (1977). Flavonol glycosides and flavonol glucoside gallates from *Peltophorum africanum*. Planta Medica 32(2): 165-70.

Fujioka T, Kashiwada Y, Kilkuskie RE, Cosentino KM, Ballas LM, Jiang JB, Janzen WB, Chen IS, Lee KH (1994). Anti-AIDS agents, betulinic acid and platanic acid as anti-HIVprinciples from Syzygium claviforum , and anti-HIV activity of structurally related triterpenoids. J. Natl. Prod. 57: 243-247.

Haque R, Mondal D, Kirkpatrick BD, Akther S, Farr BM, Sack RB, Petri WA Jr (2003). Epidemiologic and clinical characteristics of acute diarrhea with emphasis on Entamoeba histolytica infections in preschool children in an urban slum of Dhaka, Bangladesh. Am. J. Trop. Med. Hyg. 69(4): 398-405.

Joubert FJ (1981). Purification and some properties of a proteinase inhibitor (DE-1) from *Peltophorum africanum* (weeping wattle) seed. Hoppe Seylers Z Physiol. Chem. 362(11): 1515-21.

Kamanzy AK, Kone M, Terreaux C, Traore D, Hostettmann K, Dosso M (2002). Evaluation of the antimicrobial potential of medicinal plants from the Ivory Cost. Phytother. Res. 16(5): 497–502.

Katsoulis LC, Veale DJ, Havlik I (2000). The pharmacological action of *Rhoicissus tridentata* on isolated rat uterus and ileum. Phytother. Res. 14(6):460-2.

Kraft C, jenett-Siems K, Siems K, Jakupovic J, Mavi S, Bienzle U, Eich E (2003). In vitro antiplasmodial evaluation of medicinal plants from Zimbabwe. Phytother. Res. 17 (2):123-8.

Lin J, Puckree T, Mvelase TP (2002). Anti -diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. J. Ethnopharmacol. 79(1): 53-6.

Lukwa N (1994). Do traditional mosquito repellent plants work as mosquito larvicides? Central African Journal of Medicine 40(11): 306-9.

Mabogo, DEN (1990). The ethnobotany of the Vhavenda. M.Sc. thesis, University of Pretoria.

McGaw LJ, Jager AK, van Steden J (2000). Antibacterial, antihelmintic and anti-amoebic activity in South African medicinal plants. J. of Ethnopharmacol. 72(1-2):247-63.

Mebe P, Makuhunga P (1992). 11-(*E*)-*p*-Coumaric acid ester of bergenin from *Peltophorum africanum.* Phytochem. 31(9): 3286-3287.

Meyer JJM, Dilika F (1996). Antibacterial activity of *Helichrysum pedunculatum* used in circumcision rites. J. Ethnopharmacol. 53(1):51-54.

Nakashima H, Masuda M, Murakami T, Koyanagi Y, Matsumoto A, Fujii N, Yamamoto N (1992). Anti -human immunodeficiency virus activity of a novel synthetic peptide, T 22([Tyr-5,12,Lys-7] polyphemusin II): a possible inhibitor of virus-cell fusion. Antimicrobial Agents and Chemother. 36: 1249-1255.

Nostro A, Germano MP, D'Angelo V, Marino A, Cannatelli MA (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Lett. Appl. Microbiol. 30: 379-384.

Obi CL, Potgieter N, Bessong PO, Masebe T, Mathebula H, Molobela P (2003). In vitro antibacterial activity of Venda medicinal plants. South Afr. J. Bot. 69(2):1-5.

Pegel K H, Rogers CB (*1968*): Constituents of *Bridelia micrantha.* Phytochemistry 7(4): 655-656.

Ramesh N, Viswanathan MB, Saraswathy A, Balakrishna K, Brindha P, Lakshmanaperumalsamy P (2001). Phytochemical and Antimicrobial Studies of *Bridelia crenulata*. Pharm. Biol. 39(6): 460–464.

Rios JL, Recio MC, Vilar A (1988). Screening methods for natural products with antimicrobial activity: a review of the literature. J. Ethnopharmacol. 23: 127-149.

Silva O, Duarte A, Cabrita J, Pimentel M, Diniz A, Gomes E (1996). Antimicrobial activity of Guinea-Bissau traditional remedies. J. Ethnopharmacol. 50: 55-59.

Tshibangu JN, Chifundera K , Kaminsky R, Wrightn AD, Konig GM (2002). Screening of African medicinal plants for antimicrobial and enzyme inhibitory activity. J. Ethnopharmacol. 80: 25–35.

Veale DJH, Furman KI, Oliver DW (1992). South African traditional herbal medicines used during pregnancy and childbirth. J. Ethnopharmacol. 36: 185-191.

Vermani K, Garg S (2002). Herbal medicines for sexually transmitted diseases and AIDS. J. Ethnopharmacol. 80: 49-66.

Viljoen AM, S Subramoney, SF van Vuuren, KHC Ba er, B Demirci (2005). The composition, geographical variation and antimicrobial activity of *Lippia javanica* (Verbenaceae) leaf essential oils. J. ethnopharmacol. 96(1–2): 271-277.