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Antibacterial and Anti HIV 1 Reverse Transcriptase Activity of Selected Medicinal Plants from Phalaborwa, South Africa

¹M.A. Chauke, ¹L.J. Shai, ²M.A. Mogale and ³M.P. Mokgotho

¹Department of Biomedical Sciences, Faculty of Science, Tshwane University of Technology, Private Bag X680, 0001 Pretoria, South Africa

²Department of Biochemistry, Faculty of Medicine, University of Limpopo, 0204 PO Medunsa, South Africa

³Department of Biochemistry, Microbiology and Biotechnology, University of Limpopo, 0272 Sovenga, South Africa

Abstract

Background and Objective: For years mankind has depended on medicinal plants for treatment of infectious diseases. Many medicinal plants are reported to possess antimicrobial and antiviral activity. Use of these plants as antimicrobials suggests that these plants might be potential source of antimicrobial and antiviral agents for the future. This study focused on medicinal plants collected in Mashishimale village (Phalaborwa) for treatment of infectious diseases. The aim of the study was to investigate the minimal inhibitory concentration and the presence of HIV 1 reverse transcriptase of the selected plants. **Materials and Methods:** Five solvents with different polarities (n-hexane, chloroform, ethyl acetate, water and acetone) were used for extraction of plant materials. Antimicrobial activity was screened against two Gram positive and two Gram negative test bacteria. The HIV reverse transcriptase colorimetric assay was performed according to the manufacturer's instructions. **Results:** *Enterococcus faecalis* and *Escherichia coli* were most sensitive to plant extracts with average MIC values below 0.5 mg mL⁻¹, while *Staphylococcus aureus* was the most resistant. The polar extracts of *Terminalia sericea* had good inhibitory activity against HIV reverse transcriptase, recording IC₅₀ values of about 0.08 mg mL⁻¹ for acetone and water extracts. **Conclusion:** The findings of the study indicate that studies of this nature are useful and may lead to for discovery of more plant-derived compounds with activities against bacterial and viral strains. Furthermore, the identity of the active ingredients may be important to establish the mechanisms used by some of these plants.

Key words: Medicinal plants, antibacterial, anti HIV 1 reverse transcriptase

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Corresponding Author: L.J. Shai, Department of Biomedical Sciences, Faculty of Science, Tshwane University of Technology, Private Bag X680, 0001 Pretoria, South Africa

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Antibiotics have kept the majority of infectious diseases under control. However, their indiscriminate use enabled easily treatable pathogens to develop antibiotic resistance. Furthermore, more and more pathogens that are untreatable with available antibiotics are on the increase¹. Some of these pathogens only succumb to mixture of antibiotics, leading to emergence of multi-drug resistance. Development of resistant pathogens exceeds the discovery rate of new drugs. New sources of drugs may solve problems presented by resistance, in addition to the provision of solutions to problems such as the high cost of drugs and access to primary healthcare facilities in rural areas. Plants are potential candidate sources of new chemotherapeutics².

Medicinal plants produce phytochemicals, some of which possess antimicrobial³ and antiviral activity⁴. For years mankind has depended on medicinal plants for treatment of infectious diseases⁵. Use of traditional medicinal plants as antimicrobials suggests that these plants might be good source of antimicrobial agents. Antibacterial activity is widely found in different groups of flavonoids⁶. Essawi and Srour⁷ screened about 18 plants for their antibacterial activity, where eight of the plants demonstrated varying degrees of antibacterial activity. Kudi and Myrint⁸ studied some plant extracts against four viral strains and reported that most of the plants had antiviral activity against more than one viral strain.

Cassia abbreviata, *Terminalia sericea* and *Ozoroa paniculosa* are used in mixtures to treat antibacterial activity, mostly in the form of sexually transmitted diseases and the symptoms associated with infection with the human

immune-deficiency virus (HIV). The claims made in traditional medicine circles need to be subjected to rigorous laboratory testing in order to prove efficacy and safe dosages. The aim of the study was therefore to investigate antibacterial and HIV-1 reverse transcriptase inhibitory activity of medicinal plants used for treatment of infectious diseases by villagers of Mashishimale village in Phalaborwa. Plant species used in the study are listed in Table 1.

MATERIALS AND METHODS

Antibacterial activity: The minimum inhibition concentrations (MICs) were performed according to the method described elsewhere⁹. Bacterial cultures were maintained at 4°C on Mueller-Hinton agar slants until use. Bacterial test organisms used in screening tests were *Staphylococcus aureus* (Gram positive), *Enterococcus faecalis* (Gram positive), *Pseudomonas aeruginosa* (Gram negative) and *Escherichia coli* (Gram negative). These species were selected for the assay because they are considered the most important nosocomial pathogens¹⁰. Bacterial cells were inoculated and incubated at 37°C in Mueller-Hinton broth. Plant extracts were dissolved in acetone to a concentration of 10 mg mL⁻¹. Ninety six wells of microtitre plates were each filled with 100 µL of sterile distilled water. The plant extract (100 µL) was added into wells in row A and a two-fold serial dilution performed. In negative control wells, 100 µL of methanol was used as a diluent and gentamicin used as a positive control, replacing the plant extract. Then, 100 µL of a sterile broth and bacteria mixture was added and the plates incubated at 37°C for 24 h.

Table 1: Plants used in the study and their medicinal uses

Plant species	Family	PRU No.	Medicinal uses
<i>Ficus abutilifolia</i>	Moraceae	PRU113789	Boiled in mixtures for treatment of all STI's. Management of epilepsy ²¹
<i>Grewia villosa</i>	Tiliaceae	PRU117709	Used as part of mixtures for treatment of diabetes mellitus. Boils, abscess and swelling ²²
<i>Synadenium cupulare</i>	Euphorbiaceae	PRU117708	Roots boiled and given to patients suffering from erectile dysfunction. Eye infection, blackquarter in animals ²³
<i>Sanseveria hyacinthiodes</i>	Asparagaceae	PRU117713	Used in mixtures for treatment of different ailments
<i>Gomphrena celesoides</i>	Amaranthaceae	PRU117707	Roots boiled and used in mixtures for treatment of STI's
<i>Ximenia caffra</i>	Oleaceae	PRU117906	Boiled in mixtures for all STI's, eye infection
<i>Grewia flavescens</i>	Tiliaceae	PRU117710	Used as part of mixtures for treatment of diabetes mellitus
<i>Ozoroa paniculosa</i>	Anacardiaceae	PRU115079.0	Boiled together with roots of <i>T. sericea</i> and <i>C. abbreviata</i> for treatment AIDS-associated symptoms and stomach complaints
<i>Terminalia sericea</i>	Combretaceae	PRU113817.0	Boiled together with roots of <i>C. abbreviata</i> and <i>O. paniculosa</i> to treat AIDS-associated symptoms, sexually transmitted infections and stomach complaints. Wound healing in animals ²³
<i>Cassia abbreviata</i>	Fabaceae	PRU113819.0	Boiled together with roots of <i>T. sericea</i> and <i>O. paniculosa</i> for AIDS treatment. Also, used to treat diabetes mellitus, sexually transmitted infections and stomach complaints. Dewormer in ethnoveterinary medicine ²³
<i>Hexalobus monopetalus</i>	Annonaceae	PRU117907	Roots are boiled in mixtures with other plants to treat different ailments
<i>Zanthoxylum lepriuri</i>	Rutaceae		Roots boiled and given to patient to forget (soothing or comforting) after a loss of a loved one, stomach ulcer/sores ²⁴

After 24 h incubation, 40 μL of INT (p-iodonitrotetrazolium chloride; 20 mg mL^{-1}) was added to all the wells and samples were re-incubated for a further 5 min and the plates were inspected visually.

Bioautography: Extracts of the medicinal plants were tested against four nosocomial bacterial test organisms. The plant extracts were dissolved in acetone to a concentration of 10 mg mL^{-1} . Plant extracts (10 μL) was loaded onto the TLC plate and dried under a stream of air. The plates were developed in CEF (Chloroform/ethylacetate/formic acid 5:4:1). The plates (after drying) were sprayed with concentrated cultures of test microbial organisms (*Staphylococcus aureus* (SA), *Enterococcus faecalis* (EF), *Pseudomonas aeruginosa* (PA) and *Escherichia coli* (EC) until completely soaked. The plates (moist) were incubated at 37°C in a humidified chamber for 24 h. The plates were then sprayed with 2 mg mL^{-1} of p-iodonitrotetrazolium chloride (INT) and incubated for a further 2 h. Formation of purple-red formazan colour resulting from the reduction of INT was a positive indicator of cell viability. Clearing zones (appearing white) were indicative of anti proliferative activity of plants^{11,12}.

Anti HIV reverse transcriptase: The reverse transcriptase colorimetric assay, as described by the manufacturer (Roche, SA) was used for determination of inhibitory effects of the plant extracts on reverse transcriptase activity. Sixty microliters of plant sample was transferred to the MP modules, covered with foil and incubated for 1 h at 37°C. The reaction mixture was then discarded and the plate rinsed 5 times (30 sec each wash) with washing buffer. The module was then blotted dry and 200 μL of anti-DIG-POD working solution added on and re-incubated again for another 1 h at 37°C. The solution was removed completely and the module rinsed again 5 times with 250 μL of washing buffer and the washing buffer removed completely. About 200 μL of ABTS (2,2'-Azinobis [3-ethylbenzothiazoline-6-sulfonic acid]-diammonium salt) substrate solution was added and developed). The plate was then read at 405 nm using a

microplate reader (Anthos 2000, Labotec, RSA). The signalling colour intensity is directly proportional to the reverse transcriptase activity and percentage inhibition was calculated by comparing activity of a sample that does not contain an inhibitor (untreated controls).

RESULTS

Antimicrobial activity: Minimal Inhibitory Concentration (MIC) was considered as the lowest concentration at which a plant extract inhibits the growth of test organism. The MIC result values below 0.1 mg mL^{-1} are considered as an indicator of very good inhibitory capabilities⁹. The MIC values of the plant extracts are presented in Table 2. *Hexalobus monoptetalus* (acetone; 0.071 mg mL^{-1} against *E. faecalis*), *Zanthoxylum leprieurii* (n-hexane; 0.09 mg mL^{-1} against *E. faecalis*), *Ximenia caffra* (n-hexane; 0.039 mg mL^{-1} against *P. aeruginosa*) and *Gomphrena celosioides* (n-hexane; 0.078 mg mL^{-1} against *E. coli*) were the most interesting species. *Enterococcus faecalis*, a Gram positive bacterium was the most sensitive of the test bacteria.

The average MIC values for each plant extracts (Fig. 1) indicated that *Zanthoxylum leprieurii*, *Ficus abutilifolia*, *Cordia grandicalyx* and *Synadenium cupulare* had the high average minimal inhibitory activity (MIC values above 1.0 mg mL^{-1}). However, the rest of the plants had very good inhibition activity, all resulting in average MIC's values below 1.0 mg mL^{-1} .

To evaluate which plant extract was the most active against the bacteria and the most sensitive bacterial species, average MIC values were calculated for each species. For those extracts with MIC values above 2.5 mg mL^{-1} , a value of 3 mg mL^{-1} was allocated to make calculations possible (Table 1). Based on the results obtained (Fig. 2), it would seem that most plant species had very good activity against *Enterococcus faecalis* and *Pseudomonas aeruginosa*.

Enterococcus faecalis and *Escherichia coli* both demonstrated high sensitivity to plant extracts, with low

Table 2: Antimicrobial activity of plant extracts

Plants	<i>S. aureus</i>					<i>E. faecalis</i>					<i>E. coli</i>					<i>P. aeruginosa</i>				
	H	C	A	E	W	H	C	A	E	W	H	C	A	E	W	H	C	A	E	W
<i>Zanthoxylum leprieurii</i>	2.5	2.5	1.25	1.25	0.312	0.09	0.326	0.13	0.365	2.5	≥2.5	≥2.5	≥2.5	≥2.5	≥2.5	0.625	≥2.5	0.416	0.469	2.5
<i>Hexalobus monoptetalus</i>	1.25	0.833	1.042	1.25	1.25	0.12	0.117	0.071	0.156	2.5	≥2.5	≥2.5	0.625	0.63	≥2.5	≥2.5	≥2.5	0.416	0.416	0.625
<i>Ficus abutilifolia</i>	≥2.5	2.5	2.5	2.5	≥2.5	≥2.5	≥2.5	0.26	≥2.5	2.5	≥2.5	≥2.5	≥2.5	≥2.5	≥2.5	2.5	2.5	1.25	1.25	2.5
<i>Cordia grandicalyx</i>	≥2.5	2.5	2.5	≥2.5	1.88	≥2.5	≥2.5	2.5	≥2.5	2.5	≥2.5	≥2.5	≥2.5	≥2.5	≥2.5	0.31	0.31	0.312	0.312	≥2.5
<i>Grewia villosa</i>	≥2.5	1.25	0.833	1.25	1.25	≤2.5	0.313	0.365	0.365	2.5	≥2.5	≥2.5	≥2.5	≥2.5	≥2.5	≥2.5	0.625	0.938	≥2.5	2.5
<i>Synadenium cupulare</i>	≥2.5	2.5	1.25	1.67	ND	1.25	0.938	0.26	0.169	ND	≥2.5	≥2.5	≥2.5	≥2.5	≥2.5	≥2.5	≥2.5	≥2.5	0.625	ND
<i>Sansevieria hyancinthiodes</i>	≥2.5	≥2.5	0.625	ND	ND	≥2.5	0.365	1.67	1.67	ND	≥2.5	0.625	0.625	≥2.5	≥2.5	ND	ND	ND	ND	ND
<i>Gomphrena celosioides</i>	ND	ND	ND	ND	≥2.5	0.312	0.625	0.833	1.25	2.5	0.078	0.527	0.42	0.42	≥2.5	0.313	0.313	0.23	0.078	0.625
<i>Ximenia caffra</i>	ND	ND	ND	ND	0.625	0.625	0.625	0.416	0.312	2.5	0.521	0.521	≥2.5	≥2.5	0.63	0.039	1.25	0.312	1.25	2.5
<i>Grewia flavesceus</i>	ND	ND	ND	ND	0.937	ND	ND	ND	ND	2.5	0.625	0.625	1.25	1.25	1.25	0.31	0.31	0.312	0.312	≥2.5

Gentamycin was run as a positive control and MIC values recorded were below 0.019 mg mL^{-1} ; H: n-hexane, C: Chloroform, A: Acetone, E: Ethyl acetate, W: Water, ND: Not done

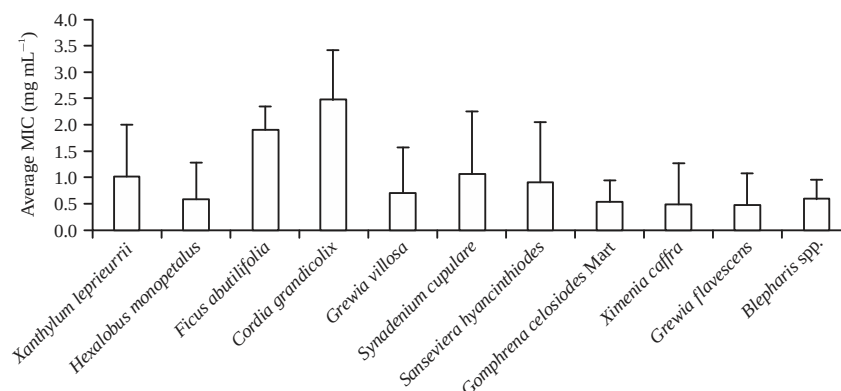


Fig. 1: Average MIC values for test plant species against four bacterial species

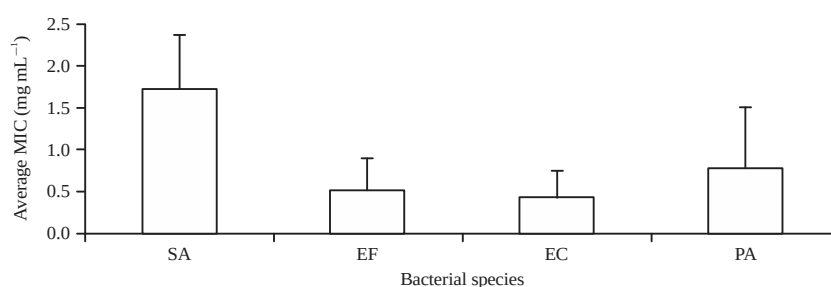


Fig. 2: Average MIC values obtained for four bacterial species. These results indicate sensitivity of bacterial species to plant extracts. *Staphylococcus aureus* (SA) *Enterococcus faecalis* (EF), *Pseudomonas aeruginosa* (PA) and *Escherichia coli* (EC)

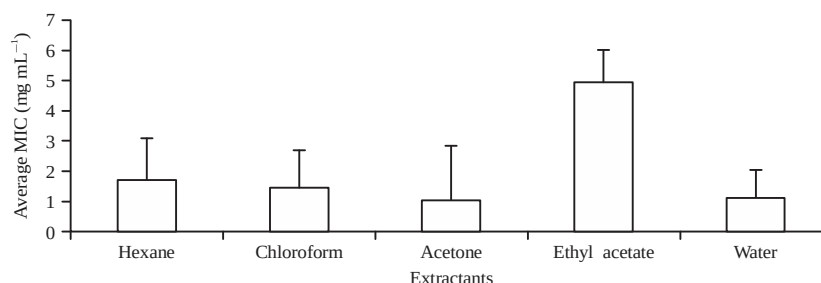


Fig. 3: Average minimal inhibitory concentrations per solvent

average MIC value. *Staphylococcus aureus* was the most resistant to plant extracts, evidenced by average MIC value about 1.6 mg mL⁻¹ (Fig. 2).

Average MIC values for each individual solvent were calculated and displayed in Fig. 3. The results demonstrate that acetone had very good activity with average MIC value below 1.0 mg mL⁻¹. On the other hand, ethyl acetate extracted the least quantity of active compounds and this is evidenced by the highest calculated average MIC value against all the bacterial species.

Bioautography: *Grewia villosa* crude extracts were active against all 4 tested bacterial cultures, indicating clearing zones on the TLC plate for the tested organism (Fig. 4). *Grewia villosa*

had at least 3 visible compounds observed against all different bacterial cultures, mostly with acetone, ethyl acetate and water extracts. The R_f values of the compounds in CEF were 0.33, 0.40 and 0.58. The antimicrobial activity of the compounds was not selective, showing activity against 4 species. The bacterial cultures were resistant to other plants species screened as indicated by fewer growth inhibiting compounds on the bioautograms.

Anti HIV reverse transcriptase activity: Three plants species (*Terminalia sericea*, *Cassia abbreviata* and *Ozoroa paniculosa*) were indicated for treatment of sexually transmitted diseases and HIV. Based on the results obtained (Fig. 5), it would seem that *Terminalia sericea* and *Cassia abbreviata* had the best

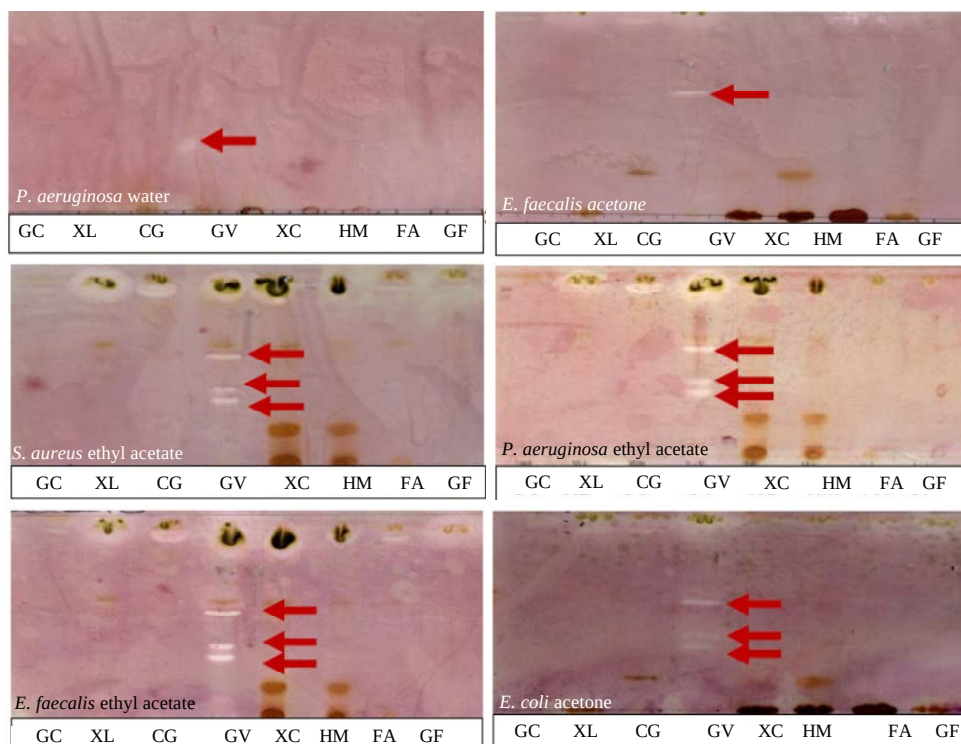


Fig. 4: Plant extracts against different bacterial cultures (Unk, GC; Mol, XL; Mtb, CG; BigM, GV; Mot, XC; Moe, HM; Mof, FA; Small M, GF). Different RF values indicated by the arrows. Plant extracts against different bacterial cultures (*Gomphrena celosioides* GC, *Zanthoxylum lepriuerii* XL, *Cordia grandicalyx* CG, *Grewia villosa* GV, *Ximenia caffra* XC, *Hexalobus monopetalus* HM, *Ficus abutilifolia* FA, *Grewia flavescens* GF). Different RF values indicated by the arrows (0.33, 0.40 and 0.58)

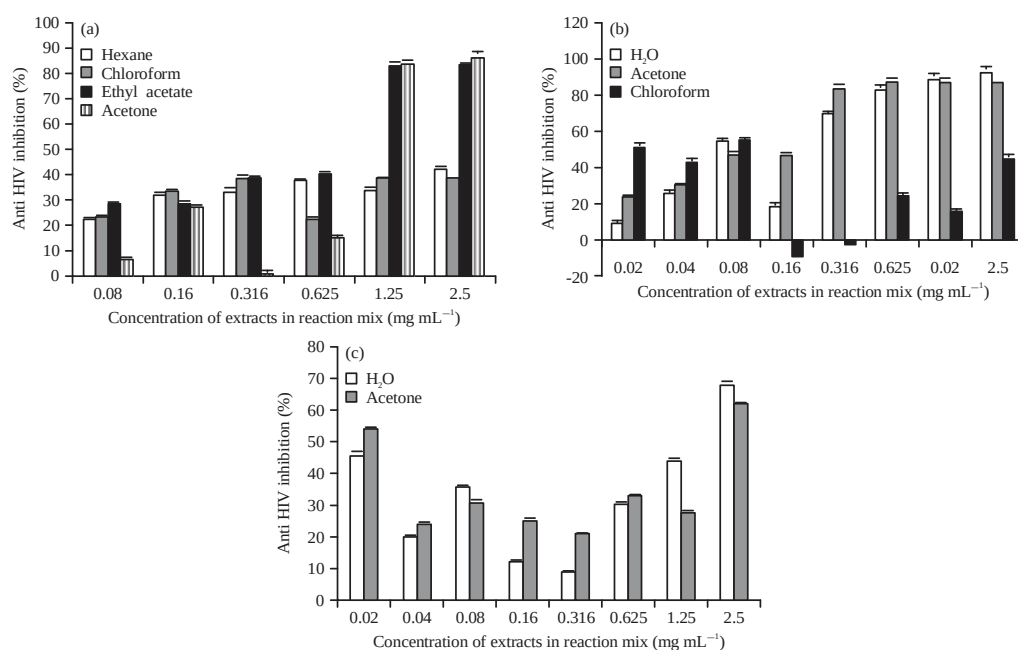


Fig. 5(a-c): Anti HIV-1 reverse transcriptase inhibition by extracts of plant species (a) *Cassia abbreviata*, (b) *Terminalia sericea* and (c) *Ozoroa paniculosa*

inhibitory activity against HIV reverse transcriptase. The IC₅₀ values of acetone and water extracts (0.08 mg mL⁻¹) for *Terminalia sericea*. The ethyl acetate and acetone extracts of *Cassia abbreviata* had the promising inhibitory activity (IC₅₀ 1.25 mg mL⁻¹). *Ozoroa paniculosa* was only active at the highest concentration of 2.5 mg mL⁻¹. Polar extracts of *T. sericea* and *C. abbreviata* were the most active.

DISCUSSION

The findings of the antibacterial activity assays indicate that the plants had varying activity ranges against the bacterial test organisms. The best activity was observed with *Zanthoxylum lepriuerii* (n-hexane) and *Hexalobus monopetalus* (acetone) against *Enterococcus faecalis*. Some of the plants investigated demonstrated very good activity, while the water extracts demonstrated very poor activity with all pathogens recording MIC values above 1.0 mg mL⁻¹. In an antibacterial study conducted by Rabe and van Staden¹³ water extracts of the 21 plants studied also demonstrated no activity with bacterial strains while most activity was observed with methanolic extracts, further validating no activity with the water extracts.

The low antibacterial activity of *Cordia grandicalyx* was hardly surprising since the plant species is not used traditionally for treatment of bacterial infections. *Ficus abutilifolia*, despite showing overall low activity against test bacterial species is used in treatment of infections. It must be noted that this plant species is used in combination with other plant species, as mixtures used to treat sexually transmitted diseases.

Zanthoxylum lepriuerii was not indicated for treatment of infections but surprisingly it was one of the plants that demonstrated the highest antibacterial activity. Activity of *Zanthoxylum lepriuerii* concurs with other studies which reported the plant to possess both antibacterial and antifungal properties^{14,15}. Essential oils from *Zanthoxylum lepriuerii* were reported to inhibit growth of some eight strains of microorganisms¹⁵, while investigation of the leaf extracts demonstrated antifungal properties¹⁴.

Activity of *Hexalobus monopetalus* was not unexpected as other members of family (Annonaceae) have antibacterial¹⁶ and antifungal activities¹⁷⁻¹⁹. It has also been indicated in literature that *Hexalobus monopetalus* contains about 95% essential oils²⁰ and a number of alkaloids, roemerine, N-formylanonaine and liriodenine were isolated from the leaves of this plant species²¹. Activity of *Hexalobus monopetalus* therefore cannot be limited to the known compounds and further isolation and studies are suggested.

The plant species listed in Table 1, except *Cordia grandicalyx* are used in treatment of various diseases associated with infections (bacterial, fungal or viral) by healers of Mashishimale village or other parts of the African continent²¹⁻²⁴. The apparent lack of activity against the test bacteria for some plants may be due to the plant part used in laboratory testing. The healers use roots and stem bark of plants. Healers also use the plant species in mixture. The laboratory tests focused on individual plant extracts, eliminating the synergistic effects presented by mixtures. The suitability of test organisms may be another reason for low activity. The results presented here require further investigations on mixtures prepared by healers as inhibitors of growth of bacterial pathogens associated with sexually transmitted infections.

The polar extracts of *C. abbreviata* and *T. sericea* had good inhibitory activity against HIV reverse transcriptase. These plants are indicated traditionally at the Mashishimale village, to treat HIV related symptoms. The healers use water as their extracting solvent. Data obtained indicate that there is some validity in the claim that these plants are useful in treating HIV and related symptoms. Though the study must be escalated to include investigation of the antiviral activity in a more inclusive study, data obtained suggests some potential of these plants in the fight against HIV and AIDS.

Based on the wide use of *Terminalia sericea*, our results corroborate with the literature that this plant possesses antimicrobial^{19,25-27} and antifungal properties²⁸. A number of compounds have been isolated from *Terminalia sericea* and they include a triterpene sericoside and resveratrol-3-O-β-d-rutinoside²⁹. The claim by traditional healers on use of this plant for treatment of sexually transmitted diseases is further validated by a study conducted by Tshikalange *et al.*³⁰, which reported that the plant was indicated for treatment of sexually transmitted diseases in Venda. Bioactive compounds were isolated from this plant³⁰. Furthermore, Steenkamp *et al.*¹⁸ confirmed the antibacterial, antioxidant activity of *Terminalia sericea*.

CONCLUSION

The selected plants had varying degrees of antibacterial and antiviral activities. The three plant species used in HIV management inhibited the activity of HIV-1 reverse transcriptase in a dose-dependent manner. However, some of the plant species indicated for treatment of bacterial infections had little activity against test bacterial species. More studies using mixtures as administered by healers are needed to prove the validity of the traditional remedies for treating

sexually transmitted diseases. The shortcoming of the study was to test individual plant extract as well as the selection of test organisms. Since the plants are used as mixtures for sexually transmitted diseases, it would be interesting to investigate their effects on pathogens causing venereal diseases. The active ingredients must be isolated from the most active plant species.

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