Available online on www.ijppr.com
International Journal of Pharmacognosy and Phytochemical Research 2016; 8(11); 1870-1876

ISSN: 0975-4873

Research Article

Anti-Microbial, Anti-Inflammatory and HIV-1 Reverse Transcriptase Activity of Selected South African Plants used to Treat Sexually Transmitted Diseases

Mamba P, Adebayo S A, Tshikalange T E*

Department of Plant and Soil Sciences, University of Pretoria, P Bag X20, Hatfield 0028.

Available Online: 15th November, 2016

ABSTRACT

The purpose of the study was to investigate the bioactivity of extracts of selected plant species used to treat sexually transmitted diseases (STD's) in southern Africa. As the emergence of drug resistance pathogens in STD's treatment and potential side effects of synthetic drugs demands the discovery of newer and safer drugs, the exploration of newer anti-microbial substances from natural sources may serve as promising alternatives. Ethanol extracts of twelve medicinal plants used traditionally to treat sexually transmitted diseases and 3 flavonoids (F1, F2 and F3) isolated from *Elaeodendron transvaalense* were evaluated for their antimicrobial properties against one fungus and three bacteria. To determined anti-inflammatory activities of the extracts and compounds, the inhibitory effect was measured on the pro-inflammatory enzyme, 15-lipoxygenase (15-LOX). The extracts and compounds were also investigated for their anti-HIV activity against recombinant HIV-1 enzyme using non-radioactive HIV-RT colorimetric assay. *Acacia karoo and Rhoicissus tridentata* extracts indicated good anti-microbial activity with MIC values ranging between 0.4 and 3.1 mg/mL. Extracts of *Jasminum fluminense, Solanum tomentosum,* F2 and F3 had good anti-inflammatory activity with IC_{50} less than positive control quercetin (IC_{50} = 48.86 µg/mL). *Acacia karoo* and F3 exhibited moderate HIV RT inhibition activity of 66.8 and 63.7% respectively. *Rhoicissus tridentata* and *Terminalia sericea* had the best RT inhibition activity (75.7 and 100%) compared to that of the positive control doxorubicin (96.5%) at 100 µg/mL. The observed activities may lead to new multi-target drug against sexually transmitted diseases.

Keywords*:* Sexually transmitted diseases, antimicrobial, anti-inflammatory, 15-LOX, HIV-1 RT.

INTRODUCTION

Microbial infections are a common public health concern especially in most developing countries. Worldwide, more than one million people acquire sexually transmitted diseases (STD's) every day[1]. Sexually transmitted diseases (STD's) have a major impact on sexual and reproductive health worldwide. Each year, the World Health Organization (WHO) estimated that 448 million new cases of curable STD's are diagnosed. STD's rank among the top five diseases for which people seek clinical care and are a major cause of morbidity[2]. Untreated infections may lead to serious health complications which may include male or female sterility, ectopic pregnancy, pelvic inflammatory disease, lower health quality of life and may also lead to increased susceptibility to human immunodeficiency virus (HIV) infection[3]. Particularly in the low and middle income countries where the health system infrastructure is least developed, the control of STD's remains a challenge[4]. As a result of these difficulties, traditional medicines remain the primary source of medical care to various healthcare needs[5]. The use of plant derived chemicals could provide alternative classes of antibiotics having different target sites than the already used antibiotics, which may be effective against drug resistant pathogens. As the emergence of drug resistance in STD related micro-organisms and potential side effects demands the discovery of newer drugs, the exploration of newer anti-microbial substances from natural sources may serve as promising alternatives[6]. Some plants can offer better prospects for the discovery of new pharmaceuticals and better anti-infective agents. This results in the increased interest in medicinal plants used by medical practitioners for various diseases[7]. Although pharmaceutical industries have produced a number of antimicrobial drugs, the increase resistance of microorganisms to these drugs is still a major problem. Antimicrobial resistance is a growing problem that impacts the treatment source of antibiotics to treat and improve human well-being[8]. One of the methods to reduce the resistance to these antibiotics is by the use of antibiotic resistance inhibitors from plants[9]. Antimicrobials originating from plant extracts can be a choice of treatment which has been reported to have therapeutic potential as treatment against infectious diseases[8-10]. Inflammation is a major risk factor for various human diseases including venereal diseases and this may often lead to treatment complications[11]. STD's ultimately occur at the mucosal surface of the genital tract, where inflammation from both non-ulcerative and ulcerative

**Author for Correspondence: Emmanuel.tshikalange@up.ac.za*

infections increases localised immune cell mobilisation, in turn enhancing susceptibility to HIV infection[12]. During inflammation, arachidonic acid is metabolized via the cyclooxygenase (COX) pathway to produce prostaglandins and thromboxane A_2, or via the Lipoxygenases (LOX) pathway to produce hydroperoxy-eicosatetraenoic acids and leukotrienes. COX/LOX metabolites play a pivotal role in cell signalling and proliferation, which can increase eicosanoids levels leading to tumour growth. The isomeric enzyme, 15-LOX is an important enzyme involved in the synthesis of leukotrienes from arachidonic acids. Biologically active leukotrienes are mediators of many pro-inflammatory and allergic reactions, therefore the inhibition of the synthesis of leukotrienes by 15-LOX is considered as one of the therapeutic strategies in the management of inflammatory conditions[13]. STD's are one of the major risk factors for HIV infection. Currently, it was reported that more than 37 million people are living with HIV worldwide[14,15]. HIV-1 reverse transcriptase (RT) contributes to the development of resistance to all anti-AIDS drugs by introducing mutations into the viral genome[16] (Das et al., 2013). Reverse transcription is an essential step in HIV-1 infection, and HIV-1 RT is the target of many anti-AIDs therapeutic drugs and there are ongoing efforts to help identify new RT inhibitors[17]. This study aims to take an in-depth look at 12 South African plants that are traditionally used for the treatment of STD's. Such effort is particularly to scientifically validate anecdotal claim for the use of these medicinal plants.

MATERIAL AND METHODS

Preparation of plant materials

The medicinal plants collected in Jongilanga community were organized into a database and ground into fine powder. The powdered plant material was then dissolved in 70% ethanol in water and vigorously shaken for 72 hours using a Labcon 3086U. Filtration was then conducted using a vacuum system and Whatman filter paper. The filtrate was then concentrated using a Rotavapor (Buchi B-480) to evaporate the solvent from the plant material. These extracts were stored in pre-weighed labelled polytops. Plants were identified at the HGWJ Herbarium by Mr Calvin Mophuting, a botanist at the department of Plant and Soil Sciences, University of Pretoria. Herbarium voucher specimen numbers are provided in Table 1. Three pure compounds/flavonoids which were previously isolated from *Elaeodendron transvaalense* were also included in the study. This follows that this plant is traditionally used in the treatment of many STD's. The isolated flavonoids are; lup-20(30)-ene-3, 29-diol, (3α)-(9Cl) (F1), lup-20(29)-ene-30-hydroxy-3-one (F2) and 4'-O-methylepigallocatechin (F3) respectively[18].

Microorganisms

STD's are a clinical syndrome caused by pathogens that can be acquired and transmitted through sexual activity. There are various pathogens known for causing STD's, in the form of bacterial, viral, fungal and protozoal and epizoal infections[19]. This study takes into account one fungal (*Candida albicans* ATCC 10231) and three of the various bacterial (*Gardnerella vaginalis* ATCC 14018, *Neisseria gonorrhoeae* ATCC 19424 *and Oligella ureolytica* ATCC 43534) sexually transmitted associated microorganisms.

Antimicrobial activity

The determination of antimicrobial susceptibility of plant extracts was performed using the serial broth micro-dilution assay as described by Eloff, 1998[20] but with slight modification. Fifty milligrams (50 mg) of each plant extract was weighed in 200 mL Eppendorf tubes. Each plant extract was dissolved in 100 µL of 10% dimethyl sulphoxide (DMSO) and 900 µL of nutrient or Muller Hilton broth, to make a final concentration of 50 mg/mL. All extracts were tested in a 96-well microtitreplate, in triplicate. Microorganisms were inoculated in sterile broth and prepared to a density of 1.5×10^8 colony forming units per mL (CFU/mL), corresponding with the 0.5 McFarland Standard. Inoculated broth (100 µL) was added to the plates. Ciproflaxacin was used as a positive control for all extracts and 10% DMSO was used as a negative control to ascertain if any growth inhibition was attributed to the solvent and culture control (pathogen growing independently). Plates were incubated for 24 hours at 37 ^oC and results were read based on the visual colour change of Presto blue dye; a pink colour change indicating microbial growth.

Anti-inflammatory activity

The method used was that described by Adebayo et al (2015)[13]. Briefly, plant extracts were prepared at 10 mg/mL and compounds at 1 mg/mL in DMSO. The enzyme (15-LOX) was made up to a final concentration of 200 units/mL in 2 M borate buffer (pH 9) and kept on ice. A volume of 12.5 µL of each plant sample and control was added to 487.5 µL of 15-LOX in a 96-well microtitre plate and incubated for 5 min at room temperature. Thereafter, 500 µL substrate solution (10 µL linoleic acid dissolved in 30 µL ethanol, made up to 120 mL with 2 M borate buffer at pH 9.0) was added to the solution and incubated for 5 min at room temperature. After incubation, the absorbance was measured with the micro-titre reader at 234 nm. Quercetin (1 mg/mL) was used as a positive control and DMSO was used as a negative control. The results were expressed as IC_{50}, i.e. concentration of the extracts and controls that resulted in 50% 15- LOX inhibition. The percentage enzyme inhibition of extract compared with negative control as 100% activity was calculated using the equation below;

$$\%\ \text{Inhibition} = \frac{\text{OD sample}}{\text{OD negative control}} \times 100\%$$

HIV-1 Reverse transcriptase activity

The activity of plant extract on RT activity was determined with recombinant HIV-1 enzyme using a non-radioactive HIV-1 RT colorimetric ELISA kit (Roche). All plant materials were weighed up to 3 mg and were dissolved in 1 mL DMSO to make a final concentration of 3 mg/mL stock solution. Ten microliters (10 µL) of stock solution was added to 90 µL of lysis buffer making a final concentration of 0.3 mg/mL. The enzyme was prepared to a stock solution of 0.764 mg/mL and 0.327 µL was added to 1000 µL lysis buffer. In appropriate wells of the

microtitre plates, 20 µL of enzyme, 20 µL diluted extract and 20 µL reaction mixture were added together. For positive control; doxorubicin at 100 µg/mL was used; (1) lysis buffer was added with DMSO and (2) lysis buffer was added with no DMSO. For negative control; only the lysis buffer and reaction mixture were added. The plates were incubated for one hour at 37 °C. The microtitre plates were washed five times with 250 µL of the washing buffer. Two hundred microlitres of Anti-Dig-POD working solution was added in each well. Thereafter, the plates were incubated for one hour at 37 °C. The microtitre plates were washed five times with 250 µL washing buffer. Two hundred microlitres of ABTS substrate solution was added in each well and allowed a 10 minute stand at room temperature. The absorbance was read on a microtitre plate reader at 405 nm with a reference wavelength of 490 nm[21]. The mean of the duplicate absorbance was analysed using the formula:

$$\% \text{ Inhibition } = \{1 - \left(\frac{\text{OD Sample}}{\text{OD negative control}}\right)\} \times 100$$

RESULTS AND DISCUSSION

Antimicrobial activity

The results of antimicrobial activity expressed in minimum inhibitory concentrations (MIC) values are shown in Table 2. The MIC value is defined as the lowest concentration of an antimicrobial agent or plant extract that will inhibit the visible growth of a microorganism upon addition of an indicator dye after overnight incubation[22]. Ciprofloxacin was used as the positive control and DMSO was used as the negative control. The concentrations of the extracts ranged between 12.5 mg/mL and 0.1 mg/mL dissolved DMSO. *Acacia karoo and Rhoicissus tridentata* had the best antimicrobial activity against *Candida albicans* with the lowest MIC value of 0.8 mg/ mL. In a study conducted by Nielsen et al (2012)[23], the leaves and stem of *A. karoo* were tested for *C. albicans* and they indicated MIC values of 0.6 mg/mL and 0.8 mg/mL respectively. The anti-Candidal activity of *R. tridentata* was tested by Hamza et al (2006)[24] and later by Samie et al (2010)[25] and these results also showed good inhibitory activities. Also showing good activity of 1.6 mg/ mL are *Elaeodendron croceum, Hilliardiella nudicaulis* and *Senna italica*. There are no previously conducted experiments on the antimicrobial activities of *H. Nudicaulis.* The anti-gonococcal and anti-candidal activities of *S. italica* were evaluated by Muladzi et al (2015)[26] and found to have a high inhibition activity and low anti-fungal activity against *C. albicans.* Little or no activity was recorded for extracts of *Solanum tomentosum* and all the isolated flavonoids. *R. tridentata* had the best activity against *Neisseria gonorrhoeae* with an MIC value of 0.4 mg/ mL. Also indicating good activity with MIC values of 0.8 mg/ mL were extracts of *A. karoo, Hilliardiella nudicaulis* and *S. italica* (Table 2). When tested for *Gardnerella vaginalis*, *R. tridentata* also had the best activity with MIC value of 0.4 mg/mL. Extracts or isolated compound with low MIC against STD causing pathogens could potentially be used as part of the template for the development of safer and potent drugs to combat these diseases. The lowest MIC against *Oligella ureolytica* (0.8 mg/ mL) were observed with extracts of *A. karoo, D. mespiliformis, R. tridentata and S. capitata*. The broad spectrum of antimicrobial activities of *D. mespiliformis* has also been confirmed by Mabona et al (2013)[27]. Extracts of *S. tomentosum* and the isolated compounds had insignificant activities against the tested microorganisms. Medicinal plants possess enormous potentials to synthesise a wide variety of bioactive secondary metabolites for defence and survival purposes. These metabolites are extracted by humans and animals for therapeutic uses. Our study was aimed at validating the anecdotal claim for the use of selected plant species for STD's and related infections. The results indicated that extracts of *Acacia karoo and Rhoicissus tridentate* had good activity against *Candida albicans*. In addition, *R. tridentata*, *A. karoo, Hilliardiella nudicaulis* and *S. italic* exhibited potent antimicrobial activity against disease causing bacteria such as *Neisseria gonorrhoeae* and *Gardnerella vaginalis*.

Anti-inflammatory activity

One of the objectives of this investigation was to evaluate the anti-inflammatory activity of selected plant extracts and compounds using the anti-15-LOX model of inhibition. Inflammation plays a vital role in the progression or resolution of many diseases, including STD's. The ability to inhibit the COX/LOX activity can be used to evaluate the anti-inflammatory activity of any given plant extract. Such plants with this ability would potentially have a therapeutic effect as an anti-inflammatory agent, and thus promote healing and tissue repair process. A plant species containing an anti-inflammatory compound with additional therapeutic activity against STD's has the potential to be developed into a product that can be used to manage the particular infection and its associated inflammation. The anti-inflammatory activities of the extracts were expressed as IC_{50} and the results for inhibition of 15-LOX enzyme are shown in Table 3. Quercetin was used as a positive control, while DMSO was used as a negative control (100% enzyme activity or no enzyme inhibition). The percentage enzyme inhibition of each extract compared with negative control as 100% enzyme activity was calculated and the results were expressed as IC_{50} (concentration of the extracts and controls that resulted in 50% 15-LOX inhibition). Quercetin had an IC_{50} value of 48.86 µg/mL. Active samples were compared with positive controls. *Acacia karoo* showed to have good 15-LOX inhibition activity. The IC_{50} value was higher than that of quercetin 48.86 µg/mL, however it can be comparable. The IC_{50} value was 62.24 µg/mL. It has also been reported that *Elaeodendron croceum* contains a naturally occurring flavonoid (naringenin) that enables it to exhibit anti-inflammatory activities[23]. Isolates of *D. mespiliformis* include tannins, steroids, anthocyanins and flavonoids[28]. Flavonoids can regulate cellular activities of inflammation related cells such as macrophages and lymphocytes[29]. Cock (2015)[30] reported that *T. sericea* is known to contain a phenolic anti-inflammatory compound, resveratrol.

Table 1: The selected plants species used in the study.

Plant species	Family	Voucher Number	Part used	Medicinal uses
Acacia Cf karoo	Fabaceae	BCM 119360	Roots	STD's
Diospyros mespiliformis	Ebenaceae	BCM 117182	Roots and Leaves	Urinary disorders, STD's
Elaeodendron croceum	Celastraceae	BC 11	Bark	HIV
Elaeodendron transvaalense	Celastraceae	BCM 117182	Bark	Inducing vomiting, STD's
Hilliardiella nudicaulis	Asteraceae	BC 236	Whole plant	STD sores
Jasminum fluminense	Oleaceae	BCM 119350	Roots	STD's
Peltophorum africanum	Fabaceae	BC 40	Roots	Body pain, TB, STD's
Rhoicissus tridentata spp cuneifolia	Vitaceae	BC 119338	Roots	Eye infections, STD's
Schotia capitata	Fabaceae	BC 99	Roots	Pulmonary infections, STD's
Senna italica spp Avachoides	Fabaceae	BCM 117179	Roots	STD's
Solanum tomentosum	Solanaceae	BCM 117177	Roots	Eye infections, STD's
Terminalia sericea	Combretaceae	BCM 118704	Roots	STD's, Tonsils

*No information

Table 2: The MIC (mg/mL) values of ten plant species tested against different microorganisms

Samples	*Candida albicans*	*Gardnerella vaginalis*	*Neisseria gonorrhoeae*	*Oligella ureolytica*
Ciprofloxacin	0.01	<0.01	<0.01	<0.01
Acacia Cf karoo	0.8	6.3	0.8	1.6
Diospyros mespiliformis	3.1	6.3	6.3	3.1
Elaeodendron croceum	1.6	12.5	1.6	3.1
Elaeodendron transvaalense	3.1	12.5	1.6	3.1
Hilliardiella nudicaulis	1.6	12.5	0.8	12.5
Jasminum fluminense	3.1	<12.5	6.3	3.1
Peltophorum africanum	3.1	12.5	1.6	1.6
Rhoicissus tridentata spp cuneifolia	0.8	0.8	0.4	1.6
Schotia capitata	3.1	12.5	1.6	3.1
Senna italica spp Avachoides	1.6	3.1	0.8	<12.5
Solanum tomentosum	<12.5	<12.5	12.5	3.1
Terminalia sericea	3.1	<12.5	1.6	<12.5
Lup-20(30)-ene-3,29-diol, (3α)-(9Cl) (F1)	<12.5	<12.5	<12.5	<12.5
Lup-20(29)-ene-30-hydroxy-3-one (F2)	<12.5	<12.5	<12.5	12.5
4'-O-methyl-epigallocatechin (F3)	3.1	<12.5	6.3	1.6

Terminalia sericea was able to inhibit cyclooxygenase COX-2 in the study. Cock (2015)[30] later reported a compound, triterpenoidal saponin sericoside to be responsible for the anti-inflammatory activities in the inhibition of COX-2 and 5-LOX. *Jasminum fluminense*, the root extract of *Solanum tomentosum*, F2 and F3 had significant inhibition on the activities of 15-LOX, with IC_{50} values of 35.22 µg/mL, 37.16 µg/mL, 39.06 µg/mL and 31.38 µg/mL respectively. These findings imply that these plants may contain compounds with higher activity than that of quercetin. More work should be done in the isolations of these active compounds.

HIV-1 Reverse transcriptase activity

Most of the plant species under study had significant anti-viral activity and therefore were all tested for activity against the HIV-1 virus and inhibition of the RT enzyme, which is vital in the lifecycle of HIV. The results are presented in a Figure 1, representing the % inhibition of the RT enzyme. The results were compared to the positive control Doxorubicin which indicated 96.5% inhibitory activity and a standard deviation of 4.83. Extracts with activity below 20% inhibition were considered to be insignificant, 20–40% low, 40–70% moderate, and 70–100% high inhibition activity[36]. *Terminalia sericea* had the highest inhibition of HIV-1 RT activity, having 102.8% inhibition. A study conducted by Krishnaveni (2012)[31] supported the use of *T. sericea* extract as an effective inhibitor of HIV-1 RT. This plant extract indicated a high inhibitory activity, with inhibition of 75.7%. *Acacia karoo* had good inhibitory activity with an inhibitory percentage of 66.8. In a study conducted by Moll et al (2013)[32], extracts of *A. karoo* was determined to have good activity against the HIV-1 RT, although, the plant species in the study were extracted with methanol which is also a polar solvent, and with similar polarity to the 70% ethanol used in our study. Thus, it is plausible that extracts of *A. karoo* possibly contained compounds with potent inhibitory activity on HIV-1 RT. The inhibitory activity of F3 was moderate, with an inhibition of 63.7%. The results however, contradict those reported by

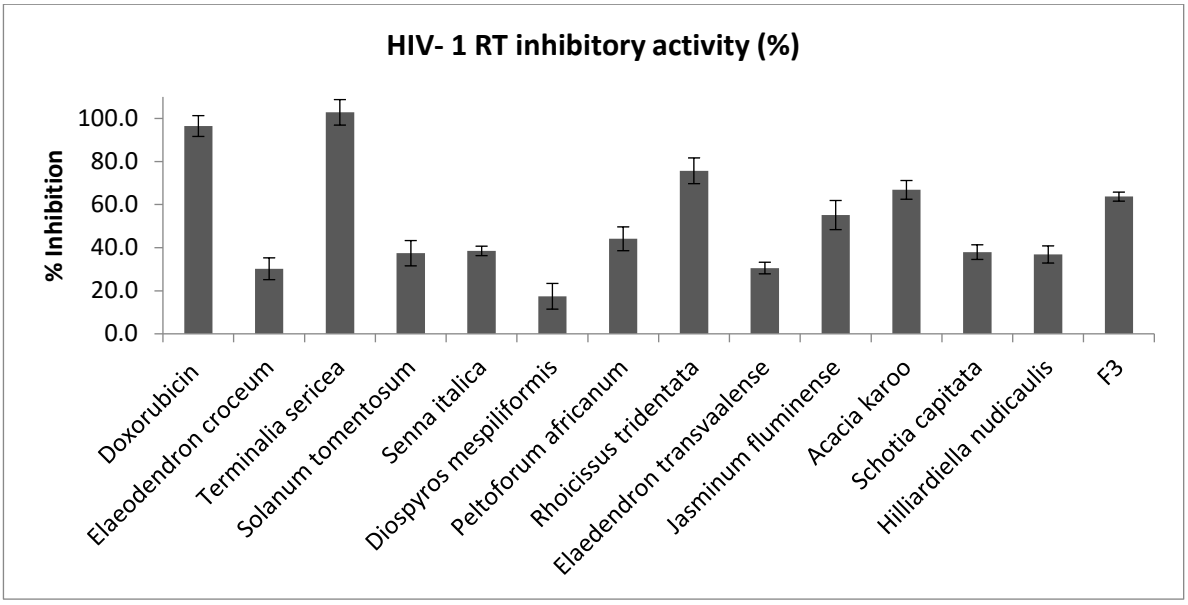

Figure 1: The inhibitory activity of extracts of the selected plant species on HIV-1 reverse transcriptase. Extracts of *T. sericea* (*D. mespiliformis* had the best inhibitory activity of HIV-1 RT when compared with the positive control.

Table 3: The inhibition of15-LOX by the extracts and compounds expressed as IC_{50} (µg/mL).

Samples	IC_{50} (µg/mL)
Quercetin	48.86
Acacia karoo	62.24
Diospyros mespiliformis	188.1
Elaeodendron croceum	82.51
Elaeodendron transvaalense	80.17
Hilliardiella nudicaulis	51.32
Jasminum fluminense	35.22
Peltophorum africanum	88.69
Rhoicissus tridentata spp cuneifolia	87.39
Schotia capitata	83.13
Senna italica spp Avachoides	77.89
Solanum tomentosum	37.16
Terminalia sericea	122.82
Lup-20(30)-ene-3,29-diol,(3α)-(9Cl) (F1)	69.77
Lup-20(29)-ene-30-hydroxy-3-one (F2)	39.06
4'-O-methyl-epigallocatechin (F3)	31.38

Maragesi et al (2010)[33], indicating that this phenolic compound did not show any anti-HIV activity. Unfortunately, there was insufficient material for F1 and F2 at the end of the bioassays to be tested for HIV-1 RT inhibitory activity. Extracts of *Jasminum fluminense* had moderate activity with 55.1% inhibition; however there is no previous literature report on HIV-1 RT inhibition by extracts of *J. fluminense. Diospyros mespiliformis* root extracts had low inhibitory activity with 17.4% inhibition of the HIV-1 RT. This was also reported by Hedimbi (2015)[34], where it was indicated that *D. mespiliformis* leaf extract at 0.1 mg/mL had 78.7% HIV-1 RT activity. This result indicated that the ethanol root extracts of *D. mespiliformis* is a less effective inhibitor. However, the reaffirms our observation that extracts of *D. mespiliformis* possesses activity against HIV-1 RT depending on the part of the plant extracted. Bacterial agents of STDs constitute a major public health burden in many parts of the World, especially in developing countries. One of the common consequences of STDs is the risk of acquiring a viral STI such as HIV (2015). HIV-1 reverse transcriptase (RT) is a very important enzyme in the HIV life-cycle, in which it recodes the viral genetic material and converts RNA into DNA. Extracts from plant species has been reported to inhibit the activity of HIV-1 RT in previous studies[31-34]. These extracts with good inhibitory activity on HIV-1 RT, in addition to extracts of *T. sericea* and *A. karoo* could be provide a good source of potent compounds for therapeutic strategy against HIV-1 RT.

CONCLUSION

After ethno-botanical evaluation of 12 medicinal plants used to treat STD's, some of the selected plant species had interesting potential as alternative therapeutic strategy to treat these diseases. Most of the plants had promising activity against organisms and enzymes implicated in STD's and propagation of inflammation respectively. These results may further validate the anecdotal claims for the use of these medicinal plants in the treatment of STD's. Additional work is required to isolate and identify the bioactive compounds that are responsible for the observed ethno-pharmacological activities.

CONFLICT OF INTEREST

All the authors declare no competing interests.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the National research Foundation (Grant: 99022) for financial support. We appreciate the support we received from the Tribal

Councils, Traditional healers and Department of Plant and Soil Sciences, University of Pretoria.

REFERENCES

1. Chen W, Zhou F, Hall BJ, Wang Y, Latkin C, Ling L, Tucker JD.Spatial distribution and cluster analysis of risky sexual behaviours and STD's reported by Chinese adults in Guangzhou, China: a representative population-based study. Sexually transmitted infections. Sex Transm infect, 2016
2. Shaukat S, Kazmi AH. Sexually transmitted infections and syndromic management. J Pak Assoc Dermatol, 2015; 25: 159-161.
3. Ruddock, P.S., Charland, M., Ramirez, S., López, A., Towers, G.N., Arnason, J.T., Dillon JAR. Antimicrobial activity of flavonoids from Piper lanceaefolium and other Colombian medicinal plants against antibiotic susceptible and resistant strains of Neisseria gonorrhoeae. Sex Trans Disease, 2011; 38: 82-88.
4. Gottlieb SL, Low N, Newman LM, Bolan G, Kamb M, Broutet N. Toward global prevention of sexually transmitted infections (STIs): the need for STI vaccines. Vaccine, 2014; 32:1527-1535.
5. Houngbeme AG, Gandonou C, Yehouenou B, Kpoviessi SD, Sohounhloue D, Moudachirou M, Gbaguidi FA. Phytochemical analysis, toxicity and antibacterial activity of Benin medicinal plants extracts used in the treatment of sexually transmitted infections associated with HIV/AIDS. Int J Pharm Sci Res,2014; 5: 1739.
6. Jadhav N, Kulkarni S, Mane A, Kulkarni, R, Palshetker A, Singh K, Kulkarni S. Antimicrobial activity of plant extracts against sexually transmitted pathogens. Nat Prod Res,2015; 29: 1562-6.
7. Gail H, Tarryn B, Oluwaseyi A, Denver D, Oluchi M, Diana G. An Ethnobotanical survey of medicinal plants used by traditional health practitioners to manage HIV and its related opportunistic infections in Mpoza, Eastern Cape province, South Africa. J Ethnopharmacol, 2015; 171:109-15.
8. Lorna M, Barbara P, Mukhlesur R. Antimicrobial resistance and synergy in herbal medicine. J Herbal Med, 2016; 6: 53-58.
9. Oyedemi SO, Oyedemi BO, Prieto JM, Coopoosamy RM, Stapleton PS, Gibbons S. In-vitro assessment of antibiotic-resistance reversal of a methanol extract from Rosa canina L. South Afr J Bot, 2016;105: 337-342.
10. Pawar MM, Patil SD, Jadhav AP, Kadam VJ. In-vitro antimicrobial activity and phytochemical screening of selected Indian medicinal plants. Indo American J Pharm Res, 2015; 5: 593-600.
11. Mulaudzi RB, Ndhlala AR, Kulkarni MG, Finnie JF, Van Staden J. Anti-inflammatory and mutagenic evaluation of medicinal plants used by Venda people against venereal and related diseases. J Ethnopharmacol, 2013; 146: 173-179.
12. Ferreira VH, Nazli A, Dizzell SE, Mueller K, Kaushic C. The anti-inflammatory activity of curcumin protects the genital mucosal epithelial barrier from disruption and blocks replication of HIV-1 and HSV-2. PloS one, 2015; 10:4.
13. Adebayo SA, Dzoyem JP, Shai LJ, Eloff JN.The anti-inflammatory and antioxidant activity of 25 plant species used traditionally to treat pain in southern African. BMC Complement Altern Med, 2015:15, 159.
14. Platt L, Easterbrook P, Gower E, McDonald B, Sabin K, McGowan C, Yanny I, Razavi H, Vickerman P. Prevalence and burden of HCV co-infection in people living with HIV: a global systematic review and meta-analysis. Lancet, 2016; 16: 797-808.
15. Valadon ALC, Abreu CM, Dias JZ, Arantes P, Verli H, Tanuri A, de Aguiar RS. Natural plant alkaloid (Emetine) inhibits HIV-1 replication by interfering with Reverse Transcriptase activity. Mol, 2015; 20: 11474-11489.
16. Das K, Arnold E. HIV-1 reverse transcriptase and antiviral drug resistance. Part 1. Curr Opinion virol, 2013; 3: 111-118.
17. Pant K, Gorelick RJ, Rouzina I, Williams, MC. Single molecule study of HIV-1 Reverse Transcriptase polymerization activity in the presence of NC. Biophysical J, 2014; 106: 272a.
18. Tshikalange TE, Hussein A. Cytotoxicity activity of isolated compounds from *Elaeodendron transvaalense* ethanol extract. J Med Plants Resear, 2010; 4: 1695-1697.
19. Choi JY, Cho IC, Lee GI, Min SK. Prevalence and associated factors for four sexually transmissible microorganisms in middle-aged men receiving general prostate health check-ups: A polymerase chain reaction-based study in Korea. Korean J urology, 2013; 54: 53-58.
20. Eloff JN. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. Planta Medica, 1998; 64: 711–713.
21. Nair R. HIV-1 reverse transcriptase inhibition by Vitex negundo L. leaf extract and quantification of flavonoids in relation to anti-HIV activity. J Cell Mol Biol, 2012; 10: 53-59.
22. de Boer M, Heuer C, Hussein H, McDougall S. Minimum inhibitory concentrations of selected antimicrobials against Escherichia coli and Trueperella pyogenes of bovine uterine origin. J Dairy Sci, 2015; 98: 4427–4438.
23. Nielsen TRH, Kuete V, Jäger AK, Meyer JJM, Lall N. Antimicrobial activity of selected South African medicinal plants. BMC Complement Altern Med, 2012; 12:74.
24. Hamza OJ, van den Bout-van CJ, Matee MI, Moshi MJ, Mikx FH, Selemani HO, Verweij PE. Antifungal activity of some Tanzanian plants used traditionally for the treatment of fungal infections. J Ethnopharmacol, 2006; 108: 124-132.
25. Samie A, Tambani T, Harshfield E, Green E, Ramalivhana JN, Bessong PO. Antifungal activities of selected Venda medicinal plants against Candida albicans, Candida krusei and Cryptococcus neoformans

isolated from South African AIDS patients. Afr J Biotech, 2010; 9:20.
26. Mulaudzi RB, Ndhlala AR, van Staden J. Ethnopharmacological evaluation of a traditional herbal remedy used to treat gonorrhoea in Limpopo province, South Africa. South Afr J Bot, 2015; 97: 117-122.
27. Mabona U, Viljoen A, Shikanga E, Marston A, van Vuuren S. Antimicrobial activity of southern African medicinal plants with dermatological relevance: From an ethno-pharmacological screening approach, to combination studies and the isolation of a bioactive compound. J Ethnopharmacol, 2013; 148: 45-55.
28. Belemtougri RG, Constantin B, Cognard C, Raymond G, Sawadogo L. Effects of two medicinal plants Psidium guajava L. (Myrtaceae) and Diospyros mespiliformis L. (Ebenaceae) leaf extracts on rat skeletal muscle cells in primary culture. J Zhejiang Univer Sci B, 2006; 7: 56-63.
29. Sharifi-Rad J, Hoseini-Alfatemi SM, Sharifi-Rad M, Da Silva JAT. Antibacterial, antioxidant, antifungal and anti-inflammatory activities of crude extract from *Nitraria schoberi* fruits. Biotech, 2015; 5: 677-684.
30. Cock IE. The medicinal properties and phytochemistry of plants of the genus Terminalia (Combretaceae). Inflammopharmacol, 2015; 23: 203-229.
31. Krishnaveni M. Medicinal Plants–A Boon for HIV/AIDS. J Pharm Resear, 2012; 5:12.
32. Moll A, Heyman HM, Meyer JJM. Plants with activity against the live HIV virus and the enzyme, reverse transcriptase. South Afr J Bot, 2013; 86: 148.
33. Maregesi SM, Hermans N, Dhooghe L, Cimanga K, Ferreira D, Pannecouque C, Apers, S. Phytochemical and biological investigations of Elaeodendron schlechteranum. J Ethnopharmacol, 2010; 129: 319-326.
34. Hedimbi M. Evaluation of selected Namibian ethno-medicinal plants for anti-HIV properties. PhD Dissertation. University of Namibia, 2015.