



Antimicrobial and anti-inflammatory activities of selected medicinal plants against pathogens causing sexually transmitted infections

Ndivhuho Patience Nthulane¹, Salerwe Mosebi¹, Thilivhali Emmanuel Tshikalange², Monde Alfred Nyila¹, Ledile Thabitha Mankga^{1*}

¹Department of Life and Consumer Sciences, University of South Africa, Private BagX06, Florida, 1710, South Africa

²Department of Plant and Soil Sciences, University of Pretoria, Private Bag X20, Hatfield 0028, South Africa

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ABSTRACT

Introduction: Worldwide, more than one million sexually transmitted infections (STIs) are acquired daily. The diversity and frequency of sexual infections caused by pathogenic microorganisms have increased thus becoming a major cause of illness and mortality amongst young adults. Medicinal plants have been good remedies for the treatment of STIs since ancient times. In this study, we evaluated antimicrobial, anti- Human immunodeficiency virus (HIV) and anti-inflammatory activities of five selected medicinal plants.

Methods: We determined the antimicrobial activities of plant extracts against the bacteria causing common STIs. Then, the anti-inflammatory activities were evaluated by measuring the inhibition of the pro-inflammatory enzyme, 15-lipoxygenase (15-LOX) and we further investigated the plants extracts of anti-HIV activities against the recombinant HIV-1 enzyme, reverse transcriptase.

Results: Methanol extract of *Terminalia sericea* and dichloromethane (DCM) extract of *Bidens pilosa* exhibited good activities against *Neisseria gonorrhoeae* and *Gardnerella vaginalis*. Ethyl acetate, dichloromethane and methanol extracts of *Bidens pilosa* exhibited good activities against *Candida albicans*. Ethyl acetate extract of *K. africana* and methanol extract of *B. pilosa* showed good anti-inflammatory activities. Ethyl acetate, DCM and methanol extracts of *T. sericea* exhibited promising anti-HIV-1 activities by inhibiting the reverse transcriptase whilst methanol extracts of *T. dregeana* showed low anti-HIV-1 activity.

Conclusion: These plants showed promising activity against the propagation of inflammation, displayed good antimicrobial activities against the bacteria causing STIs and could be used as potential leads and/or source for new drug candidates.

Implication for health policy/practice/research/medical education:

The studied plant extracts possess significant antimicrobial and anti-inflammatory activities and might contribute to the discovery of alternative antibiotics.

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Introduction

Sexually transmitted infections (STIs) among young adults in many rural communities have a profound impact on sexual and reproductive health and remain a major public health concern with more than one million STIs acquired daily, worldwide (1-3). The World Health Organization (WHO) identified *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, *Treponema pallidum* and *Trichomonas vaginalis* as the pathogens that cause STIs. *N. gonorrhoeae*

and *T. pallidum*, on the other hand, have been considered the most common causes of STIs in developing countries (4,5).

The STIs caused by *N. gonorrhoeae* result in women infertility and pelvic inflammatory disease, while *T. pallidum* is considered the causative agent of syphilis that increases the risk of HIV acquisition and numerous hemolysin (6-10). Syphilis leads to approximately 305 000 fatal neonatal deaths in pregnant women every year,

*Corresponding author: Ledile Thabitha Mankga,
Email: mankglt@unisa.ac.za

thus leaving around 200 000 infants with increased risk of prematurity, low-birth-weight and congenital disease (3,7).

Furthermore, infectious diseases caused by antimicrobial-resistant microbes are becoming a serious problem all over the world (11,12). Inappropriate use of antibiotics has been attributed to the development of antibiotic resistance and the global emergence of multi-drug resistant bacteria that gradually limit the efficacy of existing drugs resulting in treatment failure (13). Molecular mechanisms responsible for the development of drug resistance are a subject of great interest and have also been highlighted in various reports revealing clinical resistance to antifungal agents (14).

Medicinal plants have been touted as the possible sources of new classes of antimicrobial agents with novel modes of action and represent a viable alternative to treat and/or cure the infections that result from the resistant microbes. Medicinal plants have been used as remedies for the treatment of STIs since ancient times (15). The WHO reported that about 80% of the population in developing countries are dependent on medicinal plants as the primary source of health care (16). Accordingly, medicinal plants have been used to treat different ailments and they are also regarded as remedies with several advantages such as fewer side effects, low cost and are readily available (17-19). Plant extracts constituents of bioactive secondary metabolites such as flavonoid, alkaloids, tannins, terpenoids and phenolics act synergistically to combat the microbial growth (20). The validation and approval of medicinal plants for the treatment of diseases caused by pathogens could be of great value to most people in rural communities who have limited access to vital, life-saving and often costly modern medicine (21-23). Therefore, in this study, we aimed to evaluate the antimicrobial and anti-inflammatory activities of selected medicinal plants against bacteria or pathogens that cause STIs. Firstly, we evaluated the antimicrobial activity using different solvents. We, then determined the anti-inflammatory activities using the plant extracts on 15-lipoxygenase (15-LOX) enzyme. Lastly, we determined the total phenolic content and the inhibitory activity of plant extracts on HIV-1 reverse transcriptase (RT) enzyme.

Materials and Methods

Plants materials

Medicinal plants were selected based on their ethnomedicinal uses in the treatment of STIs as previously described (24-31). Plant materials were collected from the Walter Sisulu National Botanical Garden in Roodepoort, Gauteng province (South Africa). A voucher specimen of each plant were also prepared (Table S1).

Preparation of plant extracts

Plant materials were air-dried and ground into a fine powder. Different powdered plant materials were weighed

and dissolved in dichloromethane (DCM), ethyl acetate and methanol, respectively. The honey jars were vigorously shaken for 24 hours using a shaker at room temperature. The extracts were filtered using Whatman filter paper and the remaining plant materials were discarded. The extracts were then transferred into pre-weighed labeled bottles and placed in the fume hood to evaporate the remaining solvents.

Microbial strains

The following microorganisms were used in this study: *Neisseria gonorrhoeae* (ATCC 49226), *Gardnerella vaginalis* (ATCC14018) and *Candida albicans* (ATCC10231). All pathogens were cultured in a sterile fume hood. *N. gonorrhoea* was inoculated in Mueller-Hinton broth and incubated at 37°C for 24 hours in a humid environment enriched CO₂. *G. vaginalis* was inoculated in brain and heart infusion broth and incubated at 37°C for 24 hours. *C. albicans* was inoculated in tryptone soya broth and incubated at 37°C for 24 hours.

Antimicrobial activity

Antimicrobial activity was determined using broth micro-dilution method in 96-well plate as previously described (32). Briefly, 50 mg of each plant extract was weighed in 2 mL microcentrifuge tubes and each extract was dissolved in 1 mL of 10% dimethyl sulfoxide (DMSO). Microorganisms were inoculated in sterile broth and prepared to a density of 1.5×10^8 colony forming units (CFU) per mL, corresponding with the 0.5 McFarland standard. Then, a 100 µL of broth was added to the wells of the plate and a 100 µL of each dissolved plant extract (50 mg/mL) was added to the first well in triplicate and serially diluted. Later, a 100 µL of pathogen suspension was added to each well except control wells, ciprofloxacin that was used as a control compound for all the extracts, and 10% of DMSO used as a negative control. The plates were incubated at 37°C for 24 hours. Finally, the results were read based on colour change after adding 40 µL of 0.2 mg/mL Presto Blue dye. A pink colour change indicated microbial growth whereas clear wells indicated inhibition of growth by test plant extract. The lowest concentration that showed no visible pathogen growth was recorded as the MIC for each microorganism.

Anti-inflammatory activity

The anti-inflammatory activity of the plant extracts was performed as previously described (33). Briefly, plant extracts were prepared at 10 mg/mL in DMSO and the 15-LOX enzyme was prepared on ice up to the final concentration of 200 units/mL. A volume of 12.5 µL of each plant sample and controls were then added to 487.5 µL of 15-LOX in 96-well microtiter plate and incubated for 5 minutes at room temperature. After incubation 500 µL of substrate solution was added to 96 well microtiter plate and incubated for 5 minutes at room temperature. After

incubation, the absorbance was measured at 234 nm using the microtiter plate reader. Quercetin (1 mg/mL) was used as a control compound and DMSO as a negative control. The results were expressed as mean \pm standard error of mean obtained from two independent experiments.

HIV-1 reverse transcriptase inhibition studies

The activity of plant extracts on reverse transcriptase activity was determined using the HIV-1 RT colorimetric ELISA kit (Roche). Firstly, all plant materials were weighed up to 3 mg and dissolved in 1 mL DMSO to make a final concentration of 3 mg/mL stock solution. A stock solution of 10 μ L was added to 90 μ L of lysis buffer to make a final concentration of 0.3 mg/mL. The RT enzyme was prepared to a stock solution of 0.764 mg/mL and 0.327 μ L was added to 1000 μ L. Lastly, 20 μ L of enzyme, diluted extracts and reaction mixture (10 μ M dUTP/dTTP, template/primer hybrid) were added together into wells of the plate. Then, doxorubicin at various concentrations (25 μ g/mL, 50 μ g/mL and 100 μ g/mL) dissolved in 10% DMSO was used as a control compound, whilst lysis buffer with reaction mixture was used as a negative control. The plates were incubated for 1 hour at 37°C. After incubation, the wells were rinsed three times with 200 μ L of washing buffer per well. After washing, 200 μ L of anti-digoxigenin-peroxidase (DIG-POD) solution, prepared to a final concentration of 200 mU/mL, was added to each well, covered with foil and incubated for 1 hour at 37°C. After incubation, the solutions were removed from the plate wells and washed three times with 200 μ L of washing buffer. Thereafter, 200 μ L of 2,2-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) substrate solution was added to each well and allowed to stand for 10 minutes. The absorbance was then measured on a microtiter plate reader at a wavelength of

405 nm with a reference wavelength of 490 nm. The assay was performed in triplicate and percentage inhibition calculated using the following formula:

$$\% \text{ Inhibition} = \{1 - (OD \text{ sample} / OD \text{ negative control})\} \times 100 \quad (1)$$

where OD is the optical density of the sample at 405 nm.

Determination of total phenolic content

The amount of total phenolic content in plant samples was determined according to the Folin-Ciocalteu method as previously described but with slight modifications (34). In brief, 25 μ L of the extract was treated with 250 μ L of Folin-Ciocalteu reagent for 5 minutes. The reaction was stopped by adding 750 μ L of 20% anhydrous sodium carbonate. The volume was made up to 5 mL with distilled water and incubated at room temperature for 2 hours. After incubation, the absorbance was read at 760 nm using the spectrophotometer. The phenolic content was determined from the standard curve of different concentrations of gallic acid and results were expressed as mg/g gallic acid equivalent (GAE).

Results

Antimicrobial activity

The antimicrobial activity results are expressed in minimum inhibitory concentration (MIC) values shown in Table 1. The methanol extract of *Terminalia sericea* and DCM extract of *Bidens pilosa* showed good activity with MIC value of 0.8 mg/mL (Table 1). Ethyl acetate and DCM extracts of *K. africana* and DCM extract of *T. dregeana* displayed some antibacterial activity with MIC of 8.1 mg/mL. Methanol extract of *K. africana*, *C. papaya* and *B. pilosa* gave similar antibacterial activities with MIC value

Table 1. The minimum inhibitory concentration (MIC) values of plant species tested against different microorganisms

Sample	Extraction solvent	<i>Neisseria gonorrhoeae</i>	<i>Gardnerella vaginalis</i>	<i>Candida albicans</i>
Ciprofloxacin		<0.2	3.1	<0.2
<i>K. africana</i>	ET	8.1	12.5	8.1
	DCM	8.1	6.3	8.1
	M	6.3	6.3	8.1
<i>T. sericea</i>	ET	3.1	3.1	6.3
	DCM	3.1	3.1	6.3
	M	0.8	1.6	6.3
<i>C. papaya</i>	ET	6.3	>12.5	1.6
	DCM	3.1	6.3	1.6
	M	6.3	6.3	1.6
<i>B. pilosa</i>	ET	6.3	3.1	0.4
	DCM	0.8	3.1	0.4
	M	3.1	3.1	0.4
<i>T. dregeana</i>	ET	3.1	12.5	6.3
	DCM	8.1	6.3	12.5
	M	3.1	6.3	3.1

ET, ethyl acetate; DCM, dichloromethane; M, methanol.

of 6.3 mg/mL (Table 1).

Methanol extract of *T. sericea* was tested on *Gardnerella vaginalis* and showed good activity with the MIC value of 1.6 mg/mL. The DCM extracts of *C. papaya*, *T. dregeana* and *K. africana* showed some activity with MIC of about 1.6 mg/mL. Ethyl acetate extract of *C. papaya* indicated no activity with the MIC value higher than the tested concentrations >12.5 mg/mL.

Ethyl acetate, DCM and methanol leaf extracts of *B. pilosa* showed best antimicrobial activity for *C. albicans* with an MIC value of 0.4 mg/mL. Meanwhile, ethyl acetate, DCM and methanol leaves extracts of *K. africana* and *T. sericea* indicated some antifungal activity with the MIC value of 8.1 mg/mL and 6.3 mg/mL, respectively (Figure 1).

Anti-inflammatory activity

The ethyl acetate and DCM extracts of *K. africana* and methanol extract of *B. pilosa* contained compounds with higher activity compared to quercetin (control compound) whereas all extracts of *T. sericea* showed low inhibitory activity (Table 2).

HIV-1 reverse transcriptase inhibition

The percentage inhibition activity of plant extracts against RT enzyme are shown in Figure 1. Inhibitory activities of the plant extracts against HIV-1 RT ranged from 15.79% to 80.16% inhibition at a final concentration of 100 µg/mL. The results were compared to the control compound, doxorubicin, which has been shown and validated to inhibit the RT enzyme (Figure 1). Ethyl acetate, DCM and methanol leaf extracts of *T. sericea* exhibited high inhibition activity (>70%).

Ethyl acetate and DCM extracts of *T. dregeana* leaf extract showed moderate inhibitory activity while

Table 2. The anti-inflammatory activity of medicinal plants. Percentage inhibition of 15-lipoxygenase (15-LOX) result

Sample	Extraction solvent	Inhibition %
Quercetin	-	49.61 ± 12.5
	ET	60.49 ± 10.23
<i>K. africana</i>	DCM	45.55 ± 11.94
	M	5.79 ± 1.62
<i>T. sericea</i>	ET	33.04 ± 1.23
	DCM	14.40 ± 5.54
	M	21.71 ± 5.71
<i>C. papaya</i>	ET	18.74 ± 1.84
	DCM	33.46 ± 3.22
	M	70.94 ± 3.11
<i>B. pilosa</i>	ET	6.69 ± 1.00
	DCM	25.46 ± 0.62
	M	58.47 ± 3.83
<i>T. dregeana</i>	ET	21.37 ± 1.08
	DCM	28.49 ± 1.90
	M	70.06 ± 2.53

methanol extract of *T. dregeana* indicated low inhibitory activity (Figure 1). Ethyl acetate and DCM extracts of *C. papaya* showed moderate inhibitory activity whilst methanol extract of *C. papaya* had no inhibitory activity. Ethyl acetate extract of *B. pilosa* displayed high inhibitory activity with an inhibition percentage of 80%. DCM and methanol extracts of *B. pilosa* yielded moderate inhibitory activity of 65% and 55%, respectively. Inhibitory activities of ethyl acetate and methanol extracts of *K. africana* were moderate at 65% and 60%, respectively whilst DCM extract of *K. africana* had the highest inhibitory activity of 70 % (Figure 1).

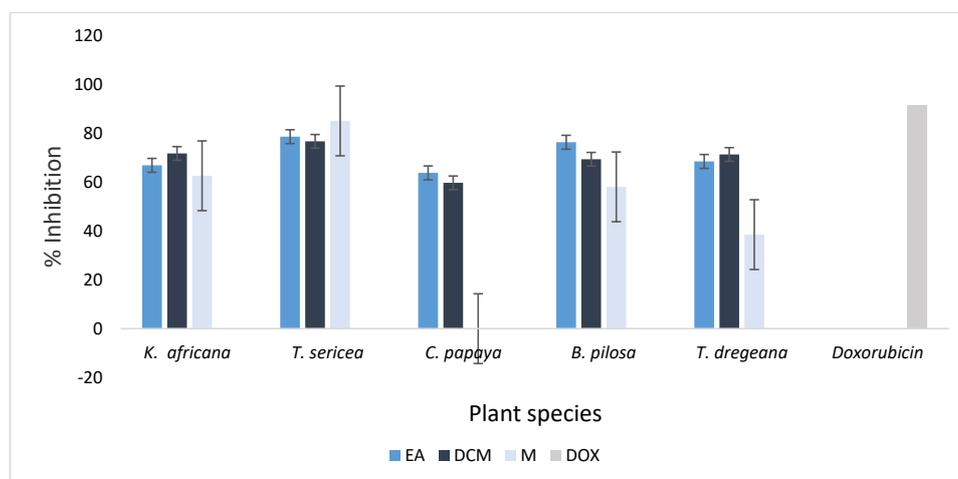


Figure 1. The activity of medicinal plant extracts on recombinant HIV-1 reverse transcriptase (RT) using HIV-1 RT colorimetric ELISA assay. The RT inhibitory activity of tested samples is expressed as percentage (%) inhibition. EA, ethyl acetate; DCM, dichloromethane; M, methanol; DOX, doxorubicin. The difference between the control (untreated) and the treated samples was determined by one-way ANOVA test (Bonferroni multiple comparison test). * $P < 0.05$ compared to control group.

Total phenolic content

The total phenolic contents of the extracts were examined using the Folin-Ciocalteu method and reported as a gallic acid equivalent (mg/g GAE) (Figure 2). The highest number of phenolic compounds were obtained from methanol extract of *T. sericea* at 270 mg/g GAE followed by ethyl acetate extract of *C. papaya* and methanol extract of *B. pilosa* with the total phenolic content of 130 mg/g GAE. Ethyl acetate extract of *K. africana* and DCM extract of *T. dregeana* had the lowest amount of phenolic compounds at 20 mg/g GAE. Interestingly, no phenolic compounds were detected in the DCM extract of both *B. pilosa* and *K. africana* and methanol extract of *K. africana*.

Discussion

Antimicrobial activity

Medicinal plants have been used as the possible source of new classes of antimicrobial agents with novel modes of action and represent a viable alternative to treat and/or cure the infections that result from resistant microbes. They have been used as remedies for the treatment of STIs since ancient times (15) and some medicinal plants have exhibited poorer or noteworthy antimicrobial efficacies against the pathogens associated with STIs. For example, in this study, the methanol extract of *T. sericea* and DCM extracts of *B. pilosa* showed good activities with the MIC value of 0.8 mg/mL. Also, the plant extracts of *T. sericea* have previously exhibited antimicrobial activity with the MIC value of 1 mg/mL while its roots tested against *N. gonorrhoeae* indicated the MIC value of 1.6 mg/mL (35,36). But, Mamba et al (36), indicated *T. sericea* having the least activity against *G. vaginalis* with the MIC value of >12.5 mg/mL in their research. The differences in MIC value results obtained in many studies are usually associated with the area of collection, environmental

conditions, the season of collection and the age of the plant (37). However, van Vuuren (35) tested *T. sericea* against anti-microbial activity and the results of aqueous extract correlated well with our findings. Furthermore, Naidoo and colleagues' study using a combination of plant extracts from *C. papaya*, *T. dregeana*, *K. africana* and *B. pilosa* demonstrated moderate antimicrobial activities that also correlated well with our findings in terms of organic extract (38). *C. papaya* has been shown to be efficacious in the treatment of STIs and has subsequently displayed good wound healing activities in the past (39). Steenkamp et al (40) tested the roots of *T. sericea* against *C. albicans* and showed inhibition of STIs bacteria. Similarly, Moshi and Mbwambo (41) tested the inhibition of *C. albicans* growth using methanol extracts of *T. sericea* and their results also indicated a notable inhibition of *C. albicans* growth that correlates with the findings of this study.

Anti-inflammatory activity

Inflammation plays an important role in the resolution of many diseases as well as the STIs (36) and the plant extracts that have anti-inflammatory activities usually inhibit the cyclooxygenase (COX) and/or LOX activity (36). Such plants usually have active therapeutic effects that promote healing and repair of tissue cells (42). *T. sericea* contains a phenolic anti-inflammatory compound, triterpenoid saponin, which can inhibit the COX-2 and 5-LOX enzymes (43). The observed anti-inflammatory activity may be due to the overall effects of plants constituents or compounds having similar action to the anti-inflammatory agent and non-steroidal anti-inflammatory drugs (48). However, all the tested leaf extracts of *T. sericea* exhibited low 15-LOX inhibitory activities. As a result, *T. sericea* might have compounds that have anti-inflammatory activities that are lower than that of quercetin. Also, the low inhibition

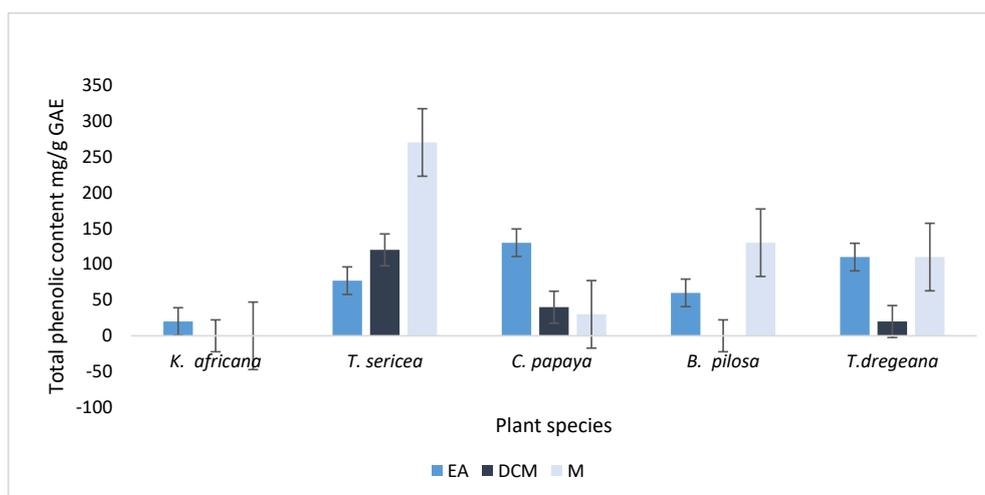


Figure 2. Total phenolic content of medicinal plants tested with the Folin-Ciocalteu method and expressed as mg/g gallic acid equivalents (GAE). EA, ethyl acetate; DCM, dichloromethane; M, methanol; DOX, doxorubicin. The difference between the control (untreated) and the treated samples was determined by one-way ANOVA test (Bonferroni multiple comparison test). * $P < 0.05$ compared to control group.

obtained could be due to impurities or low concentration of active compounds in the crude extract (44).

HIV-1 reverse transcriptase inhibition

People infected with HIV depend on antiretroviral drugs which include protease, reverse transcriptase and integrase inhibitors to prolong their lives. Nonetheless, antiretroviral drugs have disadvantages such as high costs, high toxicity profiles and development of resistance after long-term use (45). In this study, the plant extract of *T. sericea* showed the highest inhibitory activity of 102.8%. Similarly, Krishnaveni (46) and Mamba et al (36) used *T. sericea* extract, which inhibited both HIV-1 RT and COX-2 enzyme and this result concurs with those published, previously. Several plant extracts showed good inhibitory activities against HIV-1 RT and to our knowledge, there are no previous studies reporting the HIV-1 RT inhibition of *B. pilosa*, *C. papaya*, *K. africana* and *T. dregeana*.

Total phenolic content

Plant phenolic compounds have shown various medicinal properties such as anti-inflammatory, anti-diabetic, antimicrobial, anti-carcinogenic and antiviral activities (47). Due to these pharmacological properties, plant phenolic compounds have gained increasing attention in both modern and traditional medicine as a possible source of new therapeutics. In this study, methanol extract of *T. sericea* with lower crude extract yield of 2.19 % contained relatively high amounts of phenolics content which could be responsible for antimicrobial and anti-inflammatory activity observed. Anokwuru et al (49) indicated that methanol extract of *T. sericea* had the highest total phenolic content of 440 mg/g GAE. On the other hand, Singh et al (50) reported the total phenolic content of methanol extract from *B. pilosa* to be about 72 mg/g GAE. In addition, leaves of *C. papaya* were shown to have high total phenolic content that might provide the best sources of dietary antioxidants (51).

Conclusion

After evaluation of five selected medicinal plants against the pathogens causing STIs, some plants (*T. sericea* and *B. pilosa*) were shown to have good antimicrobial activity and had the potential to be used as alternative therapeutic options for the treatment of STIs. Additionally, some plants had promising activities against the propagation of inflammation. Accordingly, for a medicinal plant to be considered the candidate plant with good antimicrobial activity, it should contain the lowest MIC value in each microorganism tested. The plant extracts, which can inhibit the pro-inflammatory activities of the 15-LOX enzyme, could also be used for the discovery and development of new and novel anti-inflammatory compounds. Further characterization studies such as isolation, identification, solubility, pharmacokinetics and toxicity profiles of

bioactive compounds in these plants should be conducted to explore other pharmacological properties.

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Author's contributions

TET, MAN and LTM designed the project. NPN, MAN and LTM identified, collected and pressed plant material. NPN and TET carried out the laboratory work. NPN, LTM and SM analyzed the results. LTM, SM and NPN wrote the manuscript. All authors read the final version and confirmed its publication.

Conflict of interests

No conflict of interest.

Ethics considerations

The protocol was approved by the University of South Africa Research Committee with the ethics number: 2017/CAES/063.

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Supplementary Materials

Supplementary file 1 contains Table S1.

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