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

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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.

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A B S T R A C T

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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.

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 (ATCC149226), 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 $1.5 \times 10^8$ 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 ± 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 µL was added to 90 µL 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 µL was added to 1000 µL. Lastly, 20 µL of enzyme, diluted extracts and reaction mixture (10 µM dUTP/dTTP, template/primer hybrid) were added together into wells of the plate. Then, doxorubicin at various concentrations (25 μg/mL, 50 μg/mL and 100 μg/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 µL of washing buffer per well. After washing, 200 µL 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 µL of washing buffer. Thereafter, 200 µL 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:

\[
\% \text{ Inhibition} = \frac{1 - (\text{OD sample/OD negative control})}{1} \times 100
\]

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 µL of the extract was treated with 250 µL of Folin-Ciocalteu reagent for 5 minutes. The reaction was stopped by adding 750 µL 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. 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 of 6.3 mg/mL.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Extraction solvent</th>
<th>Neisseria gonorrhoeae</th>
<th>Gardnerella vaginalis</th>
<th>Candida albicans</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ciprofloxacin</td>
<td>ET</td>
<td>&lt;0.2</td>
<td>3.1</td>
<td>&lt;0.2</td>
</tr>
<tr>
<td><em>K. africana</em></td>
<td>DCM</td>
<td>8.1</td>
<td>12.5</td>
<td>8.1</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>6.3</td>
<td>6.3</td>
<td>8.1</td>
</tr>
<tr>
<td><em>T. sericea</em></td>
<td>ET</td>
<td>3.1</td>
<td>3.1</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>DCM</td>
<td>3.1</td>
<td>3.1</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>0.8</td>
<td>1.6</td>
<td>6.3</td>
</tr>
<tr>
<td><em>C. papaya</em></td>
<td>ET</td>
<td>6.3</td>
<td>&gt;12.5</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>DCM</td>
<td>3.1</td>
<td>6.3</td>
<td>1.6</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>6.3</td>
<td>6.3</td>
<td>1.6</td>
</tr>
<tr>
<td><em>B. pilosa</em></td>
<td>ET</td>
<td>6.3</td>
<td>3.1</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>DCM</td>
<td>0.8</td>
<td>3.1</td>
<td>0.4</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>3.1</td>
<td>3.1</td>
<td>0.4</td>
</tr>
<tr>
<td><em>T. dregeana</em></td>
<td>ET</td>
<td>3.1</td>
<td>12.5</td>
<td>6.3</td>
</tr>
<tr>
<td></td>
<td>DCM</td>
<td>8.1</td>
<td>6.3</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td>M</td>
<td>3.1</td>
<td>6.3</td>
<td>3.1</td>
</tr>
</tbody>
</table>

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 leave 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 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).

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

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 Mbambo (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 leave 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...
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. dregena*.

**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. sericca* 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.

**Funding/Support**

The University of South Africa funded this project for data collection and laboratory reagents (Departmental funding: grant code RC2015000). University of Pretoria provided the laboratory equipment and analysis of the data.

**Supplementary Materials**

Supplementary file 1 contains Table S1.

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Antimicrobial and anti-inflammatory activities of plants against STIs


