Antimicrobial, Antioxidant and Cytotoxicity Studies of Medicinal Plants Used in the Treatment of Sexually Transmitted Diseases

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ABSTRACT
Context: The World Health Organization reported that more than 1 million sexually transmitted infections (STIs) are acquired daily, with an estimated 357 million new infections caused by either chlamydia, gonorrhoea, syphils or trichomoniasis. Aim: The present study was aimed to evaluate antimicrobial, antioxidant and cytotoxicity activities of ethanol extracts of ten ethno- botanical selected plant species used to treat STD’s and related symptoms. Methods: The determination of antimicrobial susceptibility of plant extracts was done using the broth micro-dilution assay against five microorganisms. The free radical scavenging activity was determined by using DPPH (2, 2-diphenyly-1-picrylhydrazyl). Cytotoxicity activity of the plant extracts was done on Vero African monkey cells lines with 2, 3-bis [2-methoxy-4-nitro-5-sulphonyl]-2H-tetrazolium-5-carboxanilide (XTT) reagents. Results: Our results suggest that extracts of Acacia karroo and Rhoicissus tridentata subsp. cuneifolia are potential candidates with a good antimicrobial, antioxidant and low cytotoxicity activities. This results may support the anecdotal claims for the use of the selected plant species to treat venereal diseases.

Keywords: Antimicrobial, Antioxidant, cytotoxicity, sexually transmitted diseases.

INTRODUCTION
Sexually transmitted diseases are caused by microorganisms that can survive in warm, dark places including the anus, the genital areas of both males and females, and the mouth. According to the World Health Organization, more than 1 million sexually transmitted infections (STIs) are acquired every day worldwide. The report further stated that an estimated 357 million new infections with 1 of 4 STIs is caused by chlamydia, gonorrhoea, syphils or trichomoniasis annually. STIs such as gonorrhoea and chlamydia are the major causes of pelvic inflammatory disease (PID) and infertility in women. In South Africa, about 11 million cases are reported annually and young women are particularly vulnerable to STIs being one of the major contributors to the human immunodeficiency virus (HIV) epidemic. Neisseria gonorrhoeae is a Gram-negative pathogenic bacterium responsible for a range of diseases ranging from urethritis to disseminated gonococcal infections. It causes asymptomatic infections resulting in severe complications of PID. N. gonorrhoeae is a common sexually transmitted pathogen that significantly impacts female fertility, neonatal health, and transmission of HIV worldwide. Sexually transmitted and urinary tract infections (UTI’s) are believed to be the most frequent bacterial infection in women. About three quarters of the world population depend on traditional medicines for their health needs. The use of plants or plant extracts has long been used in different indigenous cultures in all parts of the world for the treatment of diseases. Herbal medicines are gaining popularity because of several advantages such as fewer side effects, better patient tolerance and acceptance due to its long history of use. The emergence of drug resistant strains has complicated the treatment of these infectious diseases. These complications have demanded the search for new antimicrobial substances from various sources. Plant extracts possess active compounds that act positively against sexually transmitted pathogens thus may be a good source for new active agents. The prevention of the spread of STDs, alongside with early detection and appropriate therapy, has the potential of reducing infections that might damage the reproductive tract. According to the WHO global survey, there are three common challenges faced in traditional medicine; the lack of sharing gathered information, lack of safety monitoring of herbal medicines and also the lack of methods to evaluate their safety and efficacy. In this study, ten plant species were selected for investigation based on their ethnomedicinal uses in the treatment STI’s and related infections. Crude ethanol extracts of the selected plant species were tested against various microorganisms; five bacteria and one fungal strain. These microorganisms have been reported to be associated with causing many sexually transmitted diseases (STDs) and urinary tract infections (UTIs).

MATERIAL AND METHODS

Plant material
Plants were selected on the basis of their ethnomedicinal uses in the treatment of sexually transmitted diseases (Table 1). All the plant material (bulbs, roots and seeds)
were collected from the Jongilanga community in Mpumalanga. Voucher specimen of each plant species were prepared and identified by Mr Calvin Mophuting at the HGJW Schweickerdt herbarium of the University of Pretoria.

**Preparation of plant extracts**

Plants parts were air dried and grounded into fine powder. The powdered plant materials were then dissolved in ethanol and vigorously shaken for 72 hr using a Labcon 3086U machine at moderate speed. The extract was filtered using a vacuum system and concentrated to dryness under reduced pressure using a Rotavapor. The plant extracts were stored in polytops at 4ºC environment before tested for different assays.

**Microbial strains**

The microorganisms used in this study include *Escherichia coli* (ATCC 8739), *Klebsiella oxytoca* (ATCC 700324), *Klebsiella pneumoniae subsp. pneumoniae* (ATCC 13883) and *Staphylococcus aureus* (ATCC 9144) grown at 37ºC on nutrient Agar. *Neisseria gonorrhoeae* ATCC 49226 was grown in chocolate agar (GC) under anaerobic conditions in a jar with anaerocult A (Merck SA (Pty) Ltd.), at 37ºC for 48 hours. *Klebsiella oxytoca* ATCC 700324 was grown at 37ºC in chocolate plates (96 wells) were seeded to determine colour change, after the addition of 50 μL of XTT reagent [1 mg/mL XTT with 0.383 mg/mL N-methyl dibenzopyrazine methyl sulphate (PMS)] to cells in the plates and incubated for 3 hours. After incubation, the absorbance of the colour was spectrophotometrically quantified using an ELISA plate reader, which measured the optical density at 490 nm with a reference wavelength of 690 nm. The assay was carried out in triplicate. Cell viability was assessed by comparing optical densities of samples with those of the negative control (DMSO control). The EC₅₀ values (concentration of sample that causes 50% cell death) were analysed using the GraphPad Prism Version 4.0, (Statistical program).

**RESULTS AND DISCUSION**

Sexually transmitted and urinary tract infections are highly prevalent in many rural areas of developing countries due to poor sanitary conditions and lack of proper hygiene. Most people in these communities depend on traditional healers and medicinal plants to treat std’s because they are too shy to talk to unknown western doctors or they don’t have access to modern medical facilities. Traditional healers also claimed their medicine are more effective and cheaper than western medicine. Several plant extracts have showed huge potential as effective measure in the treatment and management of sexually transmitted diseases including AIDS. The free radical scavenging activity was determined by using DPPH (2, 2-diphenyl-1-picrylhydrazyl), following the methods as described by Adébayo with a few modifications. For each tested sample, a dilution series was prepared in a 96-well microtiter ELISA plate, using distilled water as a dilution medium. All samples were tested in triplicate. The ten mg ethanol-extracted plant samples were tested at concentrations ranging from 500–3.91 μg/mL. Ascorbic acid (Vitamin C) was used as a positive control and ethanol was used as a solvent control (blank). Colour controls for the samples were used. DPPH was added to each test plate to determine colour change, for the presence of antioxidants. Upon the reduction of DPPH by the presence of an antioxidant, the DPPH changes from purple to colourless. After 30 min of incubation time at room temperature, the radical scavenging activity was determined by reading off absorbencies with an ELISA plate reader at 515 nm. The IC₅₀ (50%) inhibitory concentration of DPPH or turning colourless by an extract was determined using the GraphPad Prism Version 4.0. This is the amount of antioxidant that is necessary to decrease the initial absorbance of DPPH by 50%

**Antioxidant assay**

The free radical scavenging activity was determined by using DPPH (2, 2-diphenyl-1-picrylhydrazyl), following the methods as described by Adébayo with a few modifications. For each tested sample, a dilution series was prepared in a 96-well microtiter ELISA plate, using distilled water as a dilution medium. All samples were tested in triplicate. The ten mg ethanol-extracted plant samples were tested at concentrations ranging from 500–3.91 μg/mL. Ascorbic acid (Vitamin C) was used as a positive control and ethanol was used as a solvent control (blank). Colour controls for the samples were used. DPPH was added to each test plate to determine colour change, for the presence of antioxidants. Upon the reduction of DPPH by the presence of an antioxidant, the DPPH changes from purple to colourless. After 30 min of incubation time at room temperature, the radical scavenging activity was determined by reading off absorbencies with an ELISA plate reader at 515 nm. The IC₅₀ (50%) inhibitory concentration of DPPH or turning colourless by an extract was determined using the GraphPad Prism Version 4.0. This is the amount of antioxidant that is necessary to decrease the initial absorbance of DPPH by 50%.

**Antimicrobial activity**

Table 2 present antimicrobial activity results of the plant extracts. All the plant extracts showed a broad-spectrum activity against all the tested microorganisms. Most of the extracts had anti-microbial activity between the concentration ranges of 12.5 to 0.4 mg/mL. Extracts of *A. karroo* had a good antimicrobial activity and indicated the lowest MIC value against *Klebsiella oxytoca* (0.8 mg/ml), *Neisseria gonorrhoeae* (0.8 mg/ml) and *Staphylococcus*.
A. precatorius, D. mespiliformis and S. pertesiana exhibited moderate antimicrobial activity. Plant extracts of I. crassipes did not show activity against all the microorganisms, while X. caffra only showed activity against C. albicans. Previous studies have shown that plants produce secondary metabolites which have the ability inhibit the growth of microorganisms. These phytochemicals are now can be used in drug discovery for the treatment of infectious diseases\textsuperscript{24-26}.

Antioxidant activity
The antioxidant activities of the plant extracts in the study were determined by using the free radical 2, 2-diphenyly-1-picrylhydrazyl (DPPH). The free radical scavenging activity of the plant extracts indicated the presence of secondary compounds which can donate electron and reacted with free radicals to covert and terminate radical

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|}
\hline
Scientific name and family name & Voucher number & Plant part used & Medicinal uses & Secondary compound(s) & Biological activity \\
\hline
\textit{Abrus precatorius} L. Fabaceae & Mophuting 119334 & Aerial parts and seeds & Kidney problems, blood urine & Alkaloids, flavonoids, glycosides, triterpene & Antifungal and antibacterial activity\textsuperscript{11} \\
\hline
\textit{Acacia karroo} Fabaceae & Mophuting 119360 & Roots & STD’s & Anthroquinones, saponis, tannins & Antibacterial activity\textsuperscript{12} \\
\hline
\textit{Blepharis sub volubilis} subs. \textit{Sub volubilis} Acanthaceae & BC129 & Whole plant & Urinary infections & Flavonoids, saponis, steroids, tannins & \\
\hline
\textit{Diospyros mespiliformis} Ebenaceae & Mophuting 117182 & Roots & Urinary infections & Flavonoids, tannins terpenoids & Antimicrobial activity\textsuperscript{13} \\
\hline
\textit{Ipomoea crassipes} Hook Convolvulaceae & BC162 & Roots & HIV & * & * \\
\hline
\textit{Jatropha zeyheri} Euphorbiaceae & BC24 (B1) & Bulb & Testicle sores & Alkaloids, diterpenes, saponis & Antibacterial activity\textsuperscript{15} \\
\hline
\textit{Peltophorum africanaum} Fabaceae & BC40 (B1) & Roots & STD’s & Flavonoids & Antiinflammatory activity\textsuperscript{16} \\
\hline
\textit{Rhoicissus tridentata} subs. \textit{cuneifolia} Vitaceae & 49 (B1) & Roots & Bladder infections & Alkaloids, flavonoids, tannins & Antifungal activity, antioxidant\textsuperscript{17} \\
\hline
\textit{Senna petersiana} Fabaceae & BC08 (B1) & Roots & Bladder problems & Anthraquinone, glycosides & Antibacterial, antifungal activity\textsuperscript{18} \\
\hline
\textit{Ximenia caffra} Olacaceae & BC63 (B1) & Roots & HIV & Alkaloids & Antibacterial activity\textsuperscript{12} \\
\hline
\end{tabular}
\caption{The list of selected plant species and their traditional medicinal uses. Also shown are the different plant parts used and the secondary compounds found in each plant species}
\end{table}

\begin{table}[h]
\centering
\begin{tabular}{|l|c|c|c|c|c|c|}
\hline
Plant species & \textit{Candida albicans} & \textit{Escherichia coli} & \textit{Klebsiella oxytoca} & \textit{Klebsiella pneumoniae} & \textit{Neisseria gonorrhoeae} & \textit{Staphylococcus aureus} \\
\hline
\textit{Abrus precatorius} L. & 6.3 & 12.5 & 12.5 & 12.5 & 6.3 & 6.3 \\
\hline
\textit{Acacia karroo} & 1.6 & 1.6 & 0.8 & 1.6 & 0.8 & 0.4 \\
\hline
\textit{Blepharis sub volubilis} subs. \textit{Sub Volubilis} & 0.8 & 0.8 & 1.6 & 1.6 & 12.5 & 0.8 \\
\hline
\textit{Diospyros mespiliformis} & 0.8 & 3.1 & 1.6 & 1.6 & 0.8 & 3.1 \\
\hline
\textit{Ipomoea crassipes} Hook & <12.5 & <12.5 & <12.5 & <12.5 & <12.5 & <12.5 \\
\hline
\textit{Jatropha zeyheri} & 0.8 & 0.8 & 1.6 & 12.5 & 12.5 & 0.8 \\
\hline
\textit{Peltophorum africanaum} & 1.6 & 1.6 & 1.6 & 12.5 & 1.6 \\
\hline
\textit{Rhoicissus tridentata} subs. \textit{cuneifolia} & 0.8 & 0.8 & 1.6 & 3.1 & 6.3 & 0.8 \\
\hline
\textit{Senna petersiana} & 3.1 & 1.6 & 1.6 & 1.6 & 6.3 & 1.6 \\
\hline
\textit{Ximenia caffra} & 3.1 & <12.5 & <12.5 & <12.5 & <12.5 & <12.5 \\
\hline
\end{tabular}
\caption{The MIC (mg/mL) values of ten plant species tested against different microorganisms}
\end{table}
Table 3: The antioxidant activity (IC50 values) of ten tested plant extracts

<table>
<thead>
<tr>
<th>Plant species</th>
<th>IC50 values (µg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ascorbic acid</td>
<td>1.44</td>
</tr>
<tr>
<td>Abrus precatorius L.</td>
<td>2.25</td>
</tr>
<tr>
<td>Acacia karroo</td>
<td>0.83</td>
</tr>
<tr>
<td>Blepharis sub volubilis subs. Sub volubilis</td>
<td>11.77</td>
</tr>
<tr>
<td>Diospyros mespiliformis</td>
<td>1.21</td>
</tr>
<tr>
<td>Ipomoea crassipes Hook</td>
<td>10.41</td>
</tr>
<tr>
<td>Jatropha zeyheri</td>
<td>1.10</td>
</tr>
<tr>
<td>Peltophorum africanum</td>
<td>2.24</td>
</tr>
<tr>
<td>Rhoicissus tridentata subsp. cuneifolia</td>
<td>0.06</td>
</tr>
<tr>
<td>Senna petersiana</td>
<td>3.31</td>
</tr>
<tr>
<td>Ximenia caffra</td>
<td>9.21</td>
</tr>
</tbody>
</table>

Table 4: The cytotoxicity of extracts against Vero cell lines expressed in 50% inhibitory concentration (IC50) and the values of regression squared

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>IC50 (µg/mL)</th>
<th>R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinomycin D</td>
<td>0.009</td>
<td>0.07</td>
</tr>
<tr>
<td>Abrus precatorius L.</td>
<td>118.5</td>
<td>0.05</td>
</tr>
<tr>
<td>Acacia karroo</td>
<td>115.2</td>
<td>0.08</td>
</tr>
<tr>
<td>Blepharis subvolubilis subs.</td>
<td>83.35</td>
<td>0.06</td>
</tr>
<tr>
<td>Subvolubilis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diospyros mespiliformis</td>
<td>89.53</td>
<td>0.08</td>
</tr>
<tr>
<td>Ipomoea crassipes Hook</td>
<td>100.1</td>
<td>0.09</td>
</tr>
<tr>
<td>Jatropha zeyheri Sond.</td>
<td>123.4</td>
<td>0.09</td>
</tr>
<tr>
<td>Peltophorum africanum</td>
<td>133.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Rhoicissus tridentata subsp.</td>
<td>88.5</td>
<td>0.09</td>
</tr>
<tr>
<td>cuneifolia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Senna petersiana</td>
<td>70.83</td>
<td>0.05</td>
</tr>
<tr>
<td>Ximenia caffra</td>
<td>91.0</td>
<td>0.08</td>
</tr>
</tbody>
</table>

The IC50 of the extracts ranged from 11.77–0.06 µg/mL, while Vitamin C (positive control) had an IC50 value of 1.44 µg/mL. The lowest IC50 values were observed from Acacia karroo and Rhoicissus tridentata subsp. cuneifolia with IC50 values of 0.83 µg/mL and 0.06 µg/mL respectively (Table 3). The plant extracts of Diospyros mespiliformis and Jatropha zeyheri also exhibited IC50 values lower than that of vitamin C of 1.21 µg/mL and 1.10 µg/mL, respectively. The highest radical scavenging activities were observed from Blepharis subvolubilis subs. Sub volubilis, Ipomoea crassipes Hook and Ximenia caffra having IC50 values of 11.77 µg/mL, 10.41 µg/mL, and 9.21 µg/mL respectively.

Cytotoxicity of selected plant extracts

The plant extracts of A. precatorius, A. karroo, I crassipes, J. zeyheri and P. africanaum showed low toxicity with 50% viability of cells (EC50) at concentrations values of greater than 100 µg/mL (Table 4). The Lower cytotoxicity of P. africanaum extract was recently reported by Adebayo16 with an EC50 of 103 µg/mL, similar to our findings. The plant extract of Senna petersiana was found to be the most toxic among the tested plant extracts with an EC50 value of 70.83 ± 0.05 µg/mL, whereas B. subvolubilis, D. mespiliformis, R. tridentate and X. caffra showed moderate toxicity with an EC50 value of 83.35±0.06, 89.53±0.08, 88.5±0.09 and 91.0±0.08 respectively. Actinomycin D which was used as a positive control exhibited an IC50 value of 0.00932 µg/mL.

CONCLUSION

Our results indicated that, all the plant extracts exhibited significant antimicrobial and antioxidant activities and also suggest that all the extracts have little or low cytotoxicity against Vero cells. Acacia karroo and possibly Rhoicissus tridentata showed the most promising activities and have the potential to be developed as antimicrobial agents in the treatment of STDs and UTIs. The results of this study may lend credence and support to anecdotal claims for the use of the selected plant species to treat sexually transmitted diseases. However, further work is required to explore other pharmacological activities and isolation of bioactive compounds.

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