Short Communication

ANTIMICROBIAL ACTIVITY AND POTENCY OF CASSIA ABBREVIATA OLIV STEM BARK EXTRACTS

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ABSTRACT

Objective: To evaluate the potential antimicrobial activity and relative potency of aqueous and organic extracts of Cassia abbreviata Oliv stem bark against clinical isolates of Neisseria gonorrhoeae (NG), Pseudomonas aeruginosa (PA), Klebsiella pneumoniae (KP) and Candida albicans (CA).

Methods: Six extracts of Cassia abbreviata Oliv stem bark were prepared as follows: Four extracts were prepared following Soxhlet extraction procedure using ethanol, water, trichloromethane (TCM) and dichloromethane (DCM)+ethanol (1:1) as solvents, respectively. Two extracts were prepared by soaking the powdered stem bark in ethanol and water, respectively, and subjected to mechanical shaking for 8 hours. Antimicrobial activities and minimum inhibitory concentrations (MIC) of extracts were evaluated in-vitro using agar well diffusion assay.

Results: All extracts except TCM were active against NG and PA with an average MIC of 78.8 µg/ml. The cold aqueous extract had a lowest MIC (46.88 µg/ml) against PA, whereas both hot and cold ethanol extracts had a lowest MIC of 46.88 µg/ml against NG. Only hot extracts (ethanol, DCM+ethanol and TCM) were active against KP with a lowest MIC of 46.88 µg/ml of TCM extract. Both extracts of cold ethanol and DCM+ethanol were active against CA with cold ethanol extract having a lower MIC of 93.75 µg/ml compared with DCM+ethanol.

Conclusion: There were variations in in-vitro antimicrobial activities (microbial growth inhibition) of Cassia abbreviata Oliv stem bark extracts which depended on the solvent used. Organic extracts were more potent against NG, PA, KP and CA compared with aqueous extracts.

Keywords: Cassia abbreviata, Potency, Antibacterial, Antifungal.
The dry crude extracts obtained from Soxhlet extraction (ethanol, water, TCM, DCM+ethanol) and cold ethanol procedures were each reconstituted with dimethylsulfoxide to the concentration of 1500 µg/ml. The cold aqueous extract solution was diluted with distilled water to a concentration of 1500 µg/ml.

The clinical isolates of bacteria and fungi were obtained from the University Teaching Hospital Microbiology Laboratory in Lusaka. Agar well diffusion assays were performed on chocolate agar, Mueller-Hinton agar and blood agar for NG, PA and KP, respectively. On seven already prepared chocolate agar plates, pure isolates of NG were inoculated by streaking with a flame sterilized loop. The seven agar plates were each set up and marked A, B, C, D, E, F and P, respectively. Plate 'A' for ethanol extract, plate 'B' for water extract, plate 'C' for TCM extract, plate 'D' for DCM+ethanol extract, plate 'E' for cold ethanol extract, plate 'F' for cold water extract and plate 'P' for positive control, respectively. A well was made on each agar plate using a sterile borer. To each corresponding well, 0.1 ml of each diluted extract was added to the appropriate well of the agar plate. A well was made on each agar plate using a sterile borer. To each corresponding well, 0.1 ml of each diluted extract was added to the appropriate well of the agar plate.

The MIC was determined for each extract that was active against the microorganisms. Each bioactive extract was reconstituted to 1500 µg/ml and was serially diluted appropriately to six concentrations (750, 375, 187.5, 93.75, 46.88 and 23.44 µg/ml). NG was inoculated on seven chocolate agar plates marked C1, C2, C3, C4, C5, C6 and P. A well was made on each agar plate using a sterile borer. 0.1 ml of each diluted extract was added to the appropriate well of the agar plate. 0.1 ml of Ceftriaxone (100 mg/ml) was added to the well on agar plate marked 'P' as a positive control. All the agar plates were incubated for 24 hours at 37 °C. Clear zones of microbial growth inhibition were observed in comparison with positive controls and taken as indicators of antibacterial and antifungal activity [8]. The MIC was determined as the lowest concentration at which a clear zone of microbial growth inhibition was observed.

The plant was identified as Cassia abbreviata (Family: Fabaceae, Genus: Cassia, Species: Abbreviata, Author: Oliver). The yields of the extracts are shown in table 1. The results for bioactivities and MICs of extracts are presented in table 2 and 3, respectively.

### Table 1: Yields of extracts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Solvent used</th>
<th>Quantity of extracts (g)</th>
<th>Physical state</th>
<th>Powdered bark used (g)</th>
<th>Percent yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol</td>
<td>13.09</td>
<td>Brown crystals</td>
<td>69</td>
<td>19</td>
</tr>
<tr>
<td>2</td>
<td>Water</td>
<td>7.21</td>
<td>Brown crystals</td>
<td>60</td>
<td>12</td>
</tr>
<tr>
<td>3</td>
<td>TCM</td>
<td>0.13</td>
<td>Light green crystals</td>
<td>40</td>
<td>0.325</td>
</tr>
<tr>
<td>4</td>
<td>DCM+ethanol</td>
<td>4.47</td>
<td>Light brown crystals</td>
<td>60</td>
<td>12.8</td>
</tr>
<tr>
<td>5</td>
<td>Cold ethanol</td>
<td>0.13</td>
<td>Light brown crystals</td>
<td>50</td>
<td>8.9</td>
</tr>
<tr>
<td>6</td>
<td>Cold water</td>
<td>148 ml (7.2 g/l)</td>
<td>Brown liquid</td>
<td>50</td>
<td>2.13</td>
</tr>
</tbody>
</table>

*The cold water extract was quantified in the liquid state.*

### Table 2: In-vitro antibacterial and antifungal activities of plant extracts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test extract (1500 µg/ml)</th>
<th>NG</th>
<th>PA</th>
<th>KP</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Water</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>TCM</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>DCM+ethanol</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Cold ethanol</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Cold water</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Ceftriaxone</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>NT</td>
</tr>
<tr>
<td></td>
<td>Fluconazole</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
</tr>
</tbody>
</table>

Note: (+)= active (clear zone of microbial growth inhibition observed),(-)=inactive (no clear zone of microbial growth inhibition observed), (NT) = not tested.

### Table 3: MICs of bioactive plant extracts

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Test extract</th>
<th>MICs of extracts in µg/ml</th>
<th>NG</th>
<th>PA</th>
<th>KP</th>
<th>CA</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ethanol</td>
<td>46.88</td>
<td>93.75</td>
<td>187.5</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Water</td>
<td>93.75</td>
<td>93.75</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>TCM</td>
<td>NT</td>
<td>NT</td>
<td>46.88</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>DCM+ethanol</td>
<td>93.75</td>
<td>93.75</td>
<td>NT</td>
<td>93.75</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Cold ethanol</td>
<td>46.88</td>
<td>93.75</td>
<td>NT</td>
<td>93.75</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Cold water</td>
<td>93.75</td>
<td>46.88</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ceftriaxone</td>
<td>+</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ciprofloxacin</td>
<td>NT</td>
<td>+</td>
<td>+</td>
<td>NT</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fluconazole</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

Note: (+) = Active (clear zone of microbial growth inhibition observed); (NT) = Not tested; Number of agar plates for NG, PA, KP and CA were 31, 31, 19 and 13, respectively.
The highest percentage yield of extracts was achieved when ethanol was used as the extracting solvent. However, using TCM as solvent achieved the lowest percentage yield. This indicated that the majority of compounds were mostly soluble in hot ethanol and least soluble in hot TCM amongst extracting solvents used.

In the current study, five extracts (water, ethanol, DCM+ethanol, cold ethanol and cold water) showed in-vitro antibacterial activity against NG and PA by bacterial growth inhibition. Furthermore, three extracts (ethanol, TCM and DCM+ethanol) were active against KP. Our results further support the use of the decoction of Cassia abbreviata Oliv traditionally to treat gonorrhoea [9]. Similarly, Cassia abbreviata Oliv extracts (water, methanol and acetone) were found active against some gram positive and negative bacteria with MIC value range of 500–5000 µg/ml [10]. In the literature [11], the extract obtained using acetone demonstrated antibacterial activity against PA and KP with each MIC value of 80 µg/ml. In this study, the MIC values of active extracts ranged between 46.88 µg/ml and 187.5 µg/ml for PA and KP.

The differences in the MIC values of active extracts could arise from variations in solubility of specific bioactive compounds in different extracting solvents. The lower the MIC value the better the activity of the extract against micro-organisms [12]. Some scholars suggest the plant extracts MIC value of 100 µg/ml or lower as significant [12]. In this study, the hot ethanol extract had MIC value above 100 µg/ml for KP. This implies that the hot ethanol extract of Cassia abbreviata Oliv may be inferior in treating KP infections compared with other extracts.

In this study, only cold ethanol and DCM+ethanol extracts were found to have in-vitro antifungal activity against CA with cold ethanol extract being more potent than DCM+ethanol extract (MIC = 93.75 and 187.5 µg/ml, respectively). In a study by Kolaczkowski et al. [13], ethylacetate extract demonstrated antifungal activity against CA with MIC of 1200 µg/ml which was less potent than DCM+ethanol extract in the current study. Nevertheless, in another study by Hamza et al. [14], methanol extracts of root and stem bark were reported inactive against CA. Arguably, the method (maceration in 80% methanol) and duration (24 hours) of extraction employed could have affected the potency of active compounds. In the current study, Soxhlet extraction method (95% ethanol) was performed and lasted for 8 hours. It was therefore deduced that the cold ethanol and DCM+ethanol extracts of Cassia abbreviata Oliv may be useful in the treatment of superficial Candida infections.

The aqueous and organic extracts Cassia abbreviata Oliv stem bark have varying demonstrable antibacterial and antifungal activity through microbial growth inhibition. Organic extracts demonstrated more potent in-vitro activity than aqueous extracts. The extracts can potentially be applied in the treatment of community-acquired PA or KP infections and gonorrhoea. The antifungal activity of organic extracts (cold ethanol and DCM+ethanol) can be utilised in the management of CA infections like oral thrush.

ACKNOWLEDGEMENT

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CONFLICT OF INTERESTS

Authors have declared no conflict of interest

REFERENCES