Antibacterial, antibiofilm activity and cytotoxicity of crude extracts of *Ptaeroxylon obliquum* (*Ptaeroxylaceae*) used in South African ethnoveterinary medicine against *Bacillus anthracis* Sterne vaccine strain

INTRODUCTION:
Anthrax, a zoonotic disease and a potential biological weapon for terrorism is caused by *Bacillus anthracis*, a Gram-positive, spore-forming bacterium. Anthrax is endemic in South Africa and primarily affects livestock and wildlife species. Problems with the control of anthrax include growing resistance to present antibiotics and disinfectants or decontaminants coupled with low antibiotic access in rural and poor settings. Therefore, it is imperative to search for new antimicrobials, especially from natural sources that could offer effective and potent alternatives. Medicinal plants are putative sources of new antimicrobials. *Ptaeroxylon obliquum* (*Ptaeroxylaceae*) is used traditionally in South African ethnoveterinary medicine against anthrax in livestock. Here, the antibacterial and antibiofilm activity as well as cytotoxicity of the bark and fresh leaf crude extracts of *P. obliquum* was determined.

METHODOLOGY:
A two-fold serial microdilution assay was used to determine the minimum inhibitory concentration (MIC) of the acetone and chloroform extracts of bark and the chloroform extract of fresh leaves of the plant against *B. anthracis* Sterne vaccine strain. The crystal violet assay was used to determine antibiofilm activity while cytotoxicity (LC₅₀) was determined using a tetrazolium-based colorimetric (MTT) assay against Caco-2 intestinal cells.

RESULTS:
The MIC of the extracts ranged from 0.005 - 0.039 mg/mL compared to the positive control gentamicin (MIC = 4.9 X 10⁻⁵ mg/mL) with acetone bark extract being the most active (MIC = 0.005 mg/mL). Activity of extracts against *B. anthracis* biofilms was high (≥50% antibiofilm activity) at 0 h (prevention of biofilms; ranged between 87-99%), and 24 h (destruction of pre-formed biofilms; ranged between 58-93%). The chloroform fresh leaf extract had the best antibiofilm activity. The acetone bark extract had the best total antibacterial activity while cytotoxicity (LC₅₀) was determined using a tetrazolium-based colorimetric (MTT) assay against Caco-2 intestinal cells.

**Table 1:** Yield, half-maximal cytotoxicity (LC₅₀, minimum inhibition concentration (MIC), total antibacterial activity (TAA), selectivity index (SI), and antibiofilm activity of *P. obliquum* bark and fresh leaf against *B. anthracis* Sterne Vaccine strain

<table>
<thead>
<tr>
<th>Plant extract</th>
<th>Yield (mg/g)</th>
<th>LC₅₀ (mg/mL)</th>
<th>MIC (mg/mL)</th>
<th>TAA (Yield/MIC) (g/mL)</th>
<th>SI (LC₅₀/MIC)</th>
<th>Antibiofilm activity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetone (bark)</td>
<td>107.5</td>
<td>0.03</td>
<td>0.005</td>
<td>21500</td>
<td>6</td>
<td>87</td>
</tr>
<tr>
<td>Chloroform (bark)</td>
<td>108</td>
<td>0.06</td>
<td>0.013</td>
<td>8308</td>
<td>5</td>
<td>93</td>
</tr>
<tr>
<td>Chloroform (fresh leaf)</td>
<td>81.5</td>
<td>0.19</td>
<td>0.039</td>
<td>2090</td>
<td>5</td>
<td>99</td>
</tr>
<tr>
<td>Gentamycin</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

CONCLUSIONS:
The results support the use of the plant against anthrax in South African ethnoveterinary medicine. The extracts may be potential alternatives to chemicals as disinfectants or decontaminants, especially in rural areas where chemicals are not accessible to the farmers. The compounds responsible for anti-B. anthracis activity, and their mechanism(s) of action, is the focus of future studies.

References:
Elloff, 1998. Planta Med. 64, 711-713
Elisha et al, 2016. BMC Complement Altern Med. 16(1), 5
Wright et al., 2016. Phcog Commn. 5(3), 173