An Ethnobotanical approach to finding antimicrobial compounds in wooly blue curls (Trichostema lanatum) using a Kirby-Bauer disc diffusion assay

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**Abstract**

Plants can be an important source of creativity and production of new drugs. In this study, extracts of wooly blue curls (*Trichostema lanatum*) were made using DMSO and tested for antimicrobial activity on a panel of bacteria commonly found in separate ecological niches. Wooly blue curls (WBC) was chosen due to its being recorded as a strong antibacterial from previous research being a medicinal plant for the Chumash people. Therefore, shown to have antibacterial properties from previous research and was ethnobotany, which tries to characterize why cultures use bacteria and not against Gram-negative bacteria. However, wooly blue curls was chosen due to its being recorded as a strong commonly found in separate ecological niches.

**Results**

The bar graphs show the diameter of the zone of inhibition for each concentration of extract used along with a positive control (ampicillin) and a negative control (DMSO). The error bars show the standard deviation for each measurement.

**Discussion**

The resistance of Gram negative bacteria may be explained due to efflux pumps, that are able to extrude antimicrobials, and a second outer membrane that is hard for antimicrobials to penetrate. Most plant pathogens are Gram negative[1], potentially because they have greater resistance to plant antimicrobials. Gram positive bacteria also have efflux pumps[1], however they lack the outer membrane causing them to be more susceptible. Therefore the presence or absence of specific efflux pumps may explain the variation of responses between Gram positive bacteria. Due to activity against a variety of bacteria and the role of efflux pumps in resistance, the antimicrobial in WBC may have a conserved internal target among bacteria like the fatty acid cycle or a bacterial ribosome.

**Materials and Methods**

**Plant collection**

- The plant specimens were found in the Santa Monica Mtns. near Pepperdine University.
- We collected flowers, branches, and leaves of the plant sample.

**Preparation of plant extracts**

- The plant specimen was soaked in MeOH for 12-14 hrs. (3x)
- The solvent was then evaporated and the extract was re-dissolved in DMSO to its respective concentrations.

**Kirby-Bauer Disc Diffusion Assay (DDA)**

- Bacterial cultures were diluted to their respective concentrations and a cotton swab was used to inoculate an agar plate.
- A Sensi Disc Dispenser was used to introduce filter paper discs to the agar plates and 10 μL of extract was used to inoculate each disc.
- The plates were placed in a 37°C incubator for 18hrs. And the diameter of the zones of inhibition were measured in cm with a ruler. (C. xerosis was grown for 40hrs. due to a slow growth rate.)

**Figure 1:** Collection of *Trichostema lanatum*.

**Figure 2:** Example of a Kirby-Bauer disc diffusion assay. This shows the E. coli ΔtolC knockout mutant that allows for inhibition of the WBC extract.

**Table 1:** Summary of DDA data.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Niche</th>
<th>Gram (+/-)</th>
<th>125 mg/ml WBC inhibition (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus epidermidis</em></td>
<td>Skin</td>
<td>+</td>
<td>1.11 ± 0.07</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>Skin</td>
<td>+</td>
<td>0.92 ± 0.04</td>
</tr>
<tr>
<td><em>Corynebacterium xerosis</em></td>
<td>Skin</td>
<td>+</td>
<td>1.96 ± 0.19</td>
</tr>
<tr>
<td><em>Enterococcus faecalis</em></td>
<td>Gutt</td>
<td>+</td>
<td>1.16 ± 0.02</td>
</tr>
<tr>
<td><em>Bacillus subtilis</em></td>
<td>Gutt</td>
<td>+</td>
<td>1.14 ± 0.07</td>
</tr>
<tr>
<td><em>Bacillus megaterium</em></td>
<td>Env.</td>
<td>+</td>
<td>1.09 ± 0.07</td>
</tr>
<tr>
<td><em>Salmonella typhimurium</em></td>
<td>Gutt</td>
<td>-</td>
<td>0 ± 0</td>
</tr>
<tr>
<td><em>Escherichia coli WT</em></td>
<td>Gutt</td>
<td>-</td>
<td>0 ± 0</td>
</tr>
<tr>
<td><em>Escherichia coli ΔtolC</em></td>
<td>Gutt</td>
<td>-</td>
<td>1.33 ± 0.03</td>
</tr>
</tbody>
</table>

WBC extracts did not inhibit wild type Gram negative bacteria.

**Figure 3:** Comparison of the reactions of each bacteria with 125mg/ml WBC extract.

Gut bacteria have a variety of responses.

**Conclusions**

- Wooly blue curl extract does produce compounds that have antimicrobial properties.
- Wooly blue curl extracts do not affect all bacteria the same.

**References**


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