Abstract
Historically, Native Americans used Sambucus mexicana (elderberry) flowers and berries to treat bladder and kidney infections. Now, elderberry flower tea is available commercially as a health elixir. Antioxidant and antiviral effects of the berries have been demonstrated. The objective of this study was to determine the antibacterial activity of fresh S. mexicana flowers and berries. Flowers or berries were ground in acetone, 95% methanol, or sterile water. The resulting extracts were tested against Escherichia coli and Staphylococcus aureus bacteria in a disk-diffusion assay. No extracts inhibited growth of gram-negative E. coli. The acetone extracts inhibited growth of S. aureus at concentration 1.0 g/mL for berries and 0.848 g/mL for the flowers. The minimum inhibitory concentrations of the acetone extracts against S. aureus were 0.225 g/mL for flowers and 0.125 g/mL for berries. The results might explain the ethnobotanical use of Sambucus and further study may lead to a novel antimicrobial compound.

Background
Historically, Native Americans used Sambucus mexicana (elderberry) flowers and berries to treat bladder and kidney infections. Now, elderberry flower tea is available commercially as a health elixir. Antioxidant and antiviral effects of the berries have been demonstrated. The objective of this study was to determine the antibacterial activity of fresh S. mexicana flowers and berries. Flowers or berries were ground in acetone, 95% methanol, or sterile water. The resulting extracts were tested against Escherichia coli and Staphylococcus aureus bacteria in a disk-diffusion assay. No extracts inhibited growth of gram-negative E. coli. The acetone extracts inhibited growth of S. aureus at concentration 1.0 g/mL for berries and 0.848 g/mL for the flowers. The minimum inhibitory concentrations of the acetone extracts against S. aureus were 0.225 g/mL for flowers and 0.125 g/mL for berries. The results might explain the ethnobotanical use of Sambucus and further study may lead to a novel antimicrobial compound.

Materials and Methods
1. Preparation of extracts.
   - I ground the flower, berry, leaf, or stem with 95% methanol, acetone and distilled water in a mortar.
   - Extracts were centrifuged at 3360 g for 2 min. The supernatant was used for disk diffusion assays.
   - For the first trial, most of the extracts were 1.0 g/mL, with the exception of 0.5 g/mL in methanol and acetone, and 0.25 g/mL in water. barrels in acetone.
   - The second trials contained the ratios shown in Table 1.
2. Disk diffusion assay
   - Nutrient agar plates were inoculated with Staphylococcus aureus (ATCC 27665) and Escherichia coli (ATCC 11775) bacteria. Stainless filter paper disks saturated in plant extracts or solvents were placed on the cultures.
   - Cultures were incubated at 35°C for 24-48 hrs.
3. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC).
   - Serial dilutions (500-31.25 mg/mL) were made in nutrient broth and inoculated with S. aureus in cell well plates.
   - Cell well plates incubated at 35°C for 24-44 hrs.
   - Wells with no growth were subcultured in nutrient broth to determine the MBC.

Results
None of the aqueous extracts inhibited the test bacteria. None of these extracts inhibited gram-negative E. coli bacteria. Alcoholic and acetone extracts inhibited gram-positive S. aureus bacteria. The average zone of inhibition of the alcoholic and acetone extracts was 12.4 mm (Figure 2).

Discussion & Conclusion
- The results indicate that the elderberry plant inhibits growth of gram-positive bacteria. This is consistent with traditional use, as the berry and flower are the parts most used for medicinal purposes.
- A gram-positive bacterial compound is present in all plant parts. The compound is not water soluble.
- I am testing the acetone extract against the acid-fast Mycobacterium spp.
- Testing of HPLC fractions may isolate the active compound.

Table 1. Concentration of extracts for trial 2.

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Solvent</th>
<th>Concentration (g/mL)</th>
<th>MIC (g/mL)</th>
<th>MBC (g/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flower</td>
<td>Methanol</td>
<td>2.0</td>
<td>0.225</td>
<td>0.225</td>
</tr>
<tr>
<td>Leaf</td>
<td>Methanol</td>
<td>2.0</td>
<td>0.225</td>
<td>0.225</td>
</tr>
<tr>
<td>Leaf</td>
<td>Acetone</td>
<td>2.0</td>
<td>0.225</td>
<td>0.225</td>
</tr>
<tr>
<td>Leaf</td>
<td>Distilled Water</td>
<td>2.0</td>
<td>0.225</td>
<td>0.225</td>
</tr>
<tr>
<td>Stem</td>
<td>Methanol</td>
<td>1.0</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Stem</td>
<td>Acetone</td>
<td>1.0</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Stem</td>
<td>Distilled Water</td>
<td>1.0</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Berry</td>
<td>Methanol</td>
<td>1.0</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Berry</td>
<td>Acetone</td>
<td>1.0</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Berry</td>
<td>Distilled Water</td>
<td>1.0</td>
<td>0.125</td>
<td>0.125</td>
</tr>
</tbody>
</table>

Figure 3. MIC and MBC of acetone extracts against S. aureus.

Figure 2. Disk diffusion assay. Data averages of two trials. Plant parts inhibited S. aureus. Error bars = 1 standard deviation.

Literature Cited

Acknowledgements
Christina Case, Biology Professor, Skyline College
Pat Carter, Biology Lab Technician, Skyline College
Tiffany Reardon, Assistant Director, California MESA
Pat Carteer, Biology Lab Technician, Skyline College
Christine Case, Biology Professor, Skyline College
Pat Carteer, Biology Lab Technician, Skyline College
Christine Case, Biology Professor, Skyline College

Figure 1. Sambucus mexicana, blue elderberry. This shrub is in the Adoxaceae family. It is native to California and can be found in other parts of North America. I tested elderberry from Sweeney Ridge National Park in San Bruno, CA. Inset: Berries ripen in the summer.