Investigation Of The Antimicrobial Activity Of Chrysanthemum indicum

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Abstract
Pathogenic infections are one of the leading causes of death worldwide. The discovery of antibiotics aided in the fight against these infectious diseases; however, the continual increase in resistance against antibiotics has made the need for new antimicrobial compounds critical. Chrysanthemum indicum is used in traditional Chinese medicine to treat skin, throat, and eye infections. Our hypothesis is C. indicum has antimicrobial activity. Extracts were made by grinding dried flowers in water, methanol, or ethanol to a final concentration of 0.25 g/mL. Antimicrobial activity was tested using disk-diffusion assays against Escherichia coli, Staphylococcus aureus, Streptococcus mutans, and Aspergillus niger. The ethanolic extract inhibited growth of the three bacteria, but did not inhibit the fungus. The ethanol control did not inhibit the test organisms. The ethanolic extract decreased a Str. mutans culture by six log cycles. Heating the extract did not decrease its effectiveness, therefore the antibacterial compound is not likely a protein. The minimum inhibitory concentration is being determined. Additionally, the effect of C. indicum extract on bacterial capsular and, therefore, dental biofilm formation is being determined. The results of this experiment may aid in developing a new agent to fight against dental caries caused by Str. mutans.

Hypothesis
C. indicum has antimicrobial activity.

Materials & Methods
1. Dried C. indicum flowers were obtained from an Asian herbal store in San Francisco.
2. 10 g flowers were ground in 30 mL 95% ethanol and centrifuged at 4°C and 1,500 g for 10 min. The supernatant was tested for antibacterial activity.
3. Disk diffusion assays were used to test the extract against Escherichia coli (ATCC 11775), Staphylococcus aureus (ATCC 27659), methicillin-resistant S. aureus (MRSA, ATCC 43300), Streptococcus mutans (ATCC 25175), and Aspergillus niger (ATCC 16404).
4. Serial dilutions of the extract in nutrient broth (0.05 to 0.25 g/mL) were inoculated with E. coli, S. aureus, MRSA, and Str. mutans in cell well plates to determine the minimum inhibitory (MIC) and minimum bactericidal concentrations (MBC).

Results
• The ethanolic extract of C. indicum inhibits growth of E. coli, S. aureus, MRSA, and Str. mutans in disk diffusion assays (Table 1).
• The MIC and MBC against gram-positive and gram-negative bacteria was 0.25 g/mL (Table 2).
• Heating the ethanolic extract does not decrease antimicrobial activity (Table 3).
• In TSB + sucrose, Str. mutans produces capsules and sticks to the tube. The bacteria do not produce capsules or form a biofilm in TSB + sucrose + ethanolic C. indicum extract.

Discussion & Conclusion
• C. indicum is antibacterial against Str. mutans, E. coli, S. aureus, and MRSA.
• Str. mutans is a cariogenic bacterium, a major health issue around the world (10), thus it was selected for further testing.
• Results showed that heating of the extract did not reduce the effectiveness of inhibition against Str. mutans, suggesting that the antibacterial compound in the plant is not a protein.
• Capsules are the primary virulence factor of Str. mutans. The bacteria grew in the test tubes but did not form capsules.
• The results of these experiments demonstrate that an active compound in C. indicum may be developed as an antacidity ingredient for mouthwash or toothpaste.
• Further experiments are needed to isolate (HPLC) and classify and characterize (mass spectrometry) the active compounds and to test their effects against Str. mutans.
• Because the flower had been widely used to treat skin infections caused by fungi, more antifungal testing will be done.
• Identify the specific action of C. indicum against Str. mutans to develop a new agent to fight dental infection.

Table 1. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) of ethanolic C. indicum extracts (g/mL).

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>MIC g/mL</th>
<th>MBC g/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Heated Extract</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Unheated Extract</td>
<td>0.25</td>
<td>0.25</td>
</tr>
</tbody>
</table>

Table 2. Disk-diffusion assay.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Control</th>
<th>0.25 g/mL</th>
<th>Ethanol extract</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli</td>
<td>0.25</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>Str. mutans</td>
<td>0.25</td>
<td>0.15</td>
<td></td>
</tr>
<tr>
<td>S. aureus</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td>MRSA</td>
<td>0.25</td>
<td>0.25</td>
<td></td>
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</tbody>
</table>

Table 3. Effect of heat on antibacterial activity of C. indicum against Str. mutans.

<table>
<thead>
<tr>
<th>CFU/mL</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (no extract)</td>
<td>1.6 × 10⁸</td>
</tr>
<tr>
<td>Heated Extract</td>
<td>3.0 × 10⁸</td>
</tr>
<tr>
<td>Unheated Extract</td>
<td>8.0 × 10⁸</td>
</tr>
</tbody>
</table>

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Literature Cited

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