Antimicrobial Activity of Baccharis pilularis (Asteraceae), A Traditional Native American Herbal Medicine

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Abstract

Over the past 60 years, overuse of antibiotics has selected antibiotic-resistant strains of many bacterial pathogens. The emergence of these resistant pathogens demands new antibacterial compounds to deal with this crisis. The plants used for centuries by traditional healers are logical places to search for new antibacterial agents due to their proven ability to treat infections. Baccharis pilularis, coyote brush, is one such plant. It has been used to treat skin wounds and gastrointestinal ailments in Latin American and Native American traditional medicine. We have demonstrated that methanolic, ethanolic, and acetonic extracts of B. pilularis leaves and bark kill Staphylococcus aureus. We determined the minimal inhibitory concentrations of the extracts; methanolic extract (10.4 mg/mL), ethanolic extract (20.85 mg/mL), and acetonic extract (83.38 mg/mL). The purpose of this study is to isolate and characterize the antibacterial compound in the extracts. We are testing the antibacterial activity of compounds separated by chromatography. These findings may lead to development of plant-based, affordable antimicrobials to which bacteria are not resistant.

Table 1. Minimal bactericidal concentrations against S. aureus

<table>
<thead>
<tr>
<th>Extract</th>
<th>MIC (mg/mL)</th>
<th>MBC (mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetonic leaf</td>
<td>0.35</td>
<td>10.44</td>
</tr>
<tr>
<td>Ethanolic leaf</td>
<td>20.85</td>
<td>20.85</td>
</tr>
<tr>
<td>Methanolic leaf</td>
<td>41.73</td>
<td>41.73</td>
</tr>
<tr>
<td>Acetonic bark</td>
<td>83.36</td>
<td>166.77</td>
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Discussion & Conclusion

The active compound may provide an alternative treatment for antibiotic-resistant S. aureus.

Future Work

The active compound needs to be purified and characterized.

Background

There is a growing need for new antimicrobials to combat bacterial infections.

• Plants in the Baccharis genus are widely used in the Americas for their medicinal properties.
• B. dracunculifolia is used as an antiparasitic and a stomach remedy in Brazil. This plant has cytotoxic activity against leukemia cells (1).
• B. pilularis inhibits a variety of bacteria (2).
• B. dracunculifolia inhibits Staphylococcus mutans in vitro (5).
• In Paraguay, B. pseudoecheveri is commonly used as a folk remedy for the treatment of gastrointestinal disease (4).
• Native Americans used a decoction of B. douglasii to wash and disinfet wounds (3).
• The Coyote Brush, indigenous to the San Francisco Bay Area, used a B. pilularis infusion as a general remedy (6).

Antimicrobial Activity of

Baccharis pilularis (Figure 1) leaves were collected from off-road, natural sites. The plants had not been exposed to herbicides or highway emissions.

Methods

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Extract Preparation
• Fresh B. pilularis leaves and bark were ground in distilled water, 50% methanol, 80% ethanold, or 95% acetone to a final concentration of 250 mg/mL.
• Extracts were prepared by constant stirring for 48-72 hr at 23°C.
• Extracts were filtered using cheese cloth and supernatant was collected.

Agar Diffusion Assay
• Nutrient agar plates were aseptically inoculated with Escherichia coli (ATCC 11775), Staphylococcus aureus (ATCC 25923), Saccharomyces cerevisiae (ATCC 9763), and Apergillus niger (ATCC 16489).
• Sterile 10-mm filter disks were saturated with extracts and placed as inoculated plates.
• The solvents alone were used as controls.
• Plates were incubated at 37°C for 24 hr.

Minimal inhibitory concentration (MIC)
• Serial dilutions of methanolic, ethanolic, and acetonic extracts (0.16-333 mg/mL) were prepared in nutrient broth to final concentrations.
• Each dilution was inoculated with 100 L of S. aureus and incubated at 37°C for 24 hr.

Minimal bactericidal concentration (MBC)
• Methanolic, ethanolic, and acetonic extracts were separated through Sephadex G-25 beads.
• Extracts were separated through Sephadex G-25 beads.
• Each extract was used in the MIC wells showing no growth was transferred to nutrient broth and incubated at 37°C for 24 hr.

Results

B. pilularis leaf does not inhibit gram-negative E. coli, the yeast, or the mold.

B. pilularis leaf and bark extracts inhibit gram-positive S. aureus bacteria (Figure 2). The leaf and bark extracts are bactericidal (Figure 3).

• Methanolic, ethanolic, and acetonic extracts inhibited S. aureus (Table 1).

The antistaphylococcal compound was isolated by paper chromatography (Figure 4) and used for column chromatography.

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Figure 1: Baccharis pilularis, coyote brush. This Asteraceae plant is the indicator species for the coastal scrub community in northern California.

Figure 2. Disk diffusion assay of leaf extracts against S. aureus.

Figure 3: MICs and MBCs of leaf extracts against S. aureus.