An In Vitro Study of the Antimicrobial Activity of Vaccinium ovatum
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Abstract
As bacteria become increasingly resistant to antibiotics, alternative antimicrobials are needed to treat bacterial infections. For centuries, plants have been used by many cultures to treat a variety of infections. One such plant, Vaccinium ovatum, has been used by West Coast Native Americans to treat a variety of ailments. While much focus has been placed on the antimicrobial activity of F. macrocarpon, little is known about other species of Vaccinium. The purpose of our study is to investigate the antimicrobial activity of F. ovatum. An aqueous leaf extract (5.5 mL) inhibited growth of gram-positive bacteria, Staphylococcus aureus, but not gram-negative Escherichia coli bacteria in a well-diffusion assay. The crude extract is 35% less effective than a commercial penicillin disk (10 μg). The minimum inhibitory concentration of this extract against S. aureus was 0.3 g/mL. The antimicrobial activity of the plant does not appear to be caused by proteins because heat (56°C for 30 min) did not affect the extract’s activity. We are testing the aqueous extract from leaves and berries of F. ovatum against other gram-positive bacteria to ascertain its optimum potential use.

Methods
Extract Preparation
• Leaves and berries of F. ovatum were collected in California, Pacifica, California (Figure 1).
• Plant parts were ground into a homogeneous substance using mortar and pestle in the following solvents: 95% ethanol, acetone and water. Water was used to prepare berry extracts.
• Extracts had a final concentration of 500 mg/mL.
• Extracts were filtered through the cheesecloth and refrigerated at 4°C.

Well Diffusion Assay
• Nutrient agar was inoculated with Escherichia coli (ATCC 11775). Staphylococcus aureus (ATCC 29213). Streptococcus pyogenes (ATCC 12228). Streptococcus mutans (ATCC 25175) or Mycobacterium phlei (Wards 85W1691).
• Six-mm wells were made in the agar plates using a cork borer and filled with 50 μL of an extract.
• Antibiotic discs (Hardy Diagnostics) were used as positive controls: penicillin (10 μg disk), streptomycin (10 μg disk), rifampin (5 μg disk), and bacitracin (10 μg disk).
• Water, 95% ethanol, and acetone were used as negative controls.
• Nutrient agar plates were incubated at 35°C for 48-72 hr.

Minimum Inhibitory Concentration (MIC)
• Both leaf and berry aqueous extracts were serially diluted from 500 mg/mL to 19 μg/mL.
• The well diffusion assay was used for the MIC assay.
• Nutrient agar was inoculated with S. aureus, S. mutans, and M. phlei.
• All the nutrient agar plates incubated at 35°C for 48-96 hr.

Antimicrobial Peptide Assay
• Heated (50°C for 30 min), unheated aqueous leaf extracts, and sterile water (controls) were added to S. aureus in nutrient broth and incubated at 37°C for 3 hr.
• Plate counts were used to determine the number of surviving bacteria.

Results
• F. ovatum extracts had no effect on gram-negative bacteria. They did inhibit gram-positive bacteria.
• Extracts in polar solvents (water and acetone) showed the highest level of inhibition of gram-positive bacteria. Solvents alone, did not inhibit bacterial growth (Figure 2).
• The MIC against M. phlei was 200 mg/mL (Table 1).
• The antimicrobial activity of the berry extract was 51% less effective than the antibiotic rifampin against M. phlei (Figure 3).
• The leaf extract was 35% less effective than rifampin against S. aureus.
• For S. mutans the extract was 63% less effective than the antibiotic (bactericidal).
• The extract had no antimicrobial activity against S. pyogenes (Table 2).
• There was no significant difference (10%) between the antibacterial activity of heated and unheated leaf extracts. (Figure 4).

Discussion & Conclusion
• The extracts from leaves and berries of F. ovatum inhibited gram-positive bacteria.
• The aqueous extract was most effective suggesting the antibacterial compound is polar.
• Antibacterial activity is due to antimicrobial peptides.
• Aqueous leaf extract of F. ovatum may provide an alternative treatment for multi-resistant M. phlei.
• An aqueous berry extract of F. ovatum could be used in oral hygiene products.
• Aqueous leaf extract may treat S. aureus infections.
• Further studies could include investigation into the mechanism of inhibition and isolation and purification of the active compounds.

Literature Cited

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