Method Optimization for the Antimicrobial Screening of Pigmented Invasive Weed Extracts

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Introduction

New treatment options are required to combat antibiotic resistant infections (1). Plants are a promising source of new therapeutics because they produce phytochemicals that have diverse chemical structures and can inhibit bacteria (2,3). Though often overlooked in favor of traditional medicinal plants, invasive weeds produce large quantities of phytochemicals and persist in unfavorable environments by inhibiting the growth of other plants, bacteria, and fungi (4,5). Previously, Albertan invasive weed extracts were analyzed for their antimicrobial activity using Kirby-Bauer disk diffusion, broth microdilution, and drop-check assays (6). During these tests:

- Albertan invasive weeds extracts showed promising antimicrobial activity in qualitative Kirby-Bauer disk diffusion assays.
- Plant pigment molecules interfered with spectrophotometric optical density measurements of bacterial growth at 600 nm, preventing the quantification of activity in broth microdilution assays.

In these experiments, we used ultraviolet-visible (UV-Vis) spectrophotometry to develop a method for avoiding plant pigment interference during the antimicrobial screening of invasive weed extracts.

Methods

UV-Vis Spectrophotometry

- Albertan invasive weed extracts produced by sequential Soxhlet extraction with methanol, ethyl acetate, and hexane (6) were scanned at all wavelengths between 200 nm – 900 nm.

Growth Curve Analysis

- 100 mL cultures of *Escherichia coli, Bacillus subtilis, Pseudomonas aeruginosa,* and *Staphylococcus aureus* were grown in optimal growth conditions for each species.
- The optical density of these cultures was spectrophotometrically measured over time at both 750 nm and the standard wavelength, 600 nm.

Results

Antimicrobial Susceptibility Tests (6)

Extracts with Significant Activity in Qualitative Assays	Extracts with Plant Pigment Interference during Quantitative Assays
Leafy Spurge Flower/Leaf (hexane, ethyl acetate, methanol)	Leafy Spurge Flower/ Leaf (hexane)
Leafy Spurge Stem/Root (hexane, ethyl acetate)	Leafy Spurge Stem/Root (hexane)
Japanese Knotweed (hexane, methanol)	Japanese Knotweed (hexane)
Garlic Mustard (hexane, ethyl acetate)	Garlic Mustard (hexane)
Field Scabious (hexane)	Field Scabious (hexane)
Canada Thistle (hexane)	–
Yellow Toadflax (hexane, ethyl acetate)	–

Table 1. Albertan Invasive Weeds Extracts with Promising Antimicrobial Activity and Suspected Pigment Interference. Inconsistencies in the results between the quantitative broth microdilution and drop-check assays indicated that pigment molecules extracted by hexane solvents may have interfered with spectrophotometric bacterial growth measurements during the broth microdilution assay.

UV-Vis Spectrophotometry

Extraction Solvent	Ranges of Strong Absorbance	Ranges of Minimal Absorbance
Hexane	207-329 nm 383-459 nm 503-538 nm 610-668 nm	700-890 nm
Ethyl Acetate	219-340 nm 667-668 nm	400-600 nm 700-890 nm
Methanol	210-349 nm	400-900 nm

Table 2. Ranges of Light Absorbed by Invasive Weed Phytochemicals Extracted with Hexane, Ethyl Acetate, and Methanol. No absorbances were seen at wavelengths between 700-890 nm, indicating that a wavelength of 750 nm may be used to avoid interference by invasive weed pigments.

UV-Vis Absorbance Spectra

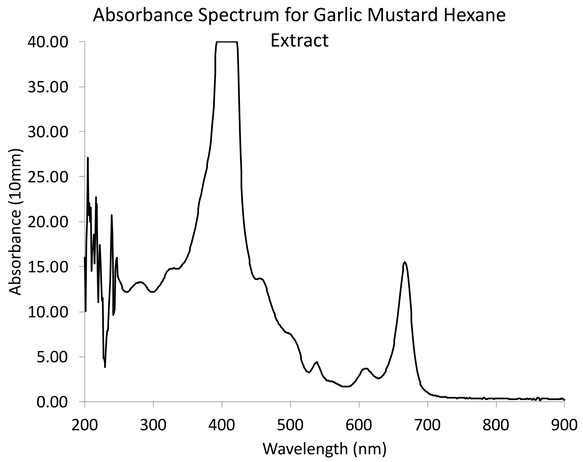

Figure 1. Representative UV-Vis Absorbance Spectrum. Absorbance peaks indicated that wavelengths of light above 700 nm were minimally absorbed by phytochemicals in garlic mustard hexane extracts. Extracts from all tested Albertan invasive weed species showed similar results, and no absorbances between 700 nm and 890 nm were observed.

Growth Curve Analysis

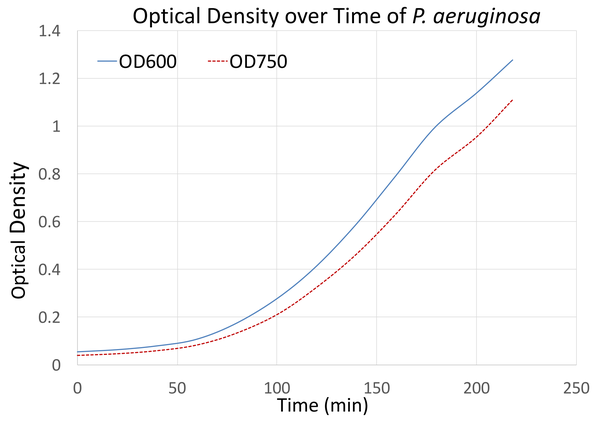

Figure 2. Representative Bacterial Growth Curve Measured at 750 nm. For bacterial cultures grown under optimal growth conditions, spectrophotometric optical density measurements at 750 nm followed the same trends as the measurements done at the standard wavelength of 600 nm. All bacterial species responded in this manner. These results indicate that 750 nm can be used to detect bacterial growth during antimicrobial susceptibility tests.

Conclusions

- Interference by invasive weed pigment molecules during spectrophotometric bacterial growth measurements may be avoided by using a wavelength of 750 nm.
- Spectrophotometric measurements done at 750 nm detect bacterial growth and may be used to quantify the antimicrobial activity of pigmented plant extracts and compounds.
- This optimized procedure for measuring bacterial growth may improve the accuracy of antimicrobial susceptibility tests for pigmented sources, aiding in the identification of plant-derived antimicrobial compounds.

Future Directions

- Quantify the antimicrobial activity of existing Albertan invasive weed extracts by using 750 nm to detect bacterial growth during broth microdilution assays.
- Extract and screen phytochemicals from additional Albertan invasive weed species using this optimized method.
- Characterize phytochemicals in active Albertan invasive weed extracts to identify the biologically active compounds.
- Test this optimized method on compounds and extracts derived from other pigmented plant sources.

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