Influence of pH on Alkaloid Extraction Efficiency and Biological Activity from *Veratrum californicum*

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Abstract. *Veratrum californicum* is a plant rich in steroidal alkaloids including cyclopamine, a known Hedgehog (Hh) pathway inhibitor. Extraction of cyclopamine from *Veratrum californicum* has been performed using benzene and recently ethanol as the Soxhlet extraction solvent under basic conditions. In this study, we analyzed the effect of pH on the extraction efficiency of cyclopamine using ethanol as the Soxhlet solvent. Separation and identification of steroidal alkaloids was achieved using HPLC-MS. Preliminary results demonstrating the effect of pH on extraction efficiency will be presented.

Overview.
- Cyclopamine is a known antagonist of the Sonic Hedgehog (Shh) signaling pathway extracted from *Veratrum californicum* (Figures 1-2).
- FDA trials for IP-926, a cyclopamine derivative, as a cancer therapeutic through Shh inhibition may increase demand for cyclopamine.
- Ethanol extraction yields nearly 3 times more cyclopamine as benzene but it was unknown if the cyclopamine was still biologically active.

Objective.
- This research compares the total yield and biological activity of cyclopamine recovered using Soxhlet extraction by using acidic, neutral, and basic ethanol.

Methods

Plant Material.
- Plant was harvested Nov. 2012 from Bogus Basin, Boise National Forest, ID
- Biomass was lyophilized to dryness, ground to powder, and lyophilized again overnight.
- All plant material was stored at -20 °C

Extraction and Isolation.
- Biomass (10 g) was subjected to Soxhlet extraction for 6 h (see Figure 3).
- Solvent conditions were:
  - EIOH:NH4OH (98:3.1:7 mL)
  - EIOH (100 mL)
  - EIOH:CH2O2 (98:3.1:7 mL)
- A liquid-liquid extraction was performed on the crude extract.
- The organic layer from the liquid-liquid extraction was subjected to liquid chromatography - mass spectroscopy (LCMS) for separation and identification of steroidal alkaloids.

Results

- LCMS chromatograms show similar alkaloid quantity and identity (Fig. 5).
- The acidic extraction has greatest alkaloid variance.
- Neutral extraction provides the best conditions for cyclopamine extraction (see Table 1).

Discussion

Neutral solvent extraction conditions provided superior quantities of cyclopamine compared to acidic or alkaline solutions. However, cyclopamine may be most soluble in the acidic solution and isomerization and degradation may be occurring as suggested in previous work (Fig. 6).

Conclusion. Biological testing will be conducted using Shh light II cells before a conclusion is reached regarding the best extraction method.

Select References


Keeler, R.F.; Cyclopamine and Related Steroidal Alkaloid Teratogens: Their Occurrence, Structural Relationship, and Biologic Effects. Lades 1976, 73, 709-716.


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<table>
<thead>
<tr>
<th>Extraction Method</th>
<th>Peak Area</th>
<th>Cyclopamine Collected (µg)</th>
<th>Extraction Efficiency (g/kg)</th>
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<tbody>
<tr>
<td>Basic</td>
<td>4804.7</td>
<td>4.88</td>
<td>1.65</td>
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<tr>
<td>Neutral</td>
<td>4940.27</td>
<td>5.01</td>
<td>1.73</td>
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<tr>
<td>Acidic</td>
<td>5220.1</td>
<td>5.30</td>
<td>1.65</td>
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