Extraction of Thyme Leaves and Analysis of Antibacterial Properties: An Adaptation for USF Analytical Chemistry Lab Course

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Introduction

- Integrated laboratory approaches are becoming increasingly popular in undergraduate chemistry education.
- Purcell et al. developed an experiment involving the extraction and analysis of thyme leaves that used an integrated laboratory approach.
- Goal: Adapt the parameters of the experiment for students taking the analytical chemistry laboratory course at the University of San Francisco and modify the procedure of the experiment to incorporate the Science Writing-Heuristic(R) (SWH) approach.

Experimental Question

- Question: Can Antibacterial Compounds be Extracted from Thyme Leaves?
- This is a multi-week lab project in which students must investigate:
  - Best extraction conditions for thyme leaves
  - Best solvents for TLC separation
  - Best mobile phase mixing program for quantitative HPLC analysis
  - Assay for antibacterial properties
  - Adapt the parameters of the experiment for students taking the analytical chemistry course.

Experimental Methods

Thin Layer Chromatography

- Purpose: To determine the best solvents to extract active compounds through multiple TLC tests and compare results with other groups.
- TLC allows compounds in a sample to be separated according to their polarity.
- Best solvent system requires visible separation and optimal Rf values as shown in Figure 3.
- A second TLC analysis is performed using unanimously chosen solvent system.

High Performance Liquid Chromatography

- Purpose: Identify the three peaks, as labeled in Figure 4, and quantitatively analyze thymol concentration.
- HPLC allows chemical compounds in a sample to be quantitatively separated by polarity and is more sensitive than TLC.
- A strict solvent ratio and gradient protocol will be given to save time.
- Students create standard addition plot (Figure 7) to determine thymol concentration.

Results

Thin Layer Chromatography

- We determined 25% Ethyl Acetate/75% Mixed Hexane to be the ideal developing solvent.
- p-Cymene was successfully separated from carvacrol and thyme extract (Table 1).
- Assay with varying concentrations to better establish the relative antibacterial potencies of thymol and carvacrol standards.
- Carvacrol had more potent antibacterial properties than thymol.

Table 1. TLC Results for 25% EA/75% MH solvent Thyme Leaf Extracts and Standards

<table>
<thead>
<tr>
<th>Lane Number</th>
<th>Distance Traveled from Starting Line (mm)</th>
<th>Rf Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lane 1: Thyme Leaf Extract</td>
<td>22</td>
<td>0.52</td>
</tr>
<tr>
<td>Lane 2: Thyme Standard</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Lane 3: Carvacrol Standard</td>
<td>23</td>
<td>0.55</td>
</tr>
<tr>
<td>Lane 4: p-Cymene Standard</td>
<td>35</td>
<td>0.83</td>
</tr>
<tr>
<td>Lane 5: co-spot</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>Solvent Front</td>
<td>42</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Future Works

- The methods of the undergraduate analytical chemistry thyme leaf experiment at UC Berkeley were successfully streamlined for USF Analytical Chemistry laboratory courses with the addition of SWH.
- p-Cymene was separated from thymol and carvacrol by TLC.
- The concentration of thymol in dried thyme leaves was approximately 2mg thymol per 1g of dried thyme leaves.
- Carvacrol was determined to have more potent antibacterial properties.

Conclusion

- The funding for this work was received through the USF Faculty Development Fund.
- Thank you to Dr. Lawrence Margerum for providing guidance and support on this project.
- Special thanks to Dr. Janet Yang and the USF Chemistry Department for providing the materials for this work.

Acknowledgements

References


Figure 1. General diagram of Purcell's overall thyme leaf experiment.

Figure 2. Structures of the main compounds found in thyme leaves.

Figure 3. General Reference Diagram of TLC Plate. Each number represents the following lane assignment: 1) Thyme Leaf Extract, 2) Thymol Standard, 3) Carvacrol Standard, 4) p-Cymene Standard, 5) co-spot. Co-spot is lane 1 through 4 combined together into a single lane that serves as a reference. Each dark circle above the starting line represents the distance the compound traveled from the starting line.

Figure 4. Example HPLC Chromatogram. The area under the peak is directly proportional to the concentration of thymol.

Figure 5. Example Kirby-Bauer Disk Diffusion Assay.

Figure 6. TLC plate from Table 1 Data.

Figure 7. Standard addition plot of increasing thymol concentration with added thymol standard. The x-axis represents the amount of thymol standard added and the y-axis represents the relative concentration of thymol in solution.

Figure 8. Concentration of thymol standard (solid line) and carvacrol standard (dotted line) plotted against zone of inhibition diameter.

High Performance Liquid Chromatography

- We performed HPLC with the following protocol: Solvent A was 70% THF:30% water and solvent B was 100% methanol.
- We calculated the concentration of thymol (Figure 7) to be 2 mg thymol/g dried thyme leaves (0.2% w/w), which did not match previous literature values due to possible differences in fresh and dried thyme leaves.

Kirby-Bauer Disk Diffusion Assay

- We tested five prepared dishes, with each dish containing different concentrations of the thymol and carvacrol standards.
- Carvacrol had more potent antibacterial properties than thymol (Figure 8).
- Data for thymol includes the concentration at 0.4 M while the data for carvacrol does not include 0.4 M, as the zone of inhibition was too large to calculate.

Concentration of thymol standard (mm)