Mushrooms have been consumed for millennia, both as food and as medicinals to treat a variety of human ailments. Over the past century, fungi have provided the raw chemical materials for most antibiotic drugs used today. In 2011, our research group began a chemistry survey of Utah mushrooms, comprising about sixty collections of almost as many species. The methanolic extracts of mushrooms that we have processed represent a library of undiscovered chemical information that we have only recently begun to decipher, by means of thin-layer and column chromatography, high performance liquid chromatography (HPLC), and nuclear magnetic resonance spectrometry (NMR).

We have been surprised by how difficult it has been to find a known poisonous mushroom species in Utah, but we will continue to seek out and collect them.

**Mushroom Extraction Process**

1. **Collect Mushroom Sample**
   - The sample is collected, weighed, and cut up to prepare it for extraction.
   - Cut-up sample is transferred into a flask.

2. **Extract compounds using Methanol**
   - The sample is submerged in 50-300mL Methanol (MeOH).
   - Placed sample in a sonicator at 40 degrees Celsius for 30 minutes to separate compounds from biomass.

3. **Filter and Isolate Extract**
   - The MeOH solution is strained out of the biomass and filtered.
   - A rotary evaporator is used to remove the MeOH, leaving dry extract which is frozen and stored.

4. **Purification Process**
   - F 250mm silica gel and 250mL chloroform.
   - Nitrogen flush station attached to column.

5. **Charge Column**
   - Mushroom extract dissolved in minimal chloroform.
   - Charge column with extract in chloroform.

6. **Chromatographic Separation**
   - Chloroform with a percentage Methanol added to column.
   - Gradient of %MeOH as follows: 1%, 2%, 4%, 10%, 20%, 100% in 250mL, eluents with chloroform.
   - Fractions collected in flasks for further research.

**Compound Isolation Procedure Using Flash Chromatography**

**HPLC Method:**

Our method is run on an Agilent 1200 system using 254 nm UV detection at a flow rate of 1 ml/min on a 150 mm Zorbax C18 column maintained at 40˚ C. We run a linear gradient from 5% acetonitrile in H₂O to 100% acetonitrile over 47.5 minutes, re-equilibrating to initial conditions prior to the next injection.

We were surprised to find that many mushroom extracts, including the edible mushrooms, show virtually no peaks following the initial injection spike. We call these “Flatliners”.

However, many shelf mushrooms, those that grow on tree trunks, as well as Polypores and *Caloscypha fulgens*, displayed multiple peaks, indicating small molecule chemistry that includes UV-absorbing chromophores. In this way we select candidates for preparative chromatographic separation.

**DIRECTIONS FOR FURTHER RESEARCH:**

- Assaying antimicrobial activity of mushroom extracts using *E. coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Candida albicans*, others.
- Partnering with industry to detect patterns and phylogenetic significance of secondary metabolite production in mushrooms (Judith Nichols, CAMAG Scientific, Inc., Wilmington, North Carolina, USA).
- Finding partners for biological assay, high field NMR & LC-MS.
- Making volatile derivatives amenable to GC-MS analysis.
- Purifying & characterizing lipoygenase enzyme(s) of *Caloscypha* & other mushrooms.

We gratefully acknowledge
Mushroom Society of Utah
UVU Scholarly Activities Committee
Nature's Way Products through donation to the UVU Foundation.