Elucidating the Antimicrobial and Anticancer Properties of *Argemone mexicana*

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Argemone mexicana

- Commonly name: \textit{Mexican prickly poppy}
- Hardy pioneer plant
- Stress tolerant
- Native to Mexico and West Indies
- Now found throughout world
Argemone mexicana

- Used in traditional medicine for centuries

- Reported medicinal properties:
  - Pain relief, anticancer & antimicrobial effects

- No bioactive compounds Id’ed to account for these activities
  - The goal of this research project!
Project Goals

- Establish which parts of *A. mexicana* have antimicrobial effects and anticancer effects
- Separate & chemically characterize responsible compound(s)
  - Could help discover new drugs to treat cancer & superbugs!
General Extraction Protocol

1. **Homogenization** ➔ Grind plant material in mortar & pestle
2. **Weigh** plant material & transfer to amber bottle (to protect from light)
3. Add extraction **solvent** (1:4 plant material:solvent ratio)
4. **Maceration** ➔ Mix at 200 rpm, 35°C for 48 hours in shaking incubator
5. **Centrifuge** at 5,000 x g for 5 minutes (to remove large cell debris)
6. **Filter** supernatant through 0.2 μM membrane (to clarify & remove most microbes & cellular organelles)
7. **Concentrate** filtrate & determine concentration
8. Test for **antimicrobial** and/or **anticancer** effects
To Test for Antimicrobial Effects

Disc diffusion (Kirby-Bauer technique)

- **Enrofloxacin 5 g**
  - (R < 17 mm; S > 22 mm)

- **Oxytetracycline 30 g**
  - (R < 17 mm; S > 22 mm)

- **Cefotaxime 30 g**
  - (R < 14 mm; S > 23 mm)

- **Gentamicin 10 g**
  - (R < 17 mm; S > 21 mm)

- **Ampicillin 10 g**
  - (R < 14 mm; S > 22 mm)

- **Chloramphenicol 30 g**
  - (R < 21 mm; S > 21 mm)

(a) R and S values differ from table 10.3 due to differing concentrations of the antimicrobials.
Microorganisms Used

- **Gram-Positive:**
  - *S. aureus*
  - *B. Cereus*

- **Gram-Negative:**
  - *E. coli*
  - *P. mirabilis*

- **Fungi:**
  - *C. albicans*
  - *S. cerevisiae*
Solvents Used

**Methanol**

H\_2\text{C-OH}

**Hexane**

H\_2\text{C-C-C-C-C-C-H}

**Non-Polar**

**Polar**

Increasing Polarity

**Hexane**
Quantification of Antimicrobial Results

- Disc diffusion assay with five biological replicates, using 1 mg sample/disc
Chemical Separation/Characterization

Hunting for a bioactive compound using organic chemistry lab instrumentation and collaboration

Thank you Dr. Pruet!!
Column Chromatography

- Common technique used to separate individual compounds from a mixture
  - Based on how they interact with the stationary and/or mobile phase
- Silica gel is used as absorbent
- Different polar molecules move through the column at different rates
Outer Root Separation

- Two main anti-microbial sub-fractions:
  - Called ‘D’ and ‘E’
Thin-Layer Chromatography (TLC)

- Rapid separation
- Solid-liquid partitioning technique
- Mixture is spotted onto the plate and developed using a solvent
  - During which different compounds are separated
- UV light and iodine stains are used as visualization methods
Outer Root Separation => TLC results

D and E were then further separated using normal-phase column chromatography & checked for purity using TLC (thin layer chromatography):
Mass Spectroscopy

- Measures the mass-to-charge ratio of ions
  - A small sample is ionized, usually to cations by loss of an electron
  - The ions are sorted and separated according to their mass and charge
  - The separated ions are then measured, and the results displayed on a chart
- Helps determine the mass & #C’s of the compounds
Outer Root Separation => MS results

- Main component:
  => Mass of 348
  => 16 or 17 C’s

- Minor component:
  => Mass of 314
  => 16 C’s

- Main component:
  => Mass 336
  => 17 or 18 C’s

- Minor components:
  => 370 & 18 C’s
  => 354 & 17 C’s
Anticancer MTT Assay

MTT (Yellow) → Formazan (Purple)

The Electromagnetic Spectrum

Quantification of Anticancer Results

- MTT assay on T84 human colon cancer cells with three biological replicates (& two tetechnical replicates each), using 1 mg sample/disc

- **Outer root methanol & seed hexane extracts have inhibitory effects against T84 cells**
Next Steps…

- Further separating => other extracts of interest
- Further characterizing ‘D’ and ‘E’ root MeOH sub-fractions via:
  - NMR
  - Tandem MS (MS/MS) => Sending samples to Notre Dame (allow comparison to library of known small molecules)
- Testing root extract effects on oncogenes in colon cancer cells using qPCR
References


