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Characterizing the Antimicrobial and Anticancer Activities and Several Associated Bioactive Compounds of Argemone mexicana

Teodora Najdeska
teodora.najdeska@valpo.edu

Danielle Orozco-Nunnelly
danielle.orozconunnelly@valpo.edu

Estefany Bocangel Gamarra
estefany.bocangelgamarra@valpo.edu

Tj Lefeber
tj.lefeber@valpo.edu

Elucidating the Antimicrobial and Anticancer Properties of *Argemone mexicana*

VALPO

Teodora Najdeska, TJ Lefeber, Estefany Bocangel

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Prepared for VU's Symposium on Undergraduate Research and Creative Expression

Argemone mexicana

- Commonly name =>

  Mexican prickly poppy

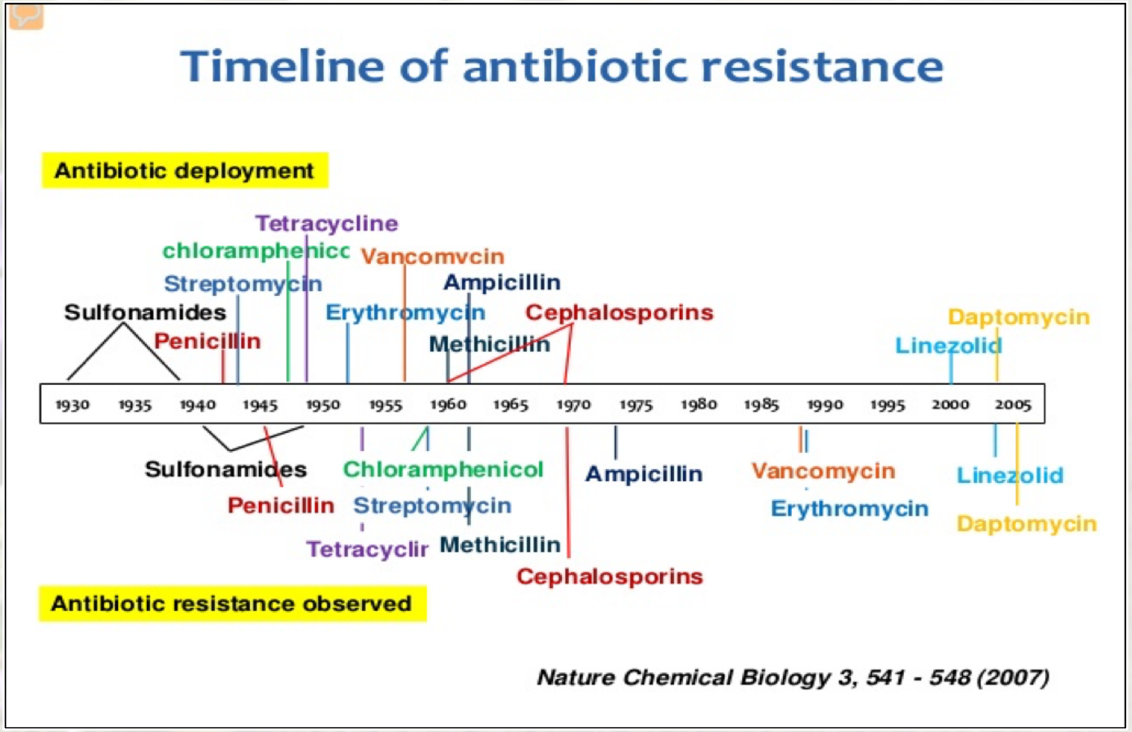
- Hardy pioneer plant
- Stress tolerant
- Native to Mexico and West Indies
- Now found throughout world

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!

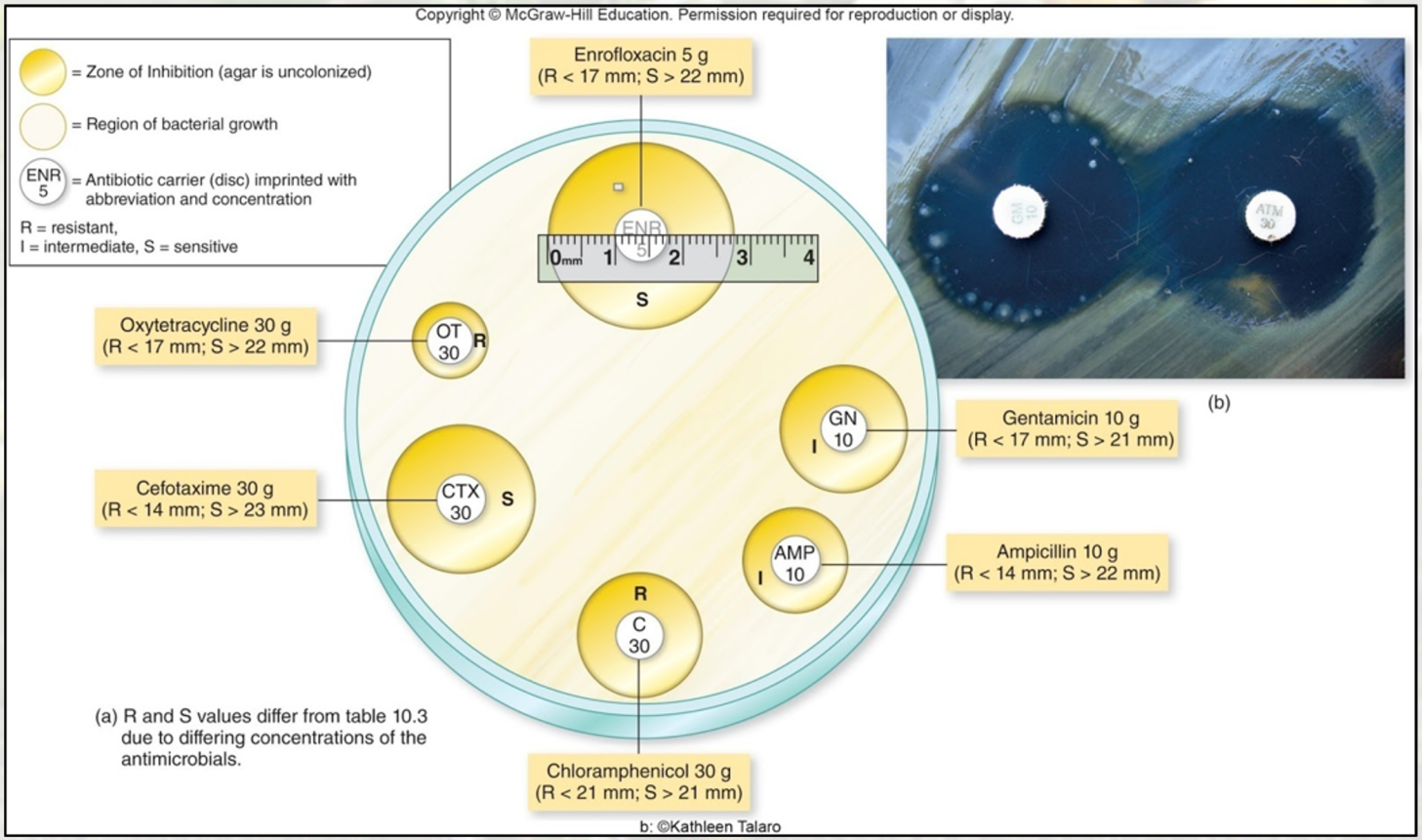


General Extraction Protocol

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^{o}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 uM membrane (to clarify & remove most microbes & cellular organelles)
7. **Concentrate** filtrate & determine concentration
8. Test for **antimicrobial** and/or **anticancer** effects

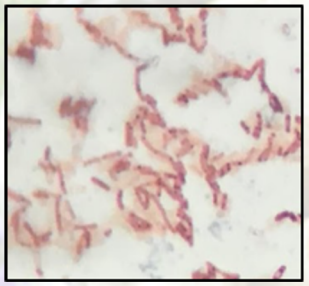
To Test for Antimicrobial Effects

Disc diffusion (Kirby-Bauer technique)

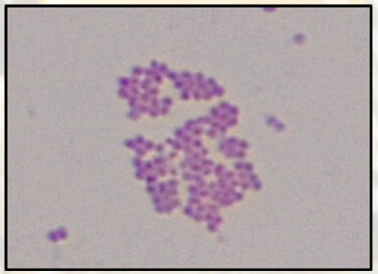


Microorganisms Used

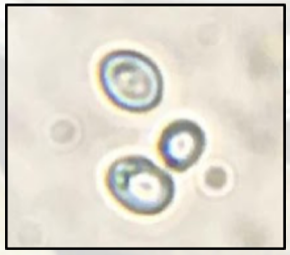
- **Gram-Positive**:
  - *S. aureus*
  - *B. Cereus*
- **Gram-Negative**:
  - *E. coli*
  - *P. mirabilis*
- **Fungi**:
  - *C. albicans*
  - *S. cerevisiae*



Gram Stain of *E. coli*; 400X total magnification



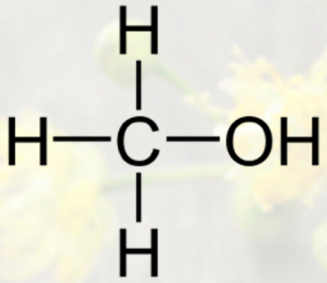
Gram stain of *S. aureus*; 1000X total magnification



Wet mount of *C. albicans*; 1000X total mag.

Solvents Used

Methanol



Non-Polar

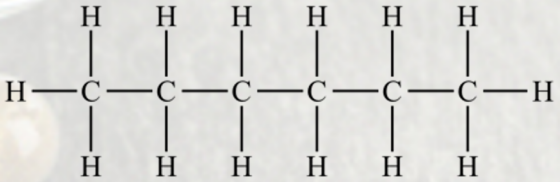
Hexane

Increasing Polarity

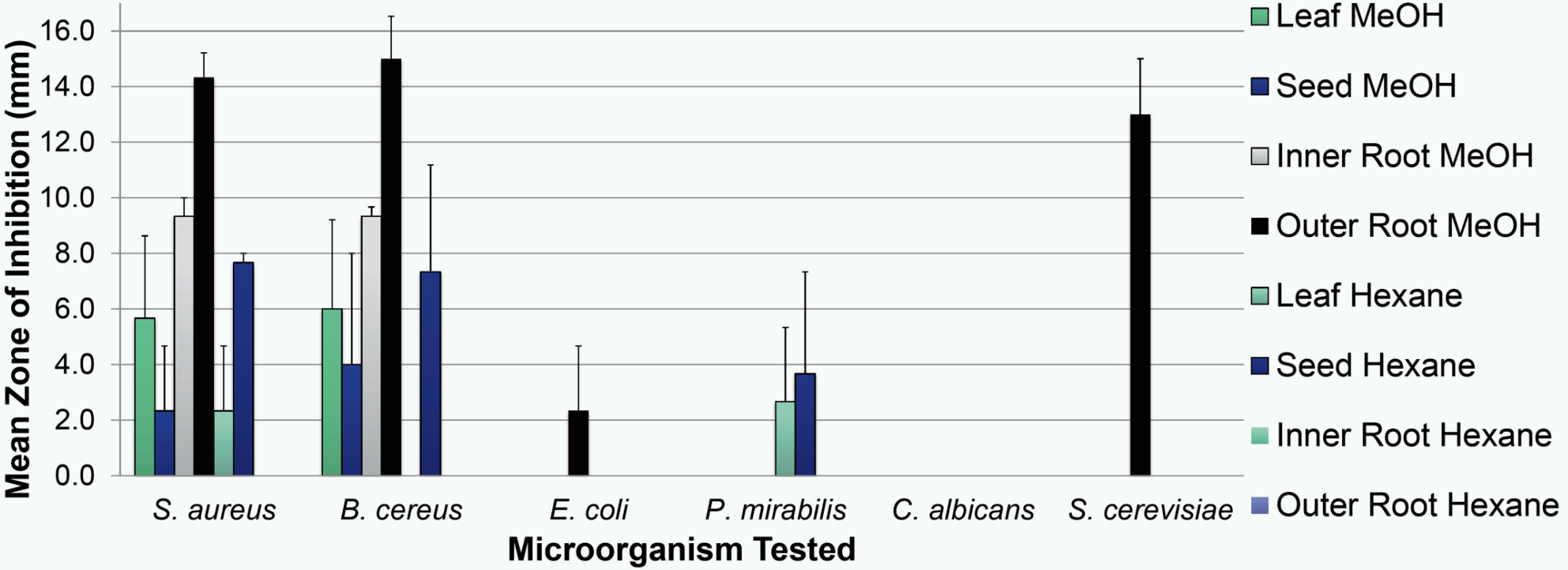
Methanol

Polar

Hexane



Quantification of Antimicrobial Results



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

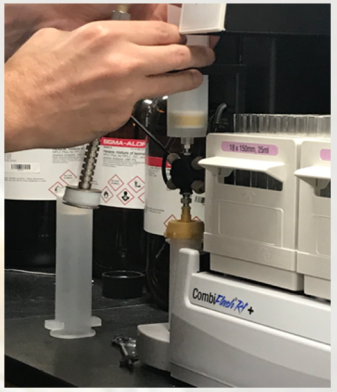
Chemical Separation/Characterization

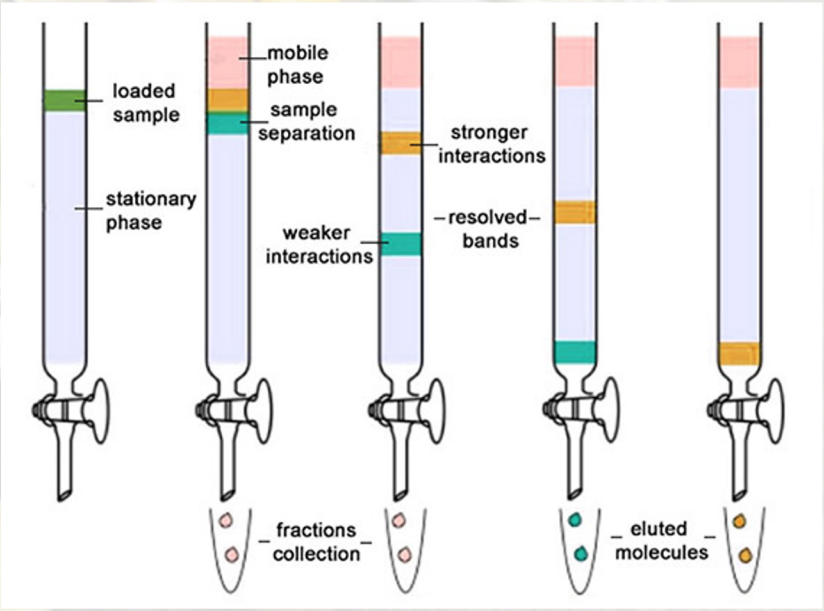
Hunting for a bioactive compound using organic chemistry lab instrumentation and collaboration

Thank you Dr. Pruet!!

Column Chromatography

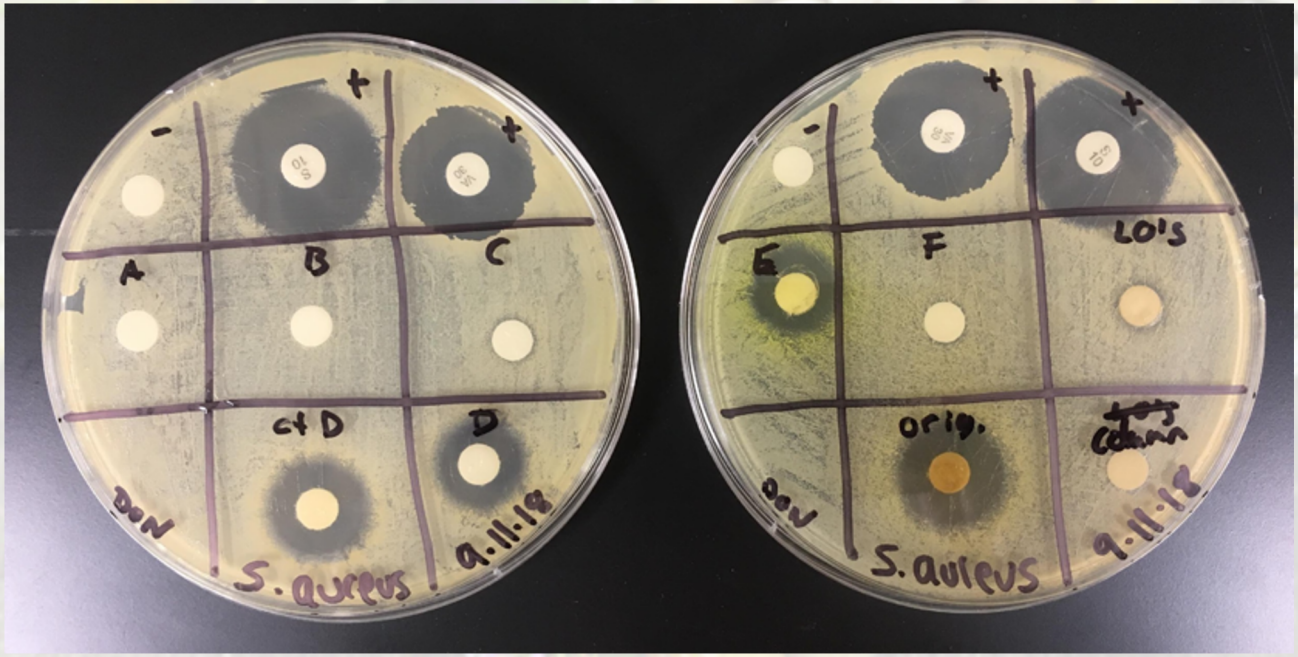
- Common technique used to separate individual compounds from a mixture
  - Based on how they interact with the stationary and/or mobile phase
- Silica gel is used as absorbent
- Different polar molecules move through the column at different rates





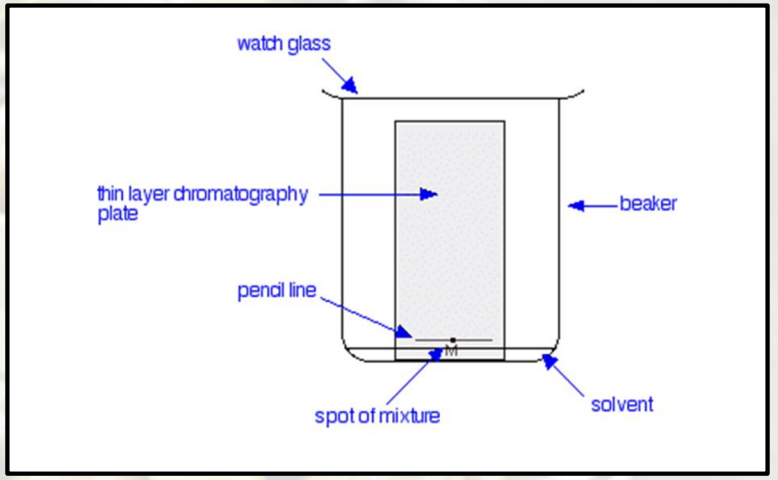
Outer Root Separation

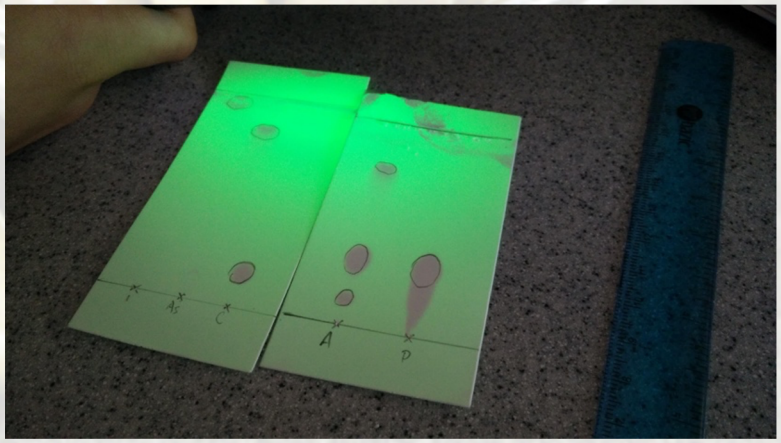
- Two main anti-microbial sub-fractions:
  - Called ‘D’ and ‘E’



Thin-Layer Chromatography (TLC)

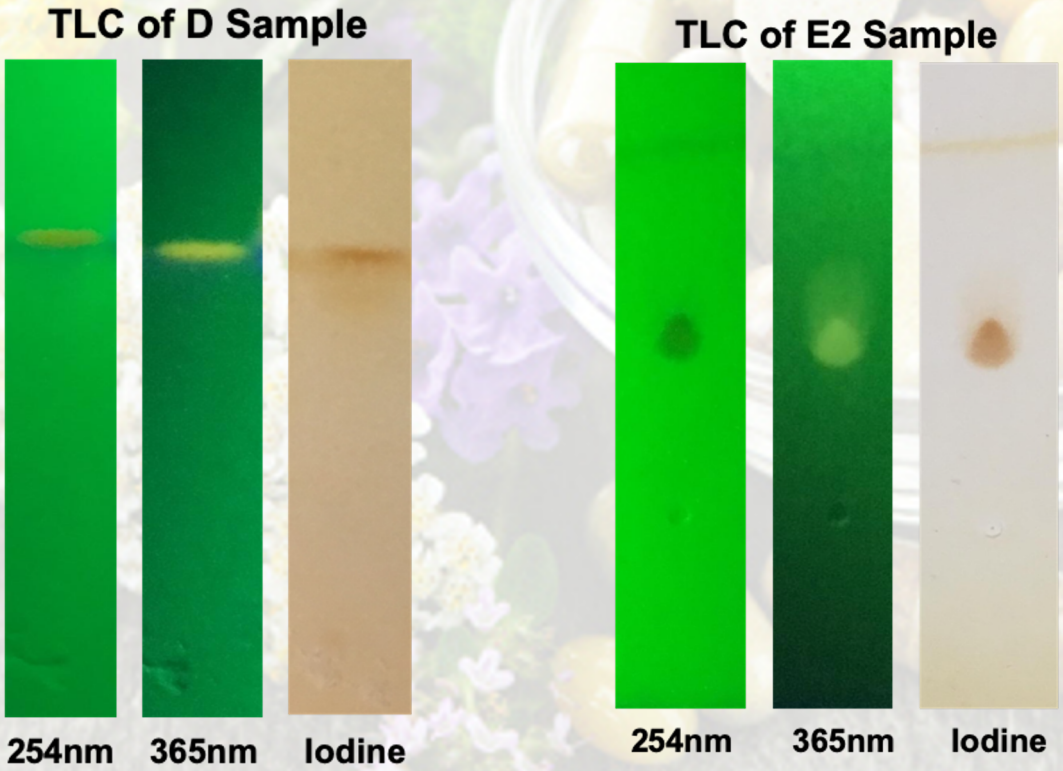
- Rapid separation
- Solid-liquid partitioning technique
- Mixture is spotted onto the plate and developed using a solvent
  - During which different compounds are separated
- UV light and iodine stains are used as visualization methods





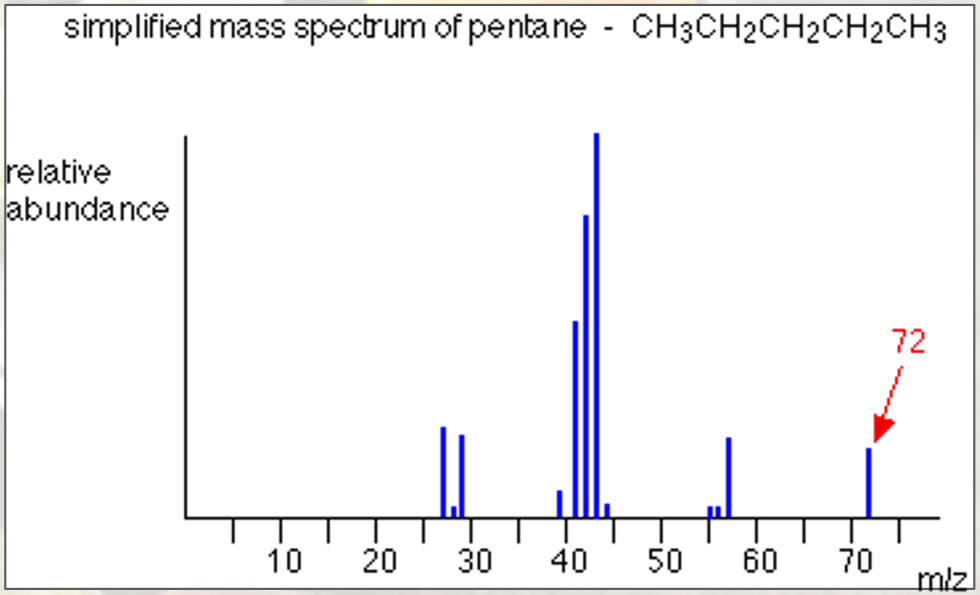
Outer Root Separation => TLC results

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



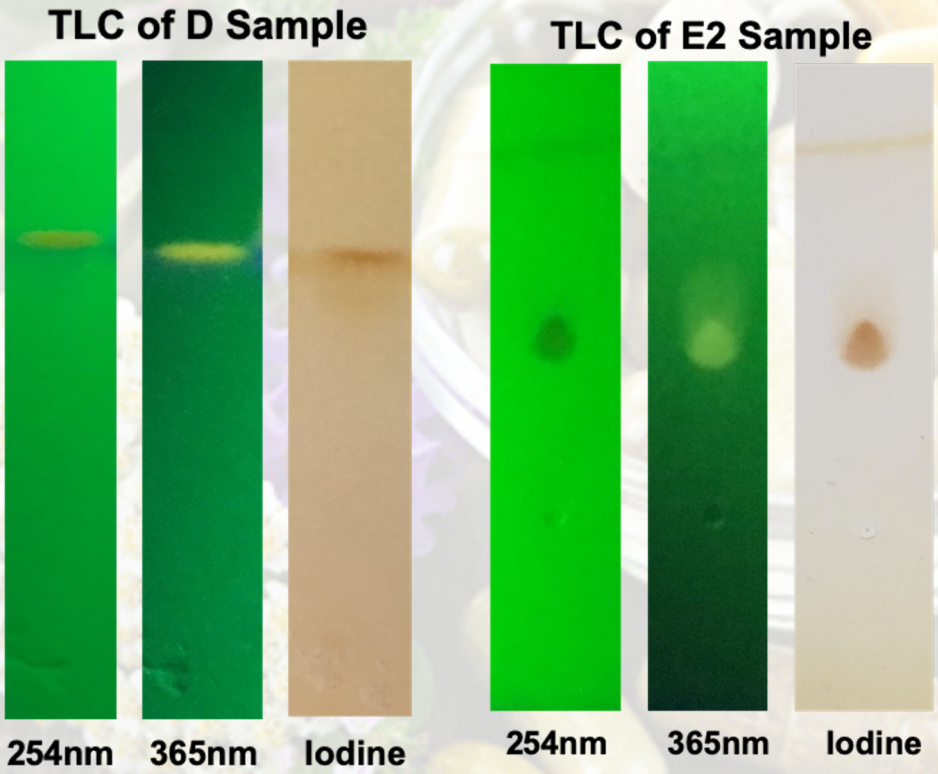
Mass Spectroscopy

- 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



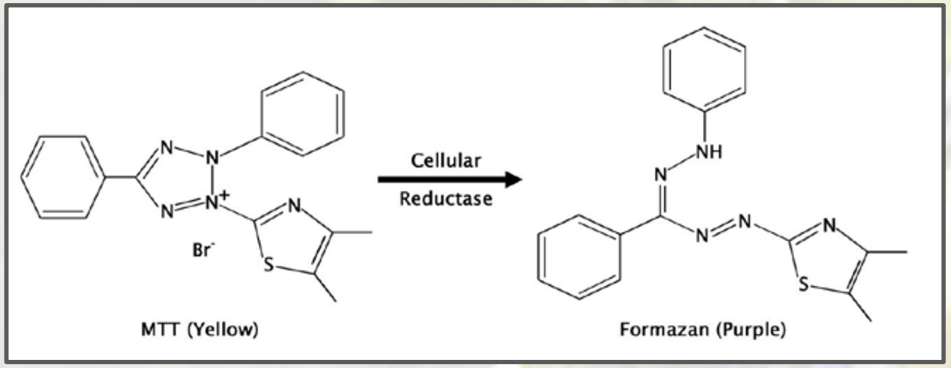
Outer Root Separation => MS results

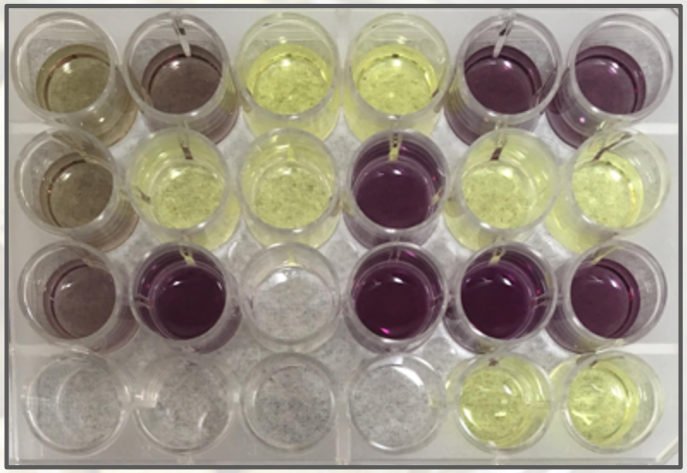
- Main component:
  => Mass of 348
  => 16 or 17 C's
- Minor component:
  => Mass of 314
  => 16 C's

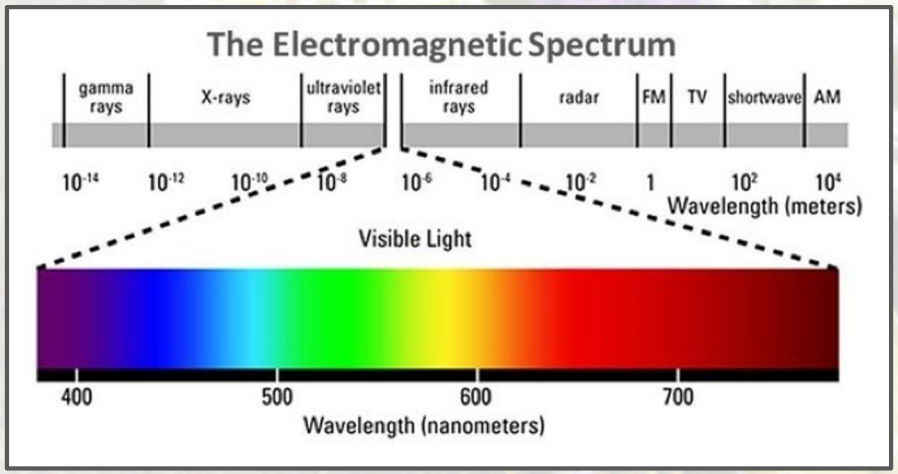


- Main component:
  => Mass 336
  => 17 or 18C's
- Minor components:
  => 370 & 18 C's
  => 354 & 17 C's

Anticancer MTT Assay

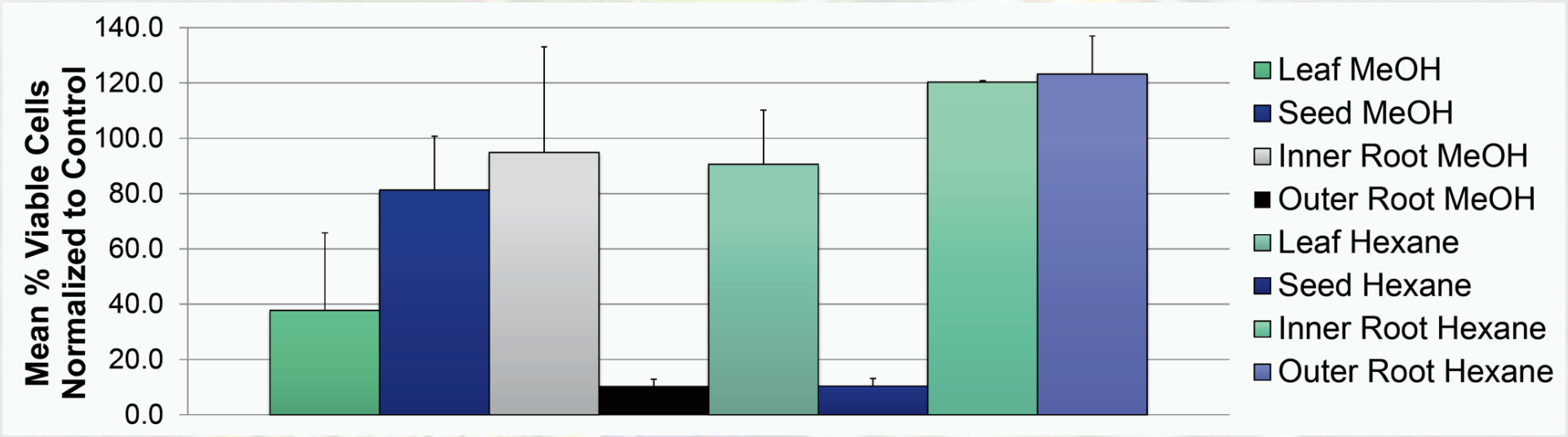






https://www.cellbiolabs.com/sites/default/files/CBA-252-mtt-cell-proliferation-assay.pdf

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

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