

## Abstract

With over two and a half million people becoming infected with an antibiotic resistant bacteria each year, antibiotic resistance has become a major world threat and new drugs are needed to combat the growing epidemic. Previous research showed that two fungal endophytes found in Arkansas-grown *Vitis aestivalis* (Norton/Cynthiana) vines – a hardy grape native to the United States – showed antimicrobial activity against two common bacteria, *S. aureus* and *E. coli*. In order to determine which compounds are responsible for the antibacterial activity, the grape endophytic fungi were grown in potato dextrose broth for two weeks in a controlled temperature and the compounds were extracted from the broth solution via an ethyl acetate wash. The ethyl acetate solutions were concentrated under reduced pressure and analyzed by GC/MS. Known compounds were identified by comparison of their mass spectra to the mass spectra located in the National Institute of Standards and Technology 2011 database

## Purpose and Hypotheses

- Purpose: To determine the structures of the antibacterial compounds present in Arkansas *Vitis aestivalis* grape tissue
- Hypotheses: The compounds found in the fungi will be isolated and identified via various chemical methods to determine the types of compounds.

## Background Information

- The CDC reports that at least two million people are diagnosed with an antibiotic resistance bacteria each year. (1)
- *V. vinifera* (and other plants) endophytes have been shown to have antibacterial activity. (6)
- In recent years, bacteria have gained an increase in antibiotic resistance cumulating in the formation of multi – drug resistant bacteria, thus leading to the need of new antibiotics. (7)
- *E. coli* (gram – negative), a common bacteria found in the human intestines, is both a leading cause of food poisoning and can cause Hemolytic Uremic Syndrome which leads to loss of kidney function. (3)
- *S. aureus* (gram – positive) causes food poisoning and health infections; there are now MRSA and VRSA strains of the bacteria (8;5)
- Species of *Xylaria*, *Coniothyrium*, and *Alternaria* have all been proven to have inhibitory properties against *S. aureus*. (2, 10, 4)
- *E. coli* is currently resistant to tetracycline, ampicillin, and gentamicin. *S. aureus* is resistant to vancomycin, methicillin, and penicillin for. (9,8)
- Previous research indicated that the inhibition by the fungi was significant and that there was at least 3 significant compounds in the ethyl acetate fraction and 5 significant compounds in the aqueous fraction.

## Methodology

- Four fungi were chosen. Two showed inhibition against *S. aureus*, one against *E. coli*, and one control. The fungi were grown for one month in potato dextrose broth before being filtered to remove the solids
- The fungal extract aqueous solution was separated via a 1:1 ethyl acetate rinse The compound containing solutions fractions were concentrated to approximately 5-10mL.
- The concentrated solutions were tested via TLC, HPLC and GC/MS.
- The GC/MS results were then compared to the National Institute of Standards and Technology 2011 Database



Picture 1: Aqueous solution being separated.



Picture 2: Solution being concentrated.

## Results

Plant Tissue	Section	Description	Fungus Name	Soluble in ethyl acetate
Leaf A	8C	Pink/White	Fungal Endophyte/ <i>Xylaria</i>	No
Stem A	7C	Matte Peridot Green	<i>Coniothyrium/Alternaria</i>	Yes
Stem B	6B	Rose	<i>Aureobasidium</i>	Yes
Leaf C	10C	Dark Gray	<i>Curvularia geniculatus</i>	Yes

Table 1: Vegetative pieces used, fungal description, likely genus species, and solubility.

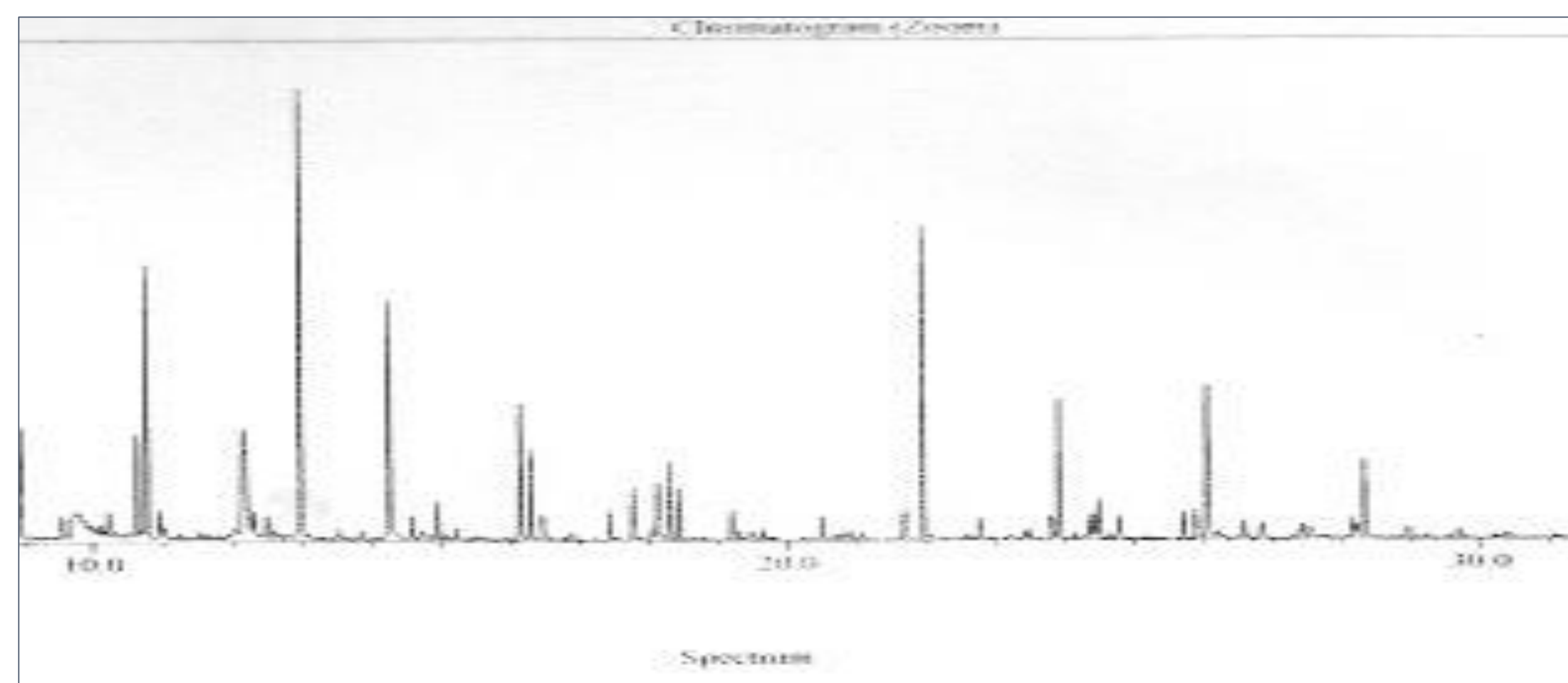


Chart 1: GC/MS of ethyl acetate fraction from Stem B

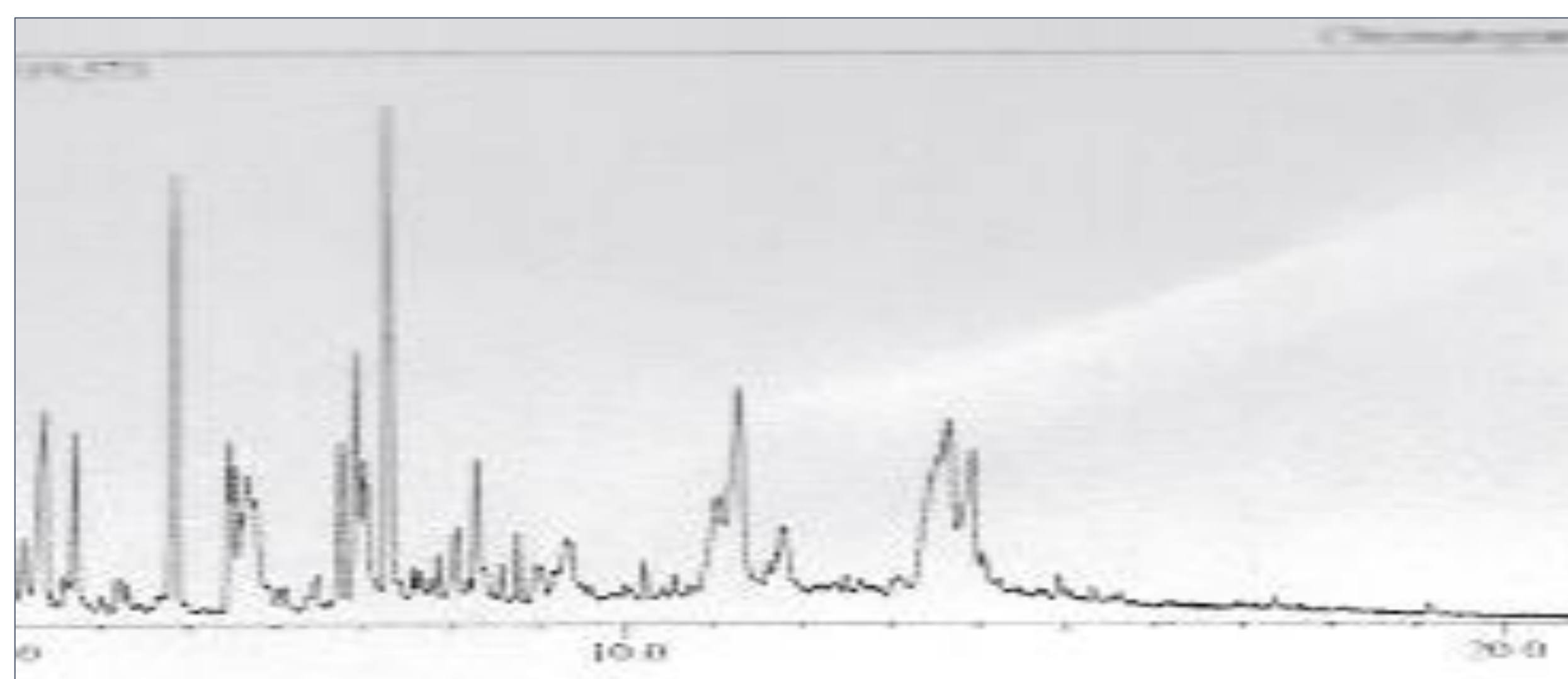
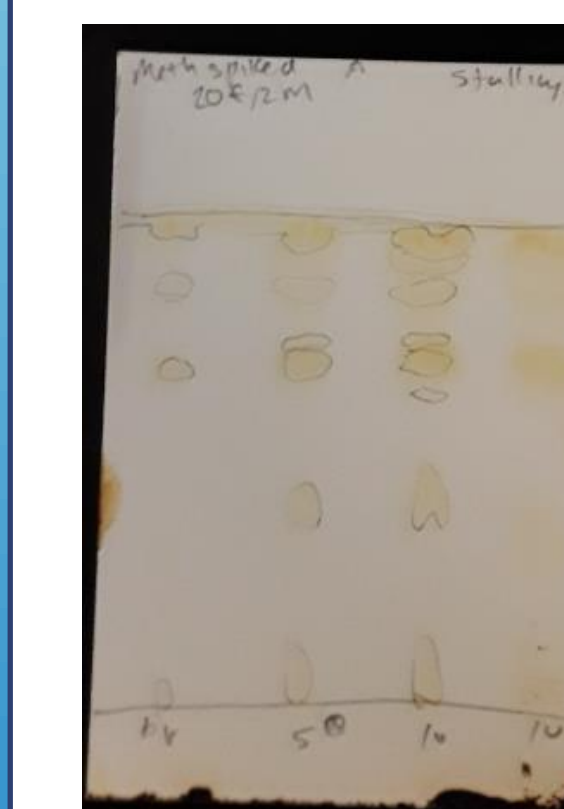


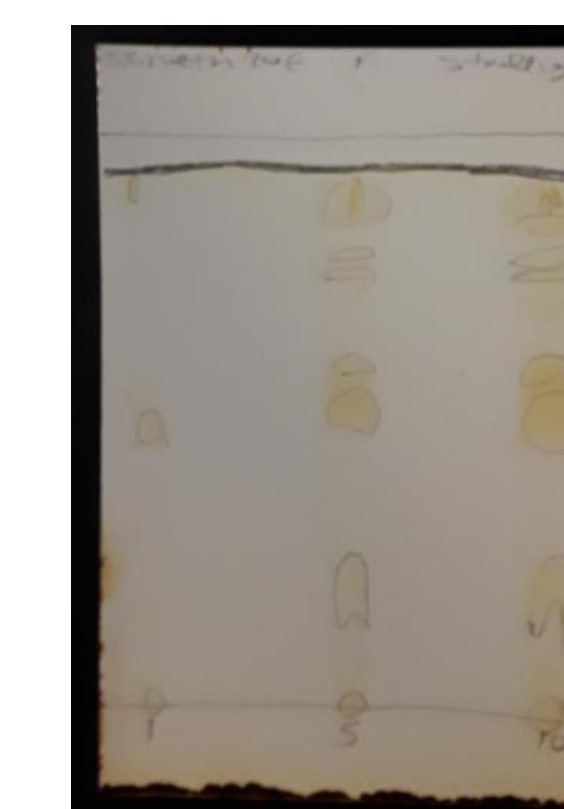
Chart 2: GC/MS of water fraction from Stem B

- Three of the four compounds moved to the ethyl acetate solution while one remained in the water solution.
- The original set of TLC plates were run with hexane, ethyl acetate, and methanol; secondary plates were run with mixtures of ethyl acetate, methanol, and methylene chloride.
- TLC Plates with a mixture of methanol and ethyl acetate showed the best separation.
- The identity of each peak is being determined by comparison of mass spectra present in the National Institute of Standards and Technology (NIST) database

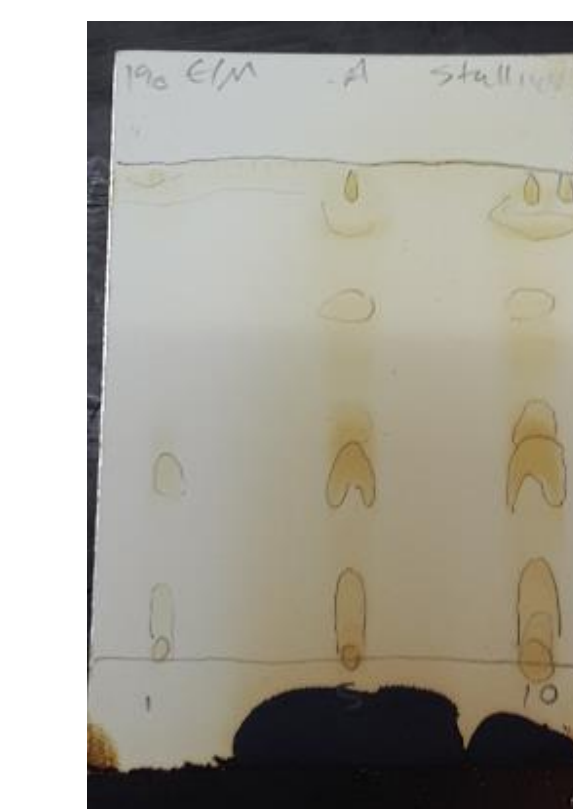
## Selected TLC Plates of Solutions



10% Methanol,  
2mL Methanol; 20mL  
Ethyl Acetate



5% Methanol,  
1mL Methanol; 20mL  
Ethyl Acetate



1% Methanol,  
0.2mL Methanol; 20mL  
Ethyl Acetate

## Discussion and Conclusions

- From the TLCs performed, it is possible that there are both polar and nonpolar compounds in the solutions.
- All ethyl acetate fractions contained compounds that were similar to known compounds in the Database
- Further research is needed to determine what compounds are found in the aqueous fraction.

## References

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