Antimicrobial Assay of *Artemisia tridentata*

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PLANTS, SOILS & CLIMATE

Introduction

- Plants and fungi secrete substances to create more favorable living environments
  - Secondary Metabolites
- Chemicals produced by plants may be used to kill competition or to modify the plant's microbiotic environment.
- Penicillin—Extracted from a mold by Alexander Fleming in the 1920s
- Aspirin—Secondary metabolite from the willow plant

Introduction

- *Artemisia tridentata*, commonly referred to as “Big Sagebrush”
  - Traditionally used by Native Americans to relieve stomach pain, colds and coughs, sore eyes, snake bites, and as an insect repellent.
- Artemisinin—Famous antimalaria drug
  - Isolated from the Chinese herb *Artemisia annua*, which belongs to the same genus as *A. tridentata*
- Antimicrobial properties of *A. tridentata*
  - Twig
  - Flower
  - Leaf
- Plant and human pathogens

Methods

- ***Sample Collection***
  - Samples of *A. tridentata* leaves, twigs, and flowers were collected from various locations in Green Canyon.

- ***Preparation of Plant Extracts***
  - The collected samples were washed and dried for a month.
  - They were then ground to a fine powder and methanol was used to create a concentrated solution.
  - The solutions were then dried using a rotary evaporator.

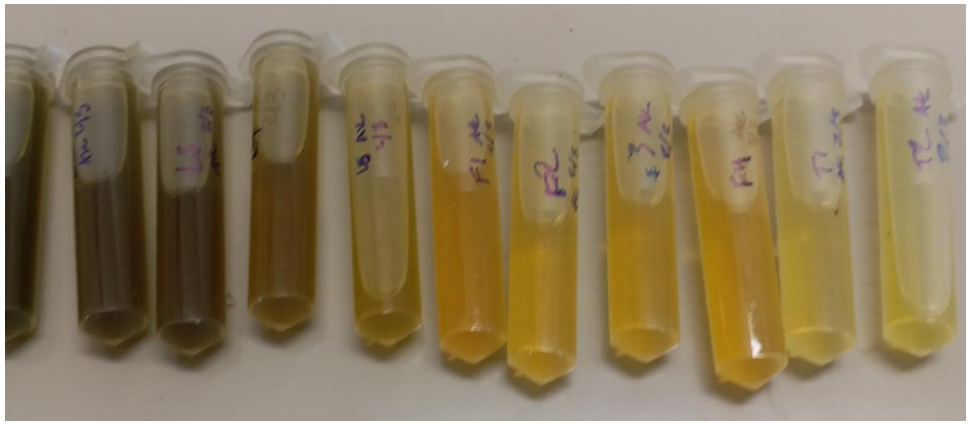
Methods

- ***Preparation of Plant Extract and Antibiotic Control Disks***

The dried extracts were resuspended in Dimethyl Sulfoxide to 50mg/ml and 200 mg/ml.

	10µl	50µl	100µl
50µg/µl	500µg (0.5mg)	2500µg (2.5mg)	5000µg (5mg)
200µg/µl	2000µg (2mg)	10000µg (10mg)	20000µg (20mg)

- Crude extract is much less potent than true antibiotics
  - High amount of extract necessary



Methods

- ***Antimicrobial Assay of Plant Extracts***

Tested for growth inhibition on Mueller Hinton Agar. All bacteria were grown and propagated on LB except *Pseudomonas*, which was grown on KB

- *E.coli* Dh5α
- *Agrobacterium tumefaciens*
- *Pseudomonas syringae* pv tabaci
- *P.syringae* pv tomato
- *Bacillus subtilis*

Plates were incubated for 12-48 hours in incubator, depending on the bacteria, and observed for zone of inhibition.

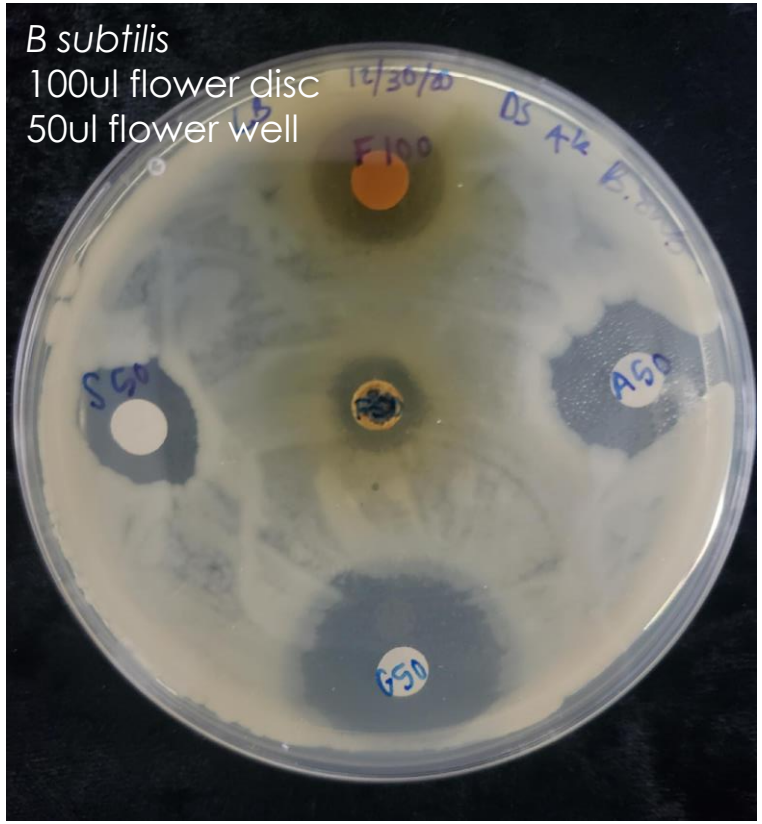
Results

- Standardization of methodology
- *E. coli* appeared to be the most resistant to extracts
- *B. subtilis* showed higher sensitivity to extracts
- *A. tumefaciens*
  - Prominent plant pathogen
  - Extract created larger Zone of Inhibition (ZOI) than antibiotics

B subtilis (OD: 0.038)

Medium zones around wells and 2.5mg disc

Large zone (larger than that around streptomycin disc) around 5mg disc



Results

	Bacillus subtilis (mm)	*Escherichia coli* Dh5a (mm)	*Agrobacterium tumefaciens* (mm)	*P. syringae* pv tabaci (mm)
Flower (2.5mg well)	**2.5**	**1**	**2**	**3.2**
Flower (0.5mg disk)	ND	**0**	ND	**0**
Flower (2.5mg disk)	**1.5**	**0**	**1**	**3**
Flower (5.0mg disk)	**5**	ND	**3**	ND
Leaf (2.5mg well)	**2**	ND	**2**	**2.7**
Leaf (0.5mg disk)	**2**	ND	ND	**0**
Leaf (2.5mg disk)	**1**	ND	**1**	**0**
Leaf (5.0mg disk)	**2**	ND	**3**	ND
Twig (2.5mg well)	**4**	ND	**3**	**3.3**
Twig (0.5mg disk)	**0**	ND	ND	ND
Twig (2.5mg disk)	**1**	ND	**2**	**0**
Twig (5.0mg disk)	**1**	ND	**1**	ND

Results

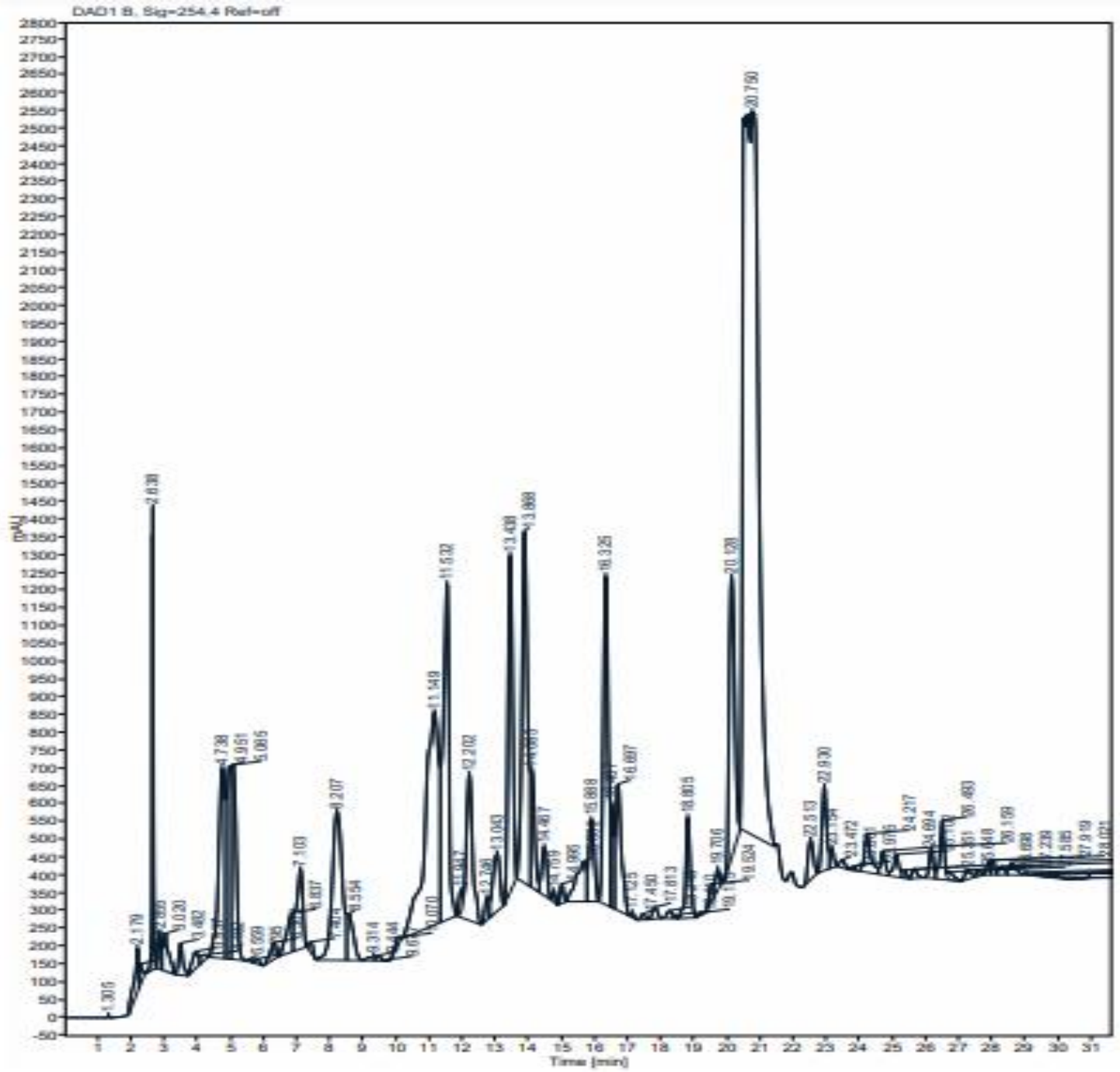
	Bacillus subtilis (mm)	*Escherichia coli* Dh5a (mm)	*Agrobacterium tumefaciens* (mm)	*P. syringae* pv tabaci (mm)
Ampicillin (10µg)	ND	**0**	ND	**2.2**
Ampicillin (50µg)	**5.7**	**1.1**	**0**	ND
Gentamycin (10µg)	ND	**6**	ND	ND
Gentamycin (50µg)	**7.2**	**3.3**	**0**	ND
Kanamycin (10µg)	ND	**5**	ND	ND
Kanamycin (50µg)	ND	**5.6**	ND	ND
Streptomycin (10µg)	ND	ND	ND	ND
Streptomycin (50µg)	**3.8**	ND	**0**	ND

HPLC Analysis

- High-Performance Liquid Chromatography
  - Separate components of our extract
  - Allow for analysis
- Non-targeted Analysis
  - Visualize chemical and physical distinctions between compounds
  - Terpenes??

HPLC Results

- Polarity decreases from left to right
- Methanol consequently evaporated from solution and replaced with DMSO (50% DMSO, 50% water with 0.1% acetic acid)



MIC Negative Control Verification

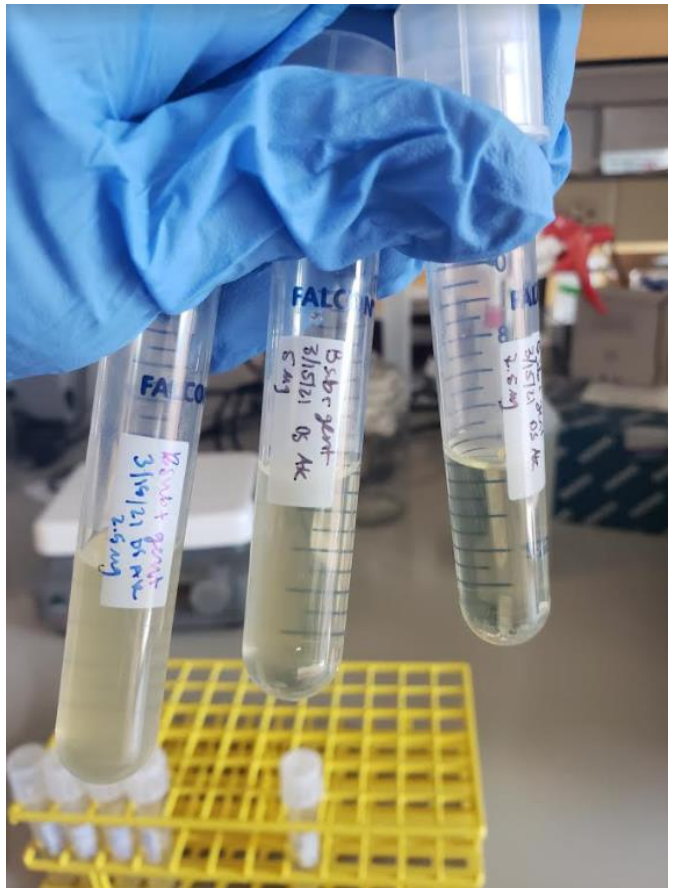
- Minimum Inhibitory Concentration
  - Streak plate (LB) with target bacteria from glycerol stock (*Bacillus subtilis*)
  - Inoculate one colony in 3mL liquid media (LB)
  - Grow 12-16 hours
  - Inoculate 5mL liquid media (LB) with 50µL culture
  - Grow (`2 hrs) and adjust OD with 0.5 McFarland standard (107-108 cfu/ml)

MIC Negative Control Verification

- Inoculate 12 tubes of 5 ml liquid media (LB) with 50 µL culture
  - 0 µg/ml, 0.1 µg/ml, 0.5 µg/ml, 1.0 µg/ml, 2.5 µg/ml, 5 µg/ml, 7.5 µg/ml, 10 µg/ml, 20 µg/ml, 30 µg/ml, 40 µg/ml, 50 µg/ml
  - Incubate overnight
  - Broth that appears turbid are indicative of bacterial growth
  - Clear broth show now growth
  - MIC for an antibiotic is the lowest concentration with no growth

MIC Negative Control Verification Results

	+ or -
No additive	+
0.1 µg gentamycin	+
0.5 µg gentamycin	+
1.0 µg gentamycin	+
2.5 µg gentamycin	+
5.0 µg gentamycin	+ (minor)
7.5 µg gentamycin	-
10 µg gentamycin	-
20 µg gentamycin	-
30 µg gentamycin	-
40 µg gentamycin	-
50 µg gentamycin	-



MIC Fraction Verification

- Inoculate 33 tubes of 5 ml liquid media (LB) with 50 µL culture (preparation described on previous slides)
  - Positive control (no addition)
  - MIC test antibiotic (gentamycin) – 7 µg
  - 0.5 ml water with 0.1% acetic acid (HPLC solvent) + 0.5 ml DMSO
  - 1 ml of each of the 30 fractions
  - Fractions which inhibit growth contain antibiotic compound

Implications

- Big Sagebrush is native to Utah
  - Production would be very cheap or even free.
- New antimicrobial compounds extremely valuable
  - Antibiotic resistance
  - Valuable to agriculture
    - Crop preservation
- Sagebrush population declining

Further Research

- Resulting fraction will be further separated by HPLC to isolate the antibiotic compound
- Resulting compound analysis will be compared with metabolite library
- We will be testing extract of *A. tridentata* on a variety of bacteria, especially human pathogens, and fungi
- *A. annua* shows promising results against cancer cells, compounds in *A. tridentata* may prove similar

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Questions
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