

# Antimicrobial Properties of Sagebrush Roots

Lauren McFadden, Amita Kaundal\*  
Plants, Soils, and Climate, College of  
Agriculture and Applied Sciences, USU

# Background

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- Native Americans have historically used Big Sagebrush to treat various illnesses.
- Plants contain secondary metabolites that have a variety of functions.
- David and Kayla Suisse have previously tested above ground parts of sagebrush.



# Methods-Harvest

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Harvested Sagebrush Roots



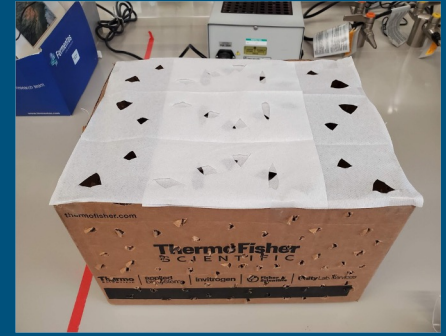
# Methods-Drying



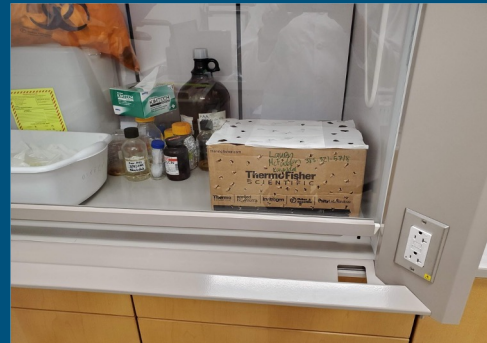
Roots Separated



Roots in Box



Box cover



Drying Box in Fume Hood

# Methods-Crushing

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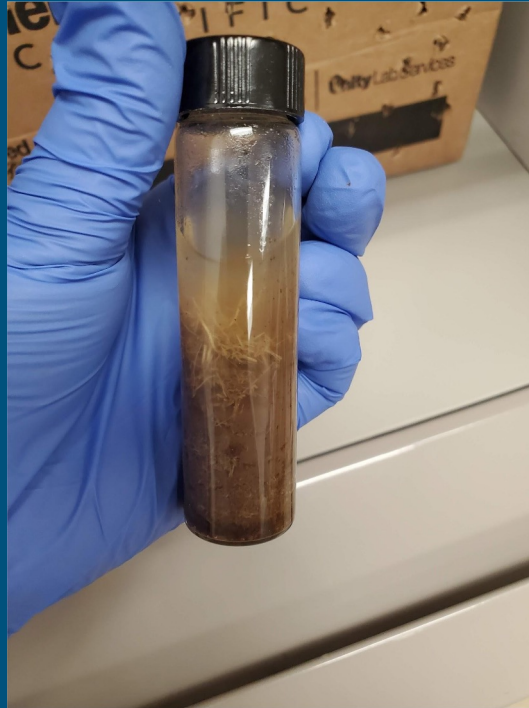


Crushed Roots

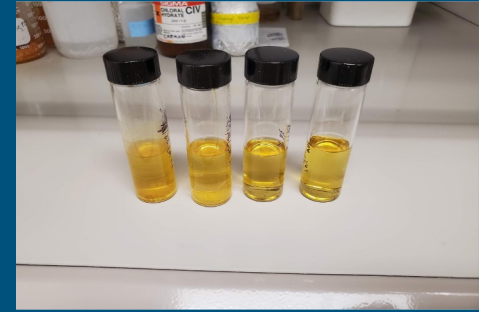
# Methods-Extraction



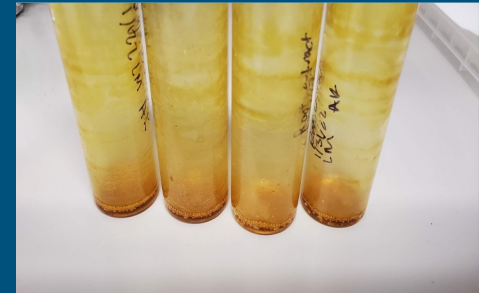
Vacuum Separation



Crushed Roots Soaking in Methanol



Filtered Extract with Methanol



Dried Extract

# Methods-Extraction

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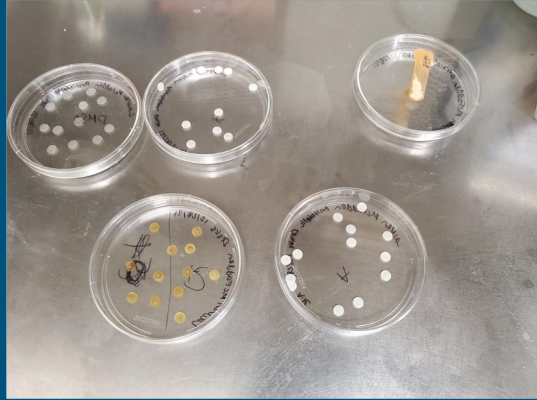
Dried Collected  
Extract



Extract Suspended in DMSO  
At 1000mg/ml  
Concentration



# Methods-Disks

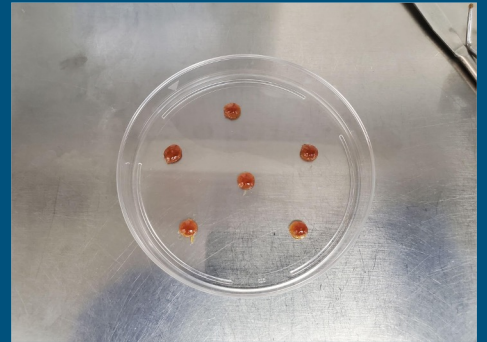


All the disks made

Ampicillin, Kanamycin, and  
Gentamycin are positive  
control  
DMSO is negative control



Filter Paper with extract on it



100mg disks



# Methods-Bacteria

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## BSL 1 Bacteria Tested

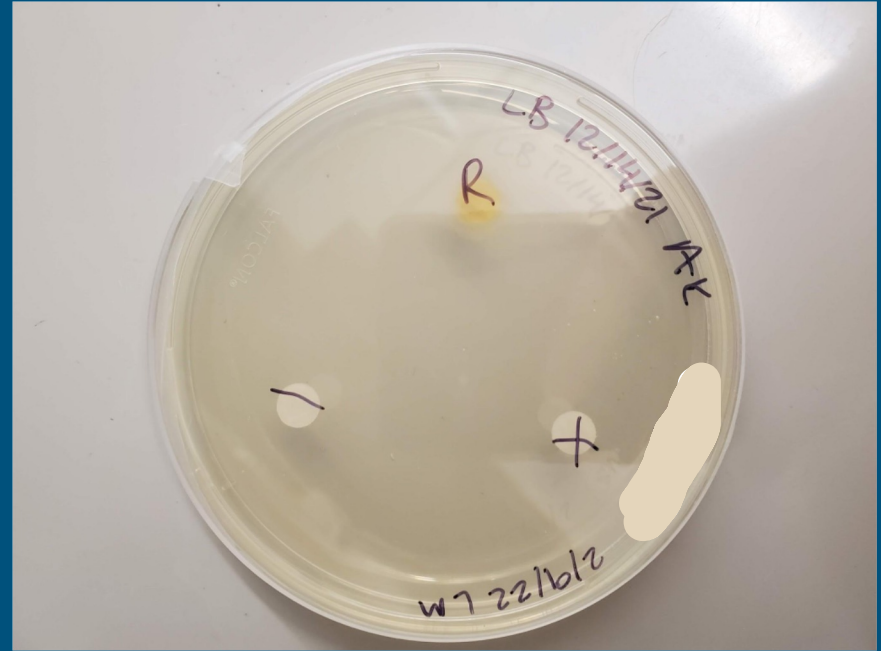
- *Bacillus subtilis*
- *Bacillus cereus*
- *E. coli* DHSα – Lab strain
- *Agrobacterium tumefaciens* – Lab strain
- *Pseudomonas syringae* pv. *tabaci* – Tobacco pathogen
- *P. syringae* pv. *tomato* DC3000 – Tomato pathogen

# Methods-Plating

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5 Disks Arrangement  
10mg Disk (Center)



3 Disks Arrangement  
20mg Disk (Top)

# Methods-Plating

Grown bacterial culture in LB broth for 16 hour

- *Bacillus subtilis*- 37°C
- *Bacillus cereus*-37°C
- *E. coli* DHS $\alpha$ - 37°C
- *Agrobacterium tumefaciens*- 28°C

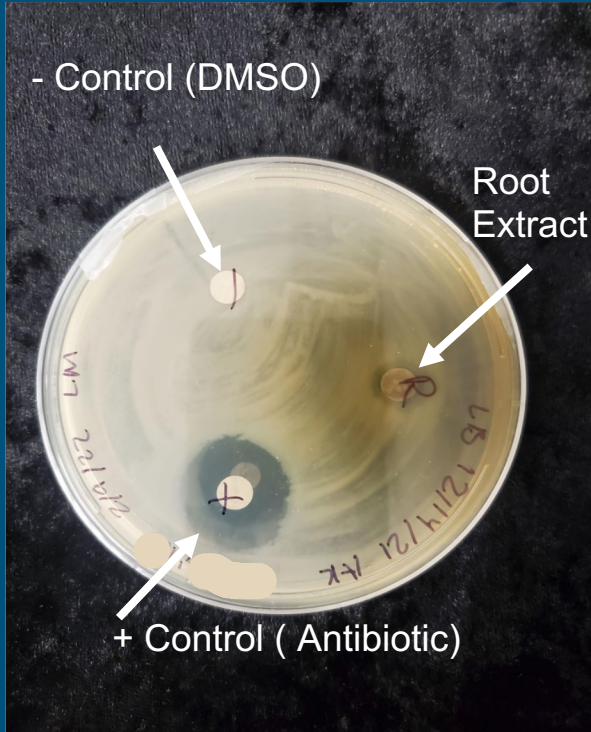
Grown bacteria culture in King's B broth for 32 hours

- *Pseudomonas syringae* pv. tabaci – 28°C
- *P. syringae* pv. tomato DC3000- 28°C

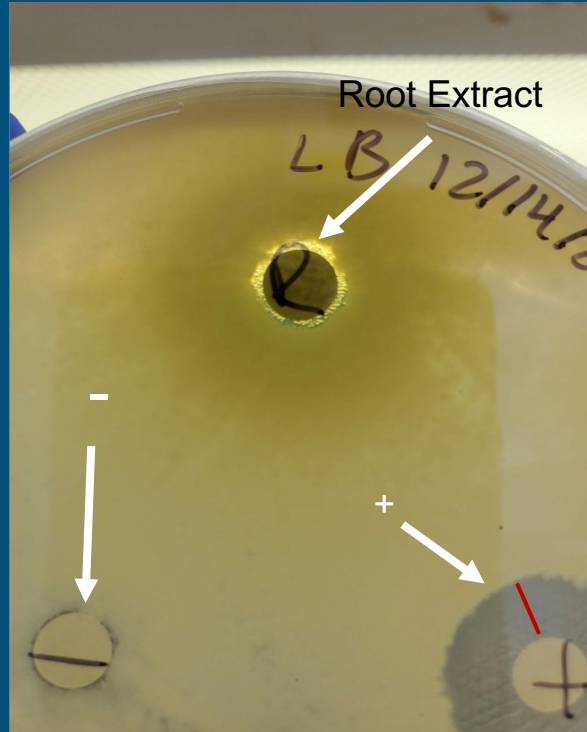
Subculture 10% in LB broth and grown for 2 hours until OD reached 0.05 at 600nm  
100 $\mu$ l of culture of OD<sub>600nm</sub> spread plated on Muller Hinton Agar plate.  
Disks placed on plate and incubated at corresponding temperatures.



# Results



20mg Extract on *Bacillus subtilis*



Close up of 20mg *Bacillus cereus*

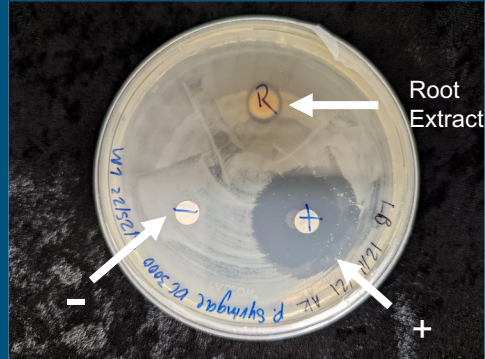


20mg Extract on *Bacillus cereus*

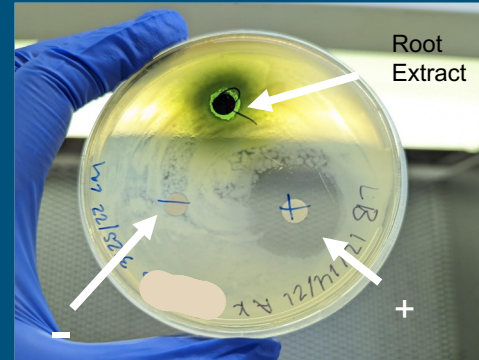


# Results

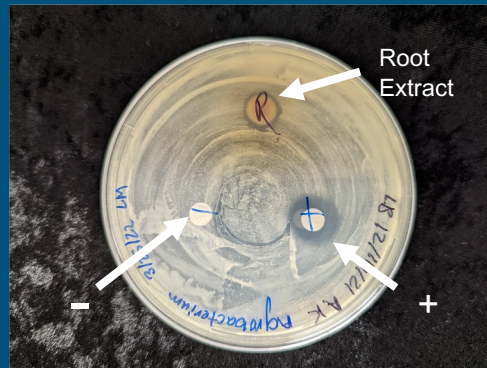
50mg Extract on  
*P. syringa* pv  
Tomato DC3000



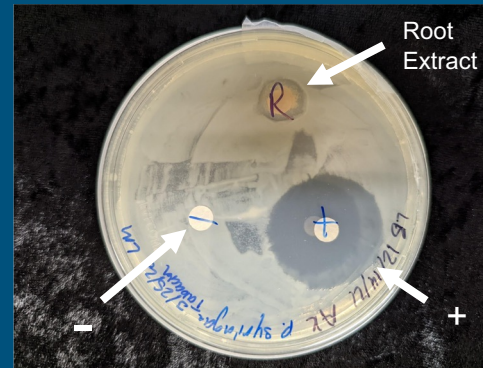
50mg Extract on  
*Bacillus subtilis*



50mg Extract on  
*Agrobacterium*



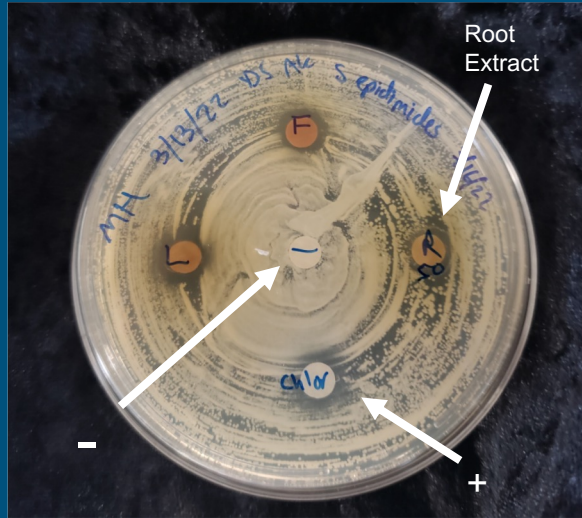
50mg Extract on  
*P. syringa* pv  
Tabacia



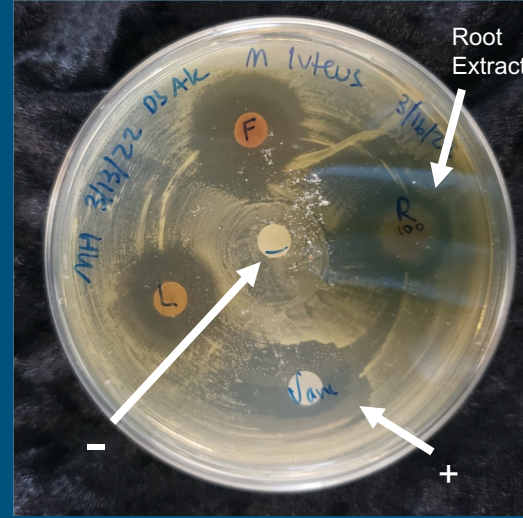
# Results

	Roots (10mg) mm	Roots (20mg) mm	Roots (30mg) mm	Roots (50mg) mm	Kanamycin (50µg) mm	Ampicillin (50µg) mm	Gentamicin (20µg) mm
<i>Bacillus subtilis</i>	1.5	1.25	1.5	1.5	8	2.5	11.75
<i>Bacillus cereus</i>	0	1	ND	ND	5.75	4	6
<i>E. coli</i> DHSα	0	0.25	ND	ND	11	7	7
<i>Agrobacterium tumefaciens</i>	1	1	1	2.5	4.75	8	7.75
<i>Pseudomonas syringae</i> pv. <i>tabaci</i>	0.5	1	1	3.5	13	0.25	13.5
<i>P. syringae</i> pv. <i>tomato</i> DC3000	1.25	1.25	1	2.5	11.75	0.25	13.5

# Results-BSL2



30mg Extract on *S. epidermidis*



30mg Extract on *M. luteus*

# Results- Human Pathogens (BSL2)

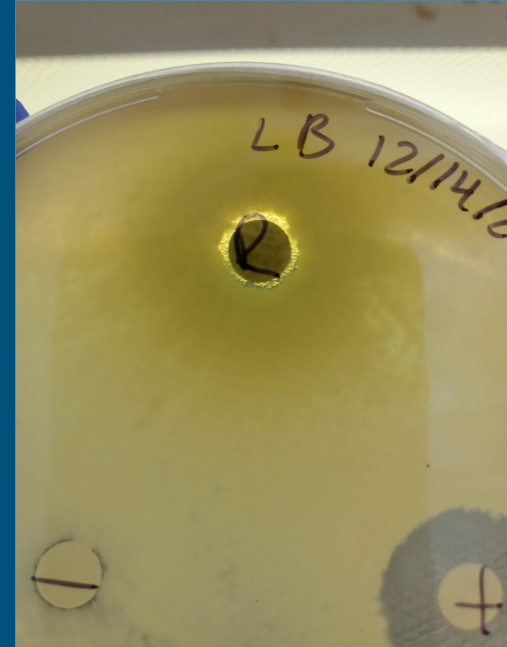
	Root (30mg) mm	Roots (100mg) mm	Ampicillin (50 $\mu$ g) mm	Vancomycin (30 $\mu$ g) mm	Chloramphenicol (30 $\mu$ g) mm
<i>Staphylococcus aureus</i>	0	0	ND	ND	2
<i>Staphylococcus epidermidis</i>	0	1	ND	ND	3
<i>Proteus vulgaris</i>	0	0	0	ND	ND
<i>Micrococcus luteus</i>	4	Contaminated	ND	9	ND



# Conclusion

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- Inhibition was shown
  - All BSL 1
  - 2 of the BSL 2
- Future Research Needed
  - Retest BSL 2
  - Test other bacteria
  - Test against fungi
  - Testing of other methods to prepare crude extract to get more concentrated extract



20mg on *Bacillus cereus*



# Acknowledgment

Dr. Amita Kaundal

Jenny Norton

David and Kayla Suisse

Erik McFadden

Utah State University

Utah State University Honors Program

PSC Department

And so many others



# Thank you!

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Questions?



# Picture Credit

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All pictures taken by me, Lauren McFadden, except Sagebrush on Slide 2 which was taken by Wikipedia editor Peemus, and plate pictures on slide 15 which were taken by David Suisse.