

An Ethnobotanical Approach to Finding Antimicrobial Compounds in Woolly Blue Curls (*Trichostema lanatum*) Using a Kirby-Bauer Disc Diffusion Assay

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Ethnobotany is the scientific study of characterizing why cultures use plants the way they do.



[http://en.m.wikipedia.org/wiki/Chumash_\(tribe\)](http://en.m.wikipedia.org/wiki/Chumash_(tribe))



<http://www.naturebridge.org/santa-monica-mountains>

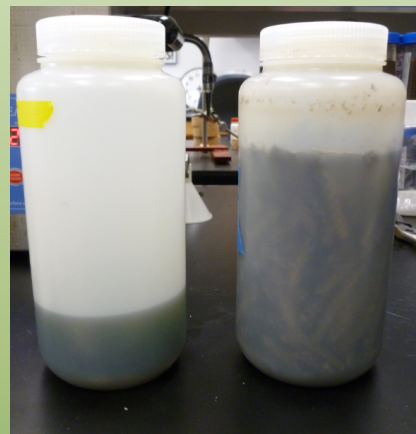
Goals of my project

1. Determine whether a woolly blue curls extract exhibits antimicrobial characteristics.
2. Determine what compound in the woolly blue curls extract works as an antimicrobial.
3. Determine how these antimicrobials effect different bacteria.

Preparation of WBC extract



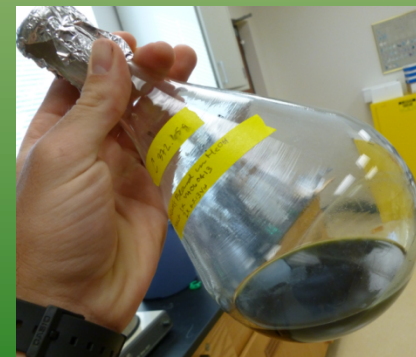
Soak in MeOH for 12-14 hrs.



Strain out solids



Evaporate solvent with a Rotevap.



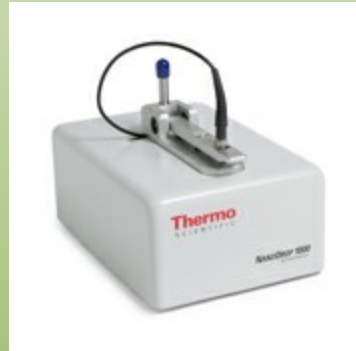
Re-dissolve in DMSO



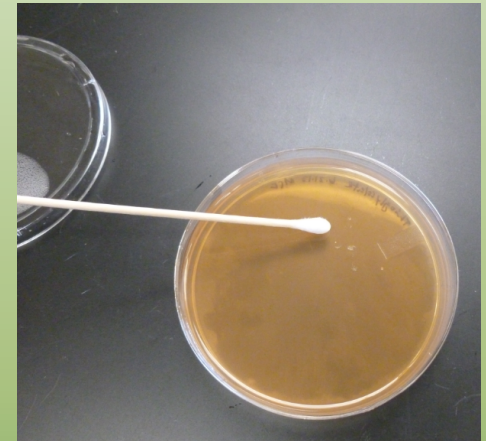
The technique used to determine antimicrobial activity was the Kirby-Bauer disc diffusion assay.



Check Density



Swab onto agar



Introduce discs



Incubate at 37°C for 18hrs.



Inoculate discs with 10uL of extract



http://www.bio-world.com/productinfo/3_43_287_683/2490/M-Medium-sterile-liquid.html

http://openwetware.org/wiki/Luckau_Protocols:NanoDrop

<http://www.grainger.com/Grainger/incubators/lab-ovens-heating-and-refrigeration/lab-supplies/catalog/N-300>

After incubation, I quantified my results by measuring the diameters of inhibition.

negative
control

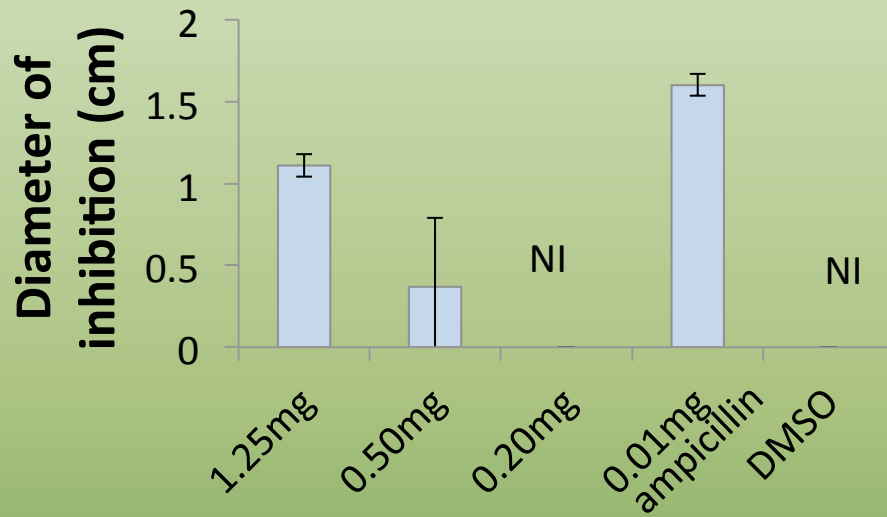
ampicillin (0.01 mg)



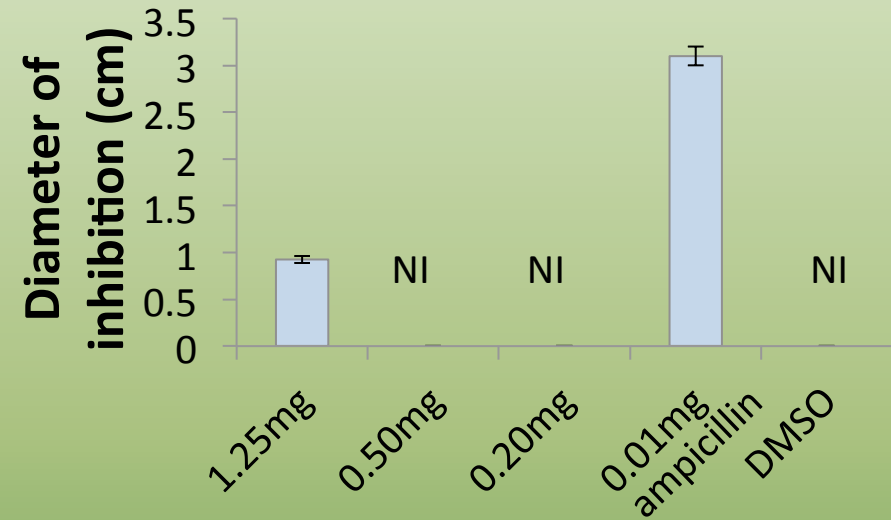
wooly blue
curl extract

All Gram + bacteria had a response to the woolly blue curls extract, but there was some variation among responses.

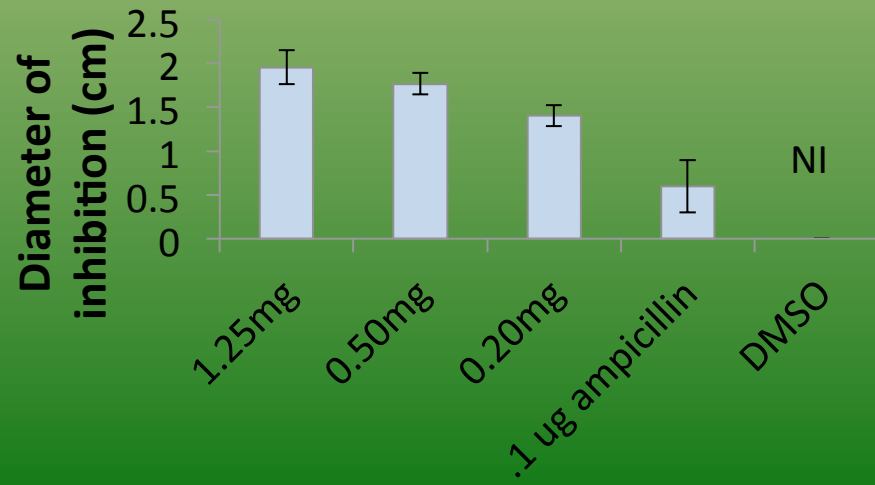
Staphylococcus epidermidis



Staphylococcus aureus



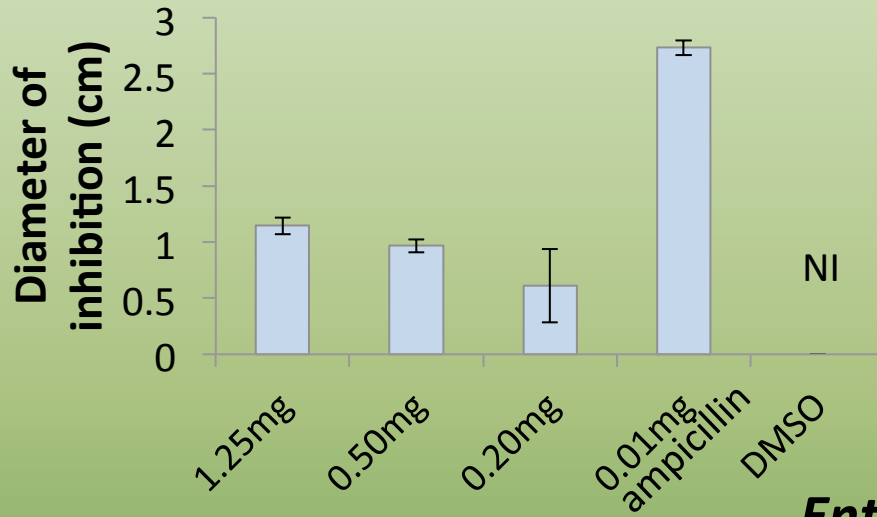
Corynebacterium xerosis



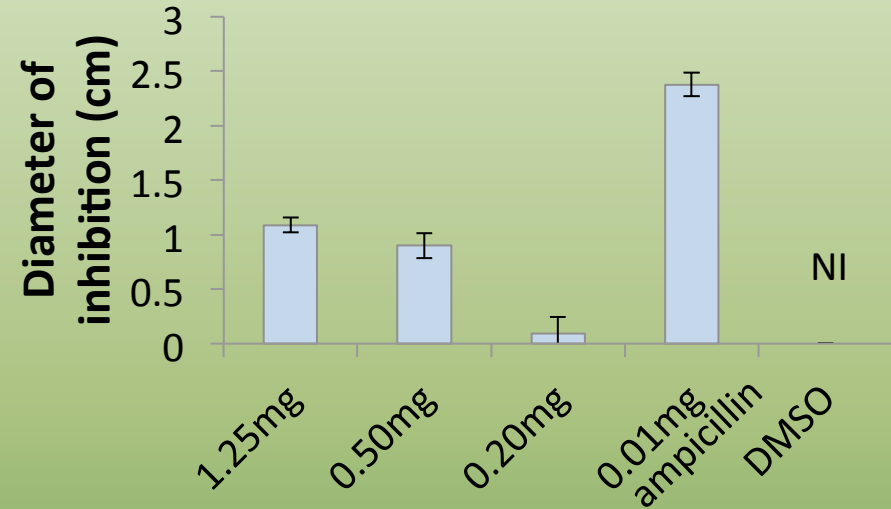
NI= No Inhibition
NT= Not Tested

Though some Gram + bacteria varied in response, others reacted very similarly.

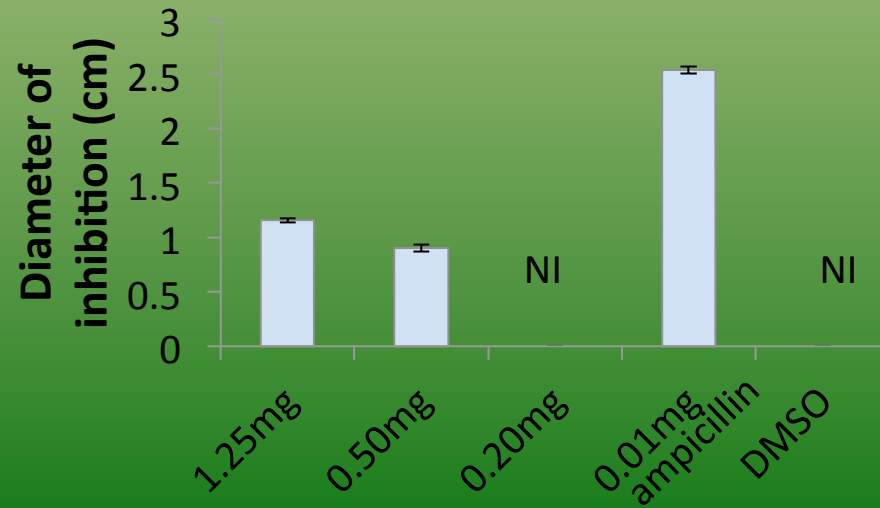
Bacillus subtilis



Bacillus megaterium



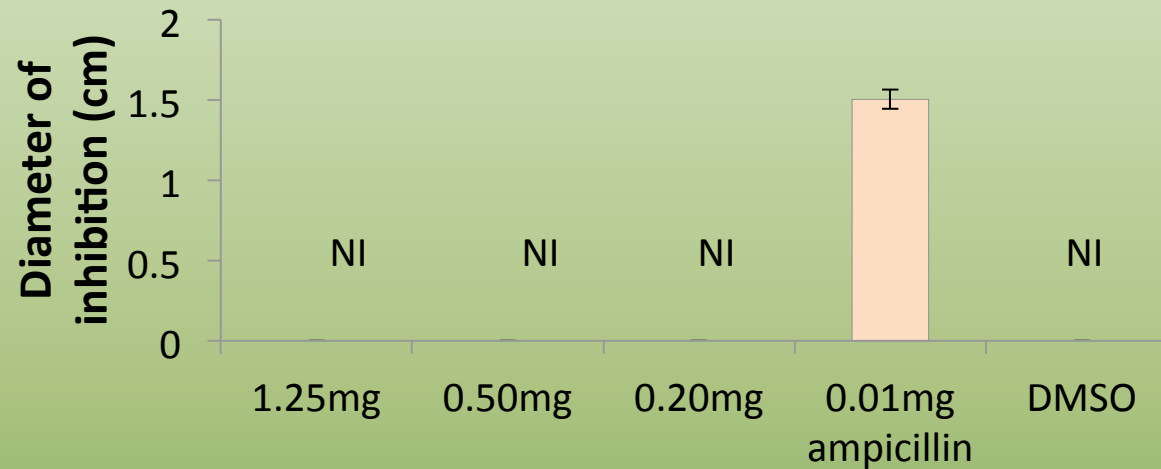
Enterococcus faecalis



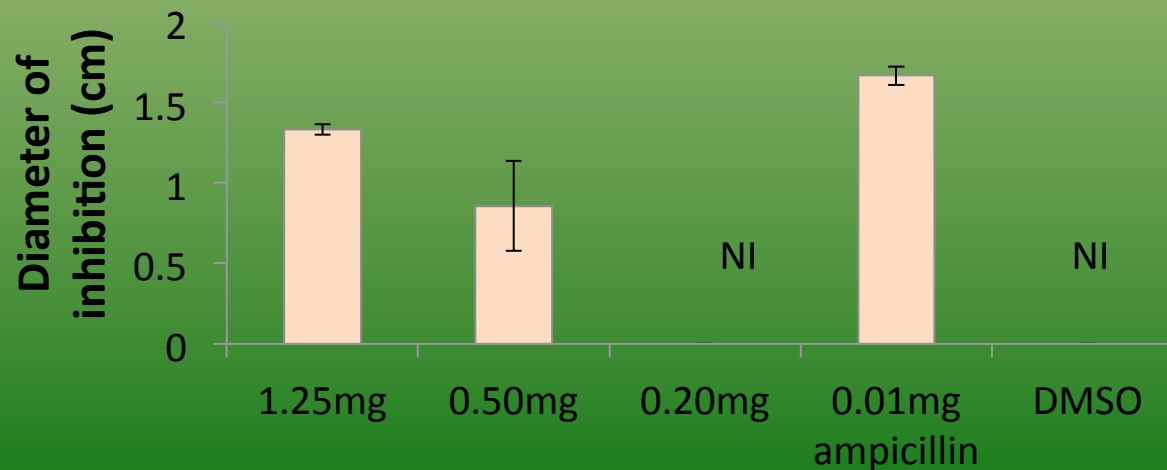
NI= No Inhibition

The *E.coli* $\Delta tolC$ strain, which does not produce the tolC drug efflux pump, was susceptible to the woolly blue curls extract!

Escherichia coli WT

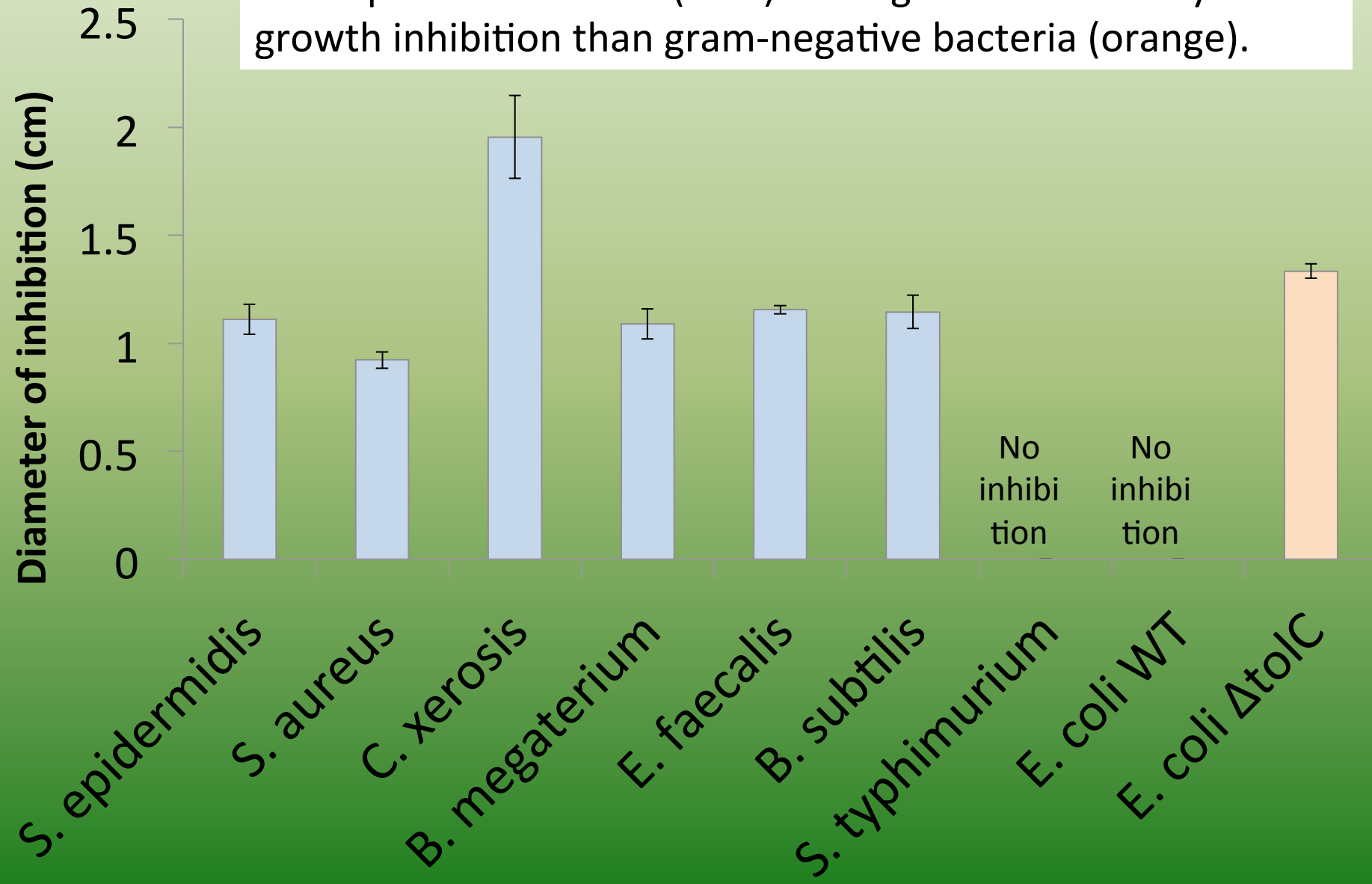


Escherichia coli $\Delta tolC$



NI= No Inhibition

Gram-positive bacteria (blue) show greater sensitivity to growth inhibition than gram-negative bacteria (orange).



All the Gram + bacteria showed positive results, whereas the Gram – had no response regardless of the niche they most commonly occupy.

Bacterium	Niche	Gram (+/-)	Diameter of inhibition (cm)
<i>Staphylococcus epidermidis</i>	Skin	+	1.11 ± 0.07
<i>Staphylococcus aureus</i>	Skin	+	0.92 ± 0.04
<i>Corynebacterium xerosis</i>	Skin	+	1.96 ± 0.19
<i>Enterococcus faecalis</i>	Gut	+	1.16 ± 0.02
<i>Bacillus subtilis</i>	Gut	+	1.14 ± 0.07
<i>Bacillus megaterium</i>	Env.	+	1.09 ± 0.07
<i>Salmonella typhimurium</i>	Gut	-	0.00 ± 0.00
<i>Escherichia coli</i> WT	Gut	-	0.00 ± 0.00
<i>Escherichia coli</i> Δ tolC	Gut	-	1.33 ± 0.03

Conclusions of goals

1. The Chumash medicinal plant woolly blue curls (*Trichostema lanatum*) does exhibit antibacterial properties.
 2. Gram - and Gram + bacteria show different susceptibility to growth inhibition by woolly blue curls extract.
 3. Resistance seen of Gram - bacteria may be due to drug efflux pumps and an extra outer membrane.
- *We were unable to determine the compound working as an antimicrobial.

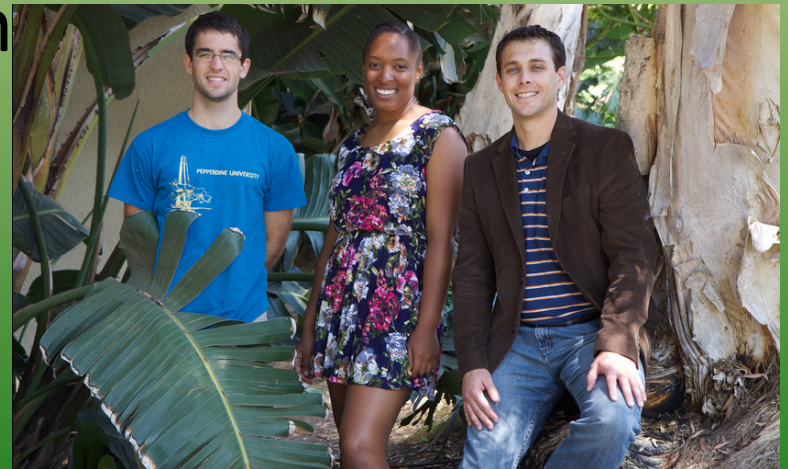
Further Study

- We would like to fractionate the extract to determine the active compound and test how effective it actually is.
- Test for synergistic properties of compounds within the woolly blue curls extract⁶.
- Screen other Chumash plants for efflux pump inhibitors.



Acknowledgments

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References

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