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An Ethnobotanical approach to finding antimicrobial compounds in wooly blue curls (Trichostema lanatum) using a Kirby-Bauer disc diffusion assay

Matthew C. Fleming
Pepperdine University

P. Matthew Joyner
Pepperdine University

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Matthew C. Fleming and P. Matthew Joyner
Department of Biology, Natural Science Division, Pepperdine University, Malibu, California, USA

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Abstract

Plants can be an important source of creativity and production of new drugs. In this study, extracts of wooly blue curls (*Trichostema lanatum*) were made using DMSO and tested for antimicrobial activity on a panel of bacteria commonly found in separate ecological niches. Wooly blue curls (WBC) was chosen due to its being recorded as a strong disinfectant by the Chumash people. It was found that WBC does exhibit antimicrobial activity against gram positive bacteria and not against gram negative bacteria. However, gram negative bacteria with reduced drug efflux function became susceptible to the WBC extract.

Introduction

This study of medicinal plants incorporates the strategy of ethnobotany, which tries to characterize why cultures use plants the way they do. Wooly blue curls is documented as being a medicinal plant for the Chumash people[1] and was shown to have antibacterial properties from previous research from our lab. In order to study WBC's antibacterial properties, a panel of bacteria commonly found in separate anatomical habitats is utilized. Differing bacteria with unique characteristics can specialize in separate niches[3]. Therefore, by using bacteria from different niches (in this study the skin niche and gut niche) we can better observe WBC's antimicrobial activity.

Materials and Methods

<u>Plant collection</u>

- The plant specimens were found in the Santa Monica Mtns. near Pepperdine University .
- We collected flowers, branches, and leaves of the plant sample.

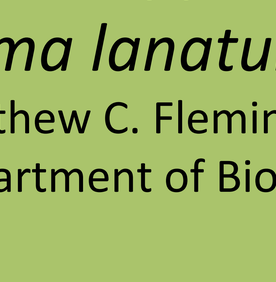


Figure 1: Collection of *Trichostema lanatum*

<u>Preparation of plant extracts</u>

- The plant specimen was soaked in MeOH for12-14hrs. (3x)
- The solvent was then evaporated and the extract was re-dissolved in DMSO to its respective concentrations.

<u>Kirby-Bauer Disc Diffusion Assay (DDA)</u>

- Bacterial cultures were diluted to their respective concentrations and a cotton swab was used to inoculate an agar plate.
- A Sensi Disc Dispenser was used to introduce filter paper discs to the agar plates and 10µL of extract were used to inoculate each disc.
- The plates were placed in a 37°C incubator for 18hrs. And the diameter of the zones of inhibition were measured in cm with a ruler. (C. xerosis was grown for 40hrs. due to a slow growth rate.)

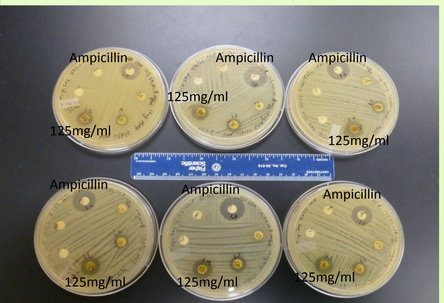
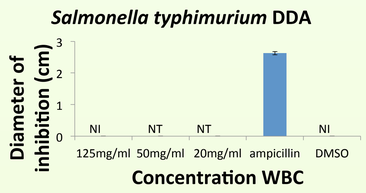


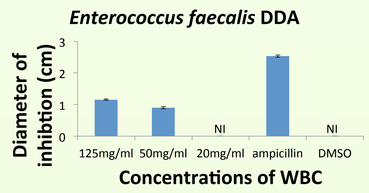
Figure 2: Example of a Kirby-Bauer disc diffusion assay. This shows the *E. coli ΔtolC* knockout mutant that allows for inhibition of the WBC extract.

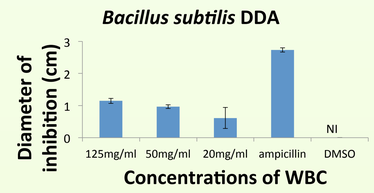
Results

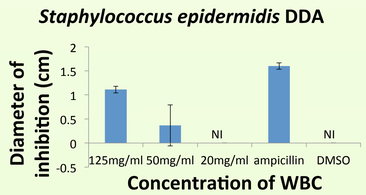
•The bar graphs show the diameter of the zone of inhibition for each concentration of extract used along with a positive control (ampicillin) and a negative control (DMSO). The error bars show the standard deviation for each measurement.

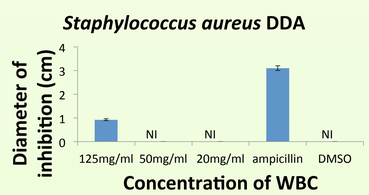
Graphs Legend:
NI= No Inhibition
NT= Not Tested

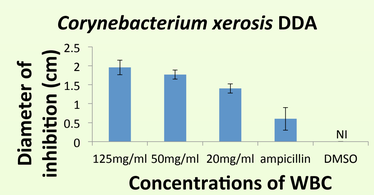


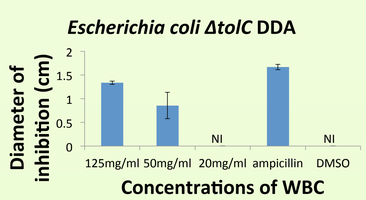


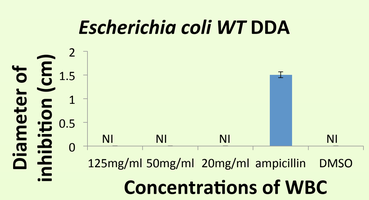












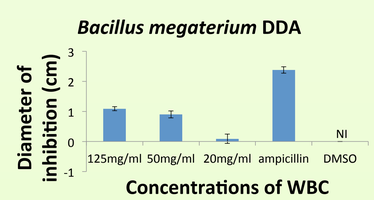


Table 1: Summary of DDA data.

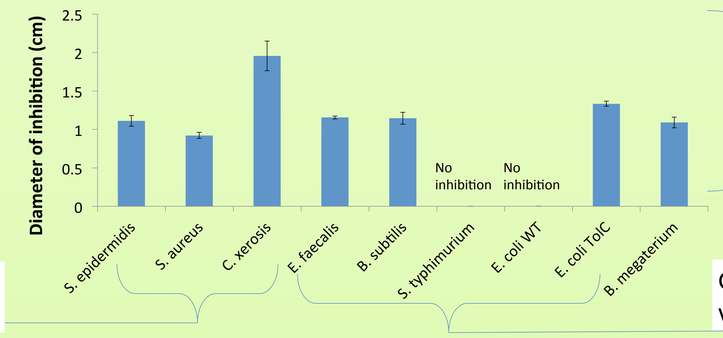
Bacteria	Niche	Gram(+/-)	125mg/ml WBC inhibition (cm)
Staphylococcus epidermidis	Skin	+	1.11 ± .07
Staphylococcus aureus	Skin	+	.92 ± .04
Corynebacterium xerosis	Skin	+	1.96 ± .19
Enterococcus faecalis	Gut	+	1.16 ± .02
Bacillus subtilis	Gut	+	1.14 ± .07
Bacillus megaterium	Env.	+	1.09 ± .07
Salmonella typhimurium	Gut	-	0 ± 0
Escherichia coli WT	Gut	-	0 ± 0
Escherichia coli ΔtolC	Gut	-	1.33 ± .03

WBC extracts did not inhibit wild type Gram negative bacteria.

Gram negative *E. coli ΔtolC* with inhibited efflux pumps becomes susceptible to WBC extracts.

Gram positive bacteria are susceptible to the WBC extract

Figure 3: Comparison of the reactions of each bacteria with 125mg/ml WBC extract.



This shows there is a broad range of reactions, from no zone of inhibition to almost a 2 cm zone of inhibition.

Skin bacteria have a variety of responses.

Gut bacteria have a variety of responses.

Conclusions

•Wooly blue curl extract does produce compounds that have antimicrobial properties.
•Wooly blue curl extracts do not affect all bacteria the same.

Discussion

The resistance of Gram negative bacteria may be explained due to efflux pumps, that are able to extrude antimicrobials, and a second outer membrane that is hard for antimicrobials to penetrate. Most plant pathogens are Gram negative[1] , potentially because they have greater resistance to plant antimicrobials. Gram positive bacteria also have efflux pumps[1], however they lack the outer membrane causing them to be more susceptible. Therefore the presence or absence of specific efflux pumps may explain the variation of responses between Gram positive bacteria. Due to activity against a variety of bacteria and the role of efflux pumps in resistance, the antimicrobial in WBC may have a conserved internal target among bacteria like the fatty acid cycle or a bacterial ribosome.

Further Study

We would like to fractionate the WBC extract to determine the active component and test how effective it is. Then we would like to test the antimicrobial along with a known efflux inhibitor to see if there is a synergistic effect between the two compounds. Another area of study that could prove beneficial is screening other Chumash medicinal plants to search for specimens that produce efflux inhibitors along with antimicrobials.

Acknowledgements

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