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Antimicrobial Activity and Germination Conditions of the Medicinal Plant Argemone mexicana

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Antimicrobial Activity and Germination Conditions of the Medicinal Plant *Argemone mexicana*.

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ABSTRACT

Commonly called the Mexican prickly poppy, *Argemone mexicana* is a stress-resistant member of the Papaveraceae family of plants that has been used in traditional medicine for centuries by indigenous communities in Mexico and Western parts of the United States. This plant has been used to treat a wide variety of ailments, including skin diseases and intestinal infections, with reported antimicrobial properties. However, these properties are poorly understood and no bioactive compounds have yet been identified in the plant to account for this antimicrobial action. Herein, we describe the growth conditions and preliminarily characterize the antibiotic effects of different parts of the *A. mexicana* plant. We report that 2 mg of *A. mexicana* methanol root extract possesses antibacterial activity against the bacteria *Bacillus cereus* and *Staphylococcus aureus*, while the same concentration has no inhibitory effect on the fungus *Candida albicans*. Moreover, the methanol root fraction displays a stronger antibacterial effect, when compared to either the methanol seed or leaf fractions at the same concentrations and normalized to background solvent alone. Additionally, we show that when supplemented with 1000 mg/L of the phytohormone gibberellic acid (GA), germination rates of *A. mexicana* are significantly increased when compared to germination with either no GA or 100 mg/L GA. These preliminary results warrant further research into defining the antimicrobial properties and chemicals produced in the roots of these plants and are especially significant given the growing global concern of antibiotic-resistant 'superbugs' and lack of new antimicrobial drug discovery.

INTRODUCTION

With the high number of antibiotic-resistant pathogenic microorganisms, there is a pressing need for the development of new classes of antibiotic drugs (Figure 1; Clatworthy *et al*., 2007). One potential source for the discovery of new anti-infection agents is from medicinal plants (reviewed by Ríos & Recio, 2005).

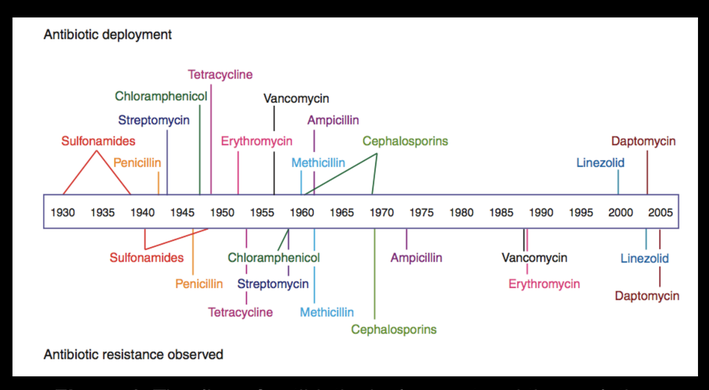

Figure 1. Timeline of antibiotic deployment and the evolution of antibiotic resistance (from Clatworthy *et al*., 2007)

One promising medicinal plant candidate is the fairly unexplored *Argemone mexicana*, commonly called the Mexican prickly poppy. *A. mexicana* is a hardy pioneer plant that has been used since the time of the Aztecs for medicinal purposes (Emmart, 1940). This plant is used in traditional medicine in different parts of the world to treat a wide variety of ailments, such as: tumors, warts, skin diseases, inflammation, rheumatism, jaundice, leprosy, microbial infections, and malaria (reviewed in Brahmachari *et al*., 2013). Some chemical and pharmacological aspects of *A. mexicana* have been identified (reviewed in Brahmachari *et al*., 2013), but, to date, no bioactive compounds have yet been identified in the plant to account most of it's medicinal effects, such as it's antimicrobial or anticancer actions. Herein, we describe the growth conditions and preliminarily characterize the antibiotic effects of different parts of the *A. mexicana* plant.

REFERENCES

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Emmart (1940) The Badianus Manuscript: an Aztec Herbal of 1552. John Hoptkins Press: Baltimore, MD.

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RESULTS & METHODS

Figure 2: Germination Experiments

Figure 2 – Gibberellic acid germination experiments.

A. Two *Argemone mexicana* seeds were planted per soil pod and watered with equal amounts of either 0 mg/L, 100 mg/L or 1000 mg/L gibberellic acid solutions. Ethanol (the solvent used to prepare the stock GA solution) was used in place of GA in the 0 & 100 mg/L GA water solutions. Seedlings were kept in small greenhouses under a 16/8 light cycle and photographed after 30 days.

B. Using the same samples as in panel A, average germination rate per pod was calculated. n=10 pods, with average percentage germination values displayed.

Figure 3: Extraction Procedure

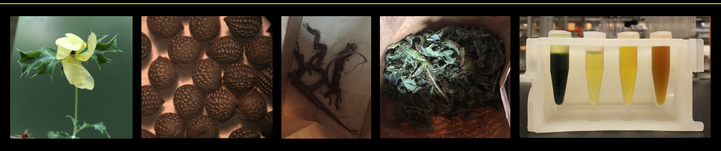

Figure 3 – Whole *Argemone mexicana* plants were separated into seeds, roots or leaves and allowed to dry in paper bags at 22°C. 2 g of each dried sample was homogenized using a mortar and pestle. The powdered sample was then macerated in methanol using a 1:4 (plant material:solvent) ratio at 200 rpm, 35°C for 48 hours. The mixture was centrifuged at 5,000 x g for 5 minutes, and the supernatant was filtered through a 0.2 uM PTFE membrane. The filtrate was then concentrated using a water bath, quantified and tested for antimicrobial effects.

Figure 4: Antimicrobial Experiments

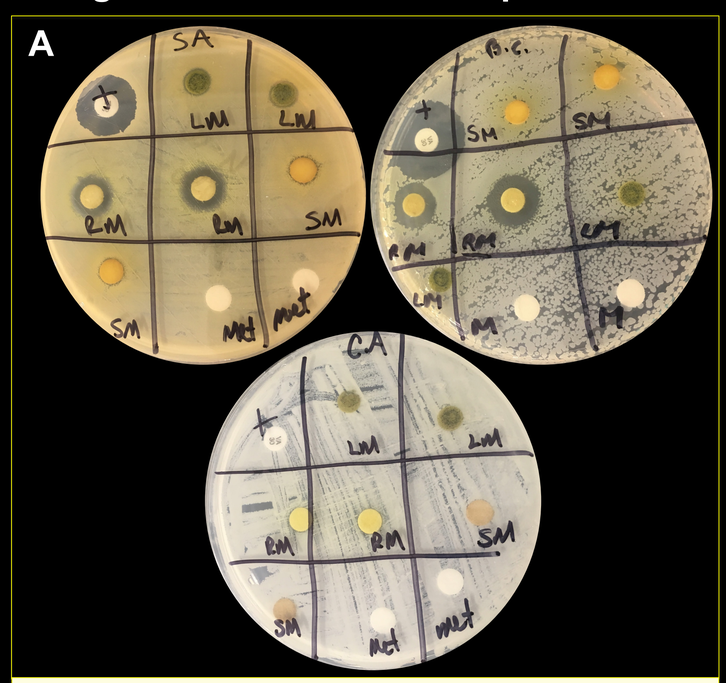

Figure 4 – Antimicrobial Disc Diffusion Assay.

A. Blank antibiotic sensitivity disks were impregnated with 2 mg of *Argemone mexicana* extract (from either the seed, root or leaf). The antibiotic vancomycin (inhibits cell wall synthesis) was used as a positive control, and the solvent alone (methanol) was used as a negative control. After the solvent completely evaporated, disks were placed onto a media plate with a lawn of the appropriate microorganism (*S. aureus*, *B. cereus* or *C. albicans*). After 48 hours of growth at 37°C, plates were photographed. One representative plate for each microorganism is show in panel A.

B. The experiment from panel A was replicated four times, and zones of inhibition were measured using a ruler for each disk. n=4, with the average zone of inhibition values displayed in millimeters.

CONCLUSIONS

- *A. mexicana* germination rates are significantly increased with the addition of 1000 mg/L gibberellic acid (Fig. 2).
- The methanol root extract displays antimicrobial activity against two bacterial species but has no effect on the fungal species tested (Fig. 4).
- The methanol root fraction displays a stronger antibacterial effect than either the methanol seed or leaf fractions (Fig. 4).
- Further experiments are being conducted to:
  - Test these extracts against different microorganisms
  - Identify the compounds in the methanol root fraction
  - Extract compounds in solvents with different polarities
  - Examine these extracts for anti-cancer properties
- These results are significant given the growing global concern of antibiotic-resistant 'superbugs' and lack of new antimicrobial drug discovery.

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