

Characterizing the cytotoxic effects and several antimicrobial phytocompounds of Argemone mexicana

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INTRODUCTION

Plants naturally produce a robust supply of novel metabolic compounds that can be used to treat a variety of human diseases. From 1981 to 2010, it is estimated that nearly 50% of all cancer drugs originated from natural products [1], many of which were derived from terrestrial plants [2]. Likewise, plants produce many antimicrobial agents, which include a wide variety of natural defense compounds, such as phenolics, terpenoids, alkaloids, polyacetylenes, lectins and polypeptides [3].

With the advent of modern antibiotic drugs mainly of bacterial, fungal and synthetic sources, many of these natural plant-derived antibiotic compounds have been left unexplored. Yet with the high number of antibiotic-resistant pathogenic microorganisms, there is a pressing need for the development of new classes of antibiotic drugs (Fig. 1).

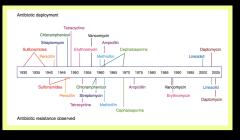


Figure 1. Timeline of antibiotic deployment and the evolution of antibiotic resistance (taken from [4]).

One promising medicinal plant candidate is the fairly unexplored Argemone mexicana, commonly called the Mexican prickly poppy. A. mexicana is a stress-resistant member of the Papaveraceae family of plants that has been used since the time of the Aztecs [5] to treat a wide variety of ailments, such as: tumors, warts, skin diseases, inflammation, rheumatism, jaundice, leprosy, microbial infections, and malaria [6]. Some chemical and pharmacological aspects of *A. mexicana* have been identified [6], but, until the publication of this research [7], very few bioactive compounds had been identified in the plant to account for its many medicinal effects. Thus, this plant possesses great potential for the discovery of novel antibiotic and anticancer compounds, which is the nature of the work herein.

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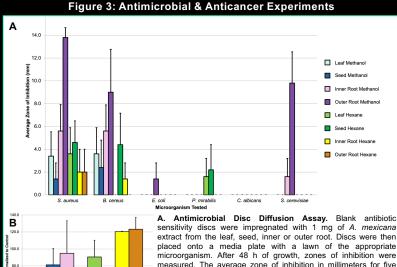
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METHODS & RESULTS

Figure 2: Extraction Procedure



Extract Preparation. Whole Argemone mexicana plants were separated into leaves, seeds, inner or outer roots and allowed to dry in paper bags at 22°C. 2 grams of each dried sample was homogenized using a mortar and pestle. The powdered sample was then macerated in methanol or hexane 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 dehydrated, quantified and tested for biological activity

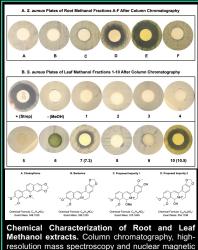


60.0 20.0

placed onto a media plate with a lawn of the appropriate microorganism. After 48 h of growth, zones of inhibition were measured. The average zone of inhibition in millimeters for five biological replicates is shown with associated SEM B. Cancer Cell Viability Assay. T84 human colon cancer cells

were treated with 1 mg of leaf, seed, inner root or outer root extract for 1 h. The MTT colorimetric assay was then used to determine cell metabolic activity after treatment with extracts. The mean percentage of viable cells normalized to the control (solvent alone) for at least three biological replicates is shown with SEM.

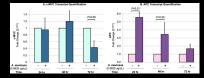
Figure 4: Chemical Characterization



Methanol extracts. Column chromatography, high-resolution mass spectroscopy and nuclear magnetic resonance were performed to separate and identify antimicrobial phytocompounds from A. mexicana root and leaves. Chelerythrine was consistent with root fraction 'D', while berberine was consistent with root fraction 'E' and with leaf fraction '10.5'.

CONCLUSIONS & ON-GOING WORK

- Outer root methanol extracts possess antimicrobial activity, with greatest effects against gram-positive bacteria (Fig. 3).
- Outer root methanol and seed hexane extracts have inhibitory effects against T84 human colon cancer cells (Fig. 3).
- mRNA levels of c-MYC (oncogene) and APC (tumor suppressor) were quantified:



- Chelerythrine and berberine were found • as main antibiotic compounds in the roots and/or leaves of A. mexicana (Fig. 4).
- Recent work on synthesizing and testing new berberine and chelerythrine variants can be found at posters #38 and #68.