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# Isolation, Identification, Modification, and Biological Analysis of extracts of *A. Mexicana*

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## Abstract

*Argemone mexicana* is a member of the Papaveraceae family of plants that has been used for centuries in traditional medicine by indigenous communities in Mexico and the United States. Through a collaborative project, we have explored separation of key components of the seeds and leaves of this plant to isolate the source of their biological activity. We have already identified two key molecules which give this plant anti-microbial properties against the gram-positive bacteria. Furthermore, we have chemically modified one of these molecules to observe variations on activity. We hope this leads to the discovery of new antibiotic drugs.

## Introduction

Microbial infections, such as bacterial or fungal, account for a persistent threat. This issue is compounded by the growing number of drug-resistant microorganisms. In 2014, global deaths due to antimicrobial resistance were approximately 700,000 per year.<sup>1</sup> *Staphylococcus aureus* is a common human pathogen, some strains of which are resistant to multiple antibiotics.<sup>2</sup>

Plants naturally produce a robust supply of novel metabolic compounds that can be used to treat a variety of human diseases. *Argemone mexicana* has been reported to possess a wide-range of biological activities, such as anticancer, antimicrobial, anthelmintic, anti-inflammatory, anthelmintic, antidiabetic and antioxidant actions.<sup>3</sup> The *A. mexicana* plant is native to the West Indies, but today it can also be found in Mexico and throughout the Americas.<sup>4</sup> It has been used in traditional medicine for centuries. Our primary goal in the project is to identify the components of this plant which are responsible for this bioactivity. By identifying these plant-derived antimicrobials, we use this to guide the development of new, unnatural compounds which can pave the way for new drugs.

Prior to this work, the Orozco-Nunnelly lab studied extracting various parts of the plant with solvents of different polarity. This work showed that methanol extracts of the root showed the strongest activity against *S. aureus* (Figure 1).

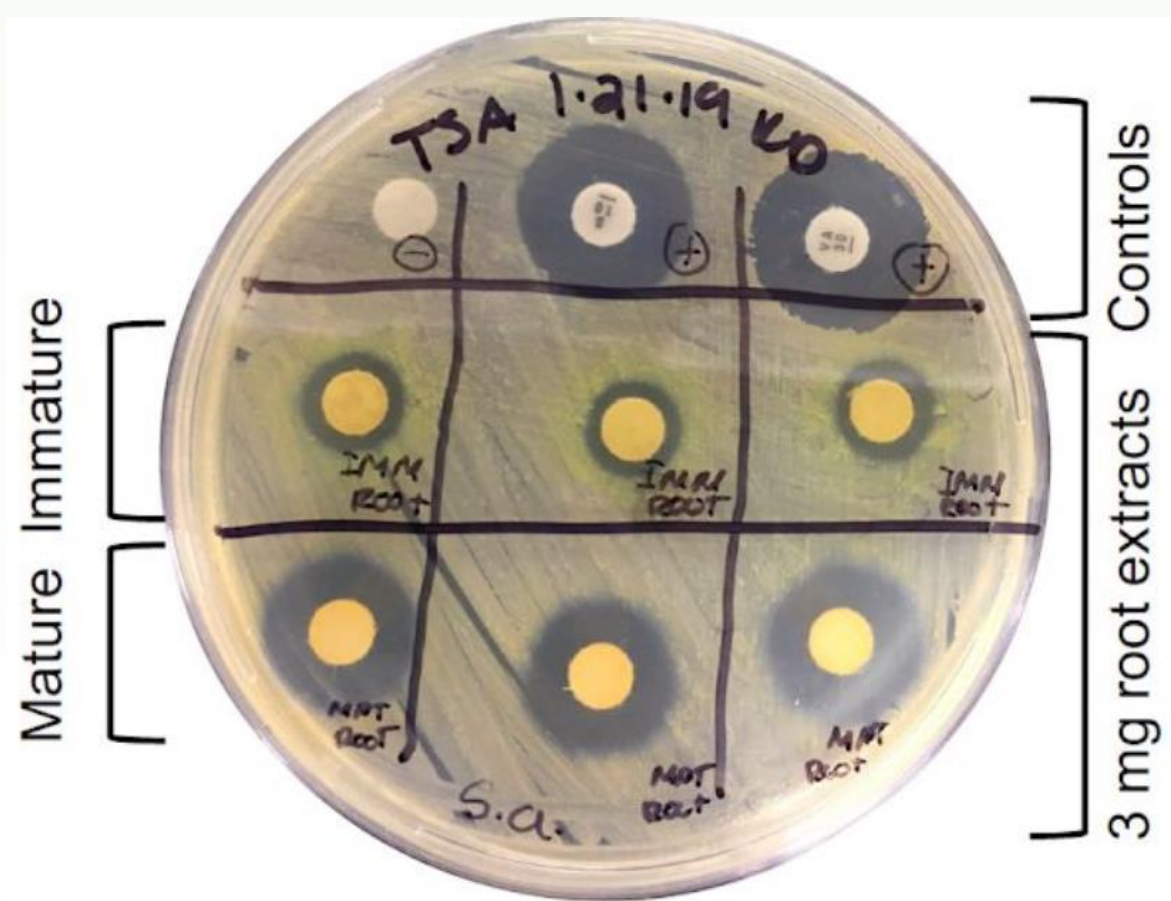


Figure 1: Preliminary tests showing antibacterial activity of the methanol-root extract

## Results

As stated, the methanol extract of the *A. mexicana* showed the highest level of bioactivity. To aid in the identification of the antimicrobial components, the crude methanol extract was separated using various chromatographic techniques, such as Pre-TLC (Figure 2).

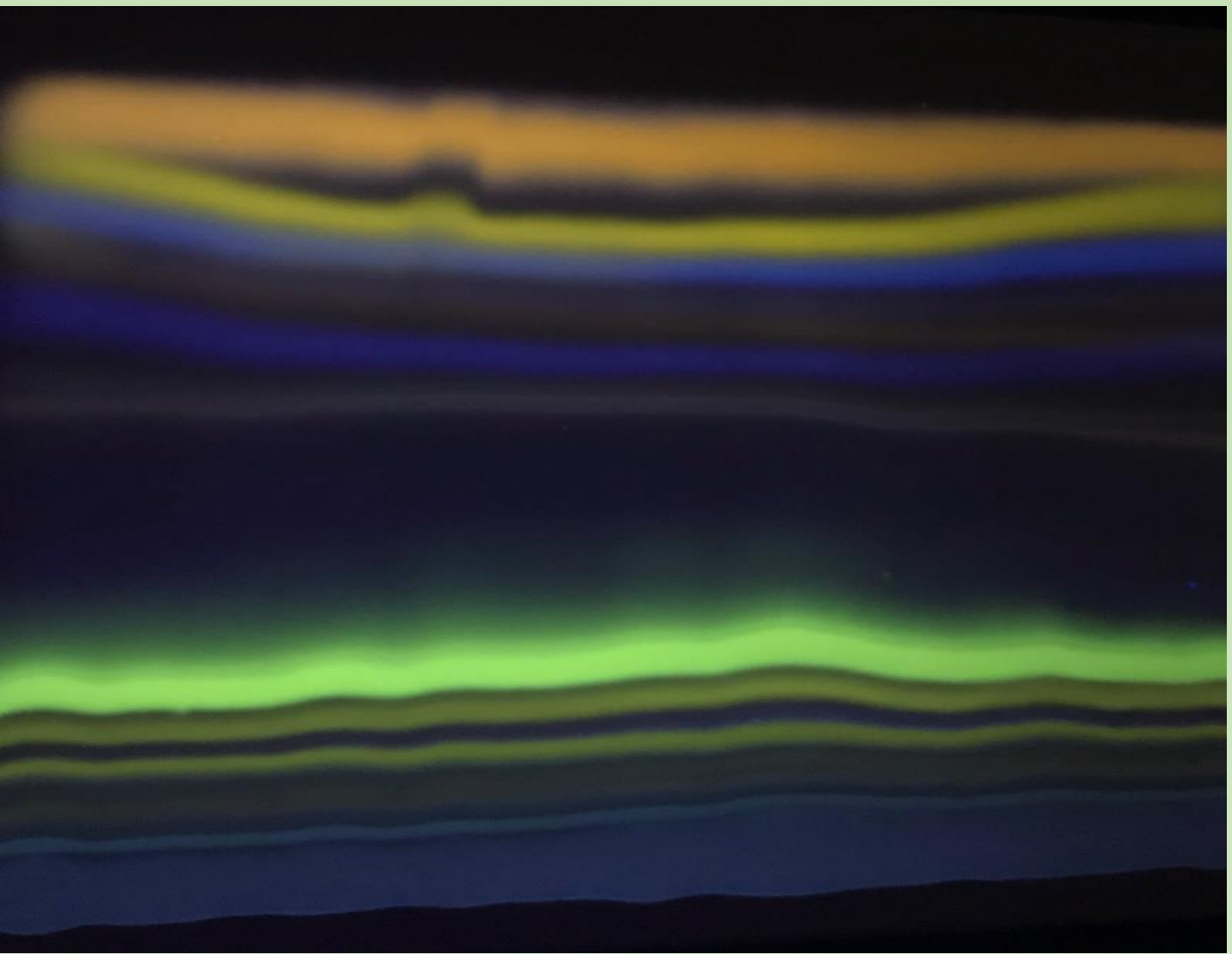


Figure 2: Prep-TLC was performed on the crude methanol leaf extract of the *A. mexicana*. The bands showed different fluorescent compounds in the extract, which were used to identify the distinct components.

The separated components, labeled fractions A-F, were examined using the Kirby-Bauer test to see zones of inhibition (Figure 3). It was determined that fractions 'D' and 'E' showed the most activity.

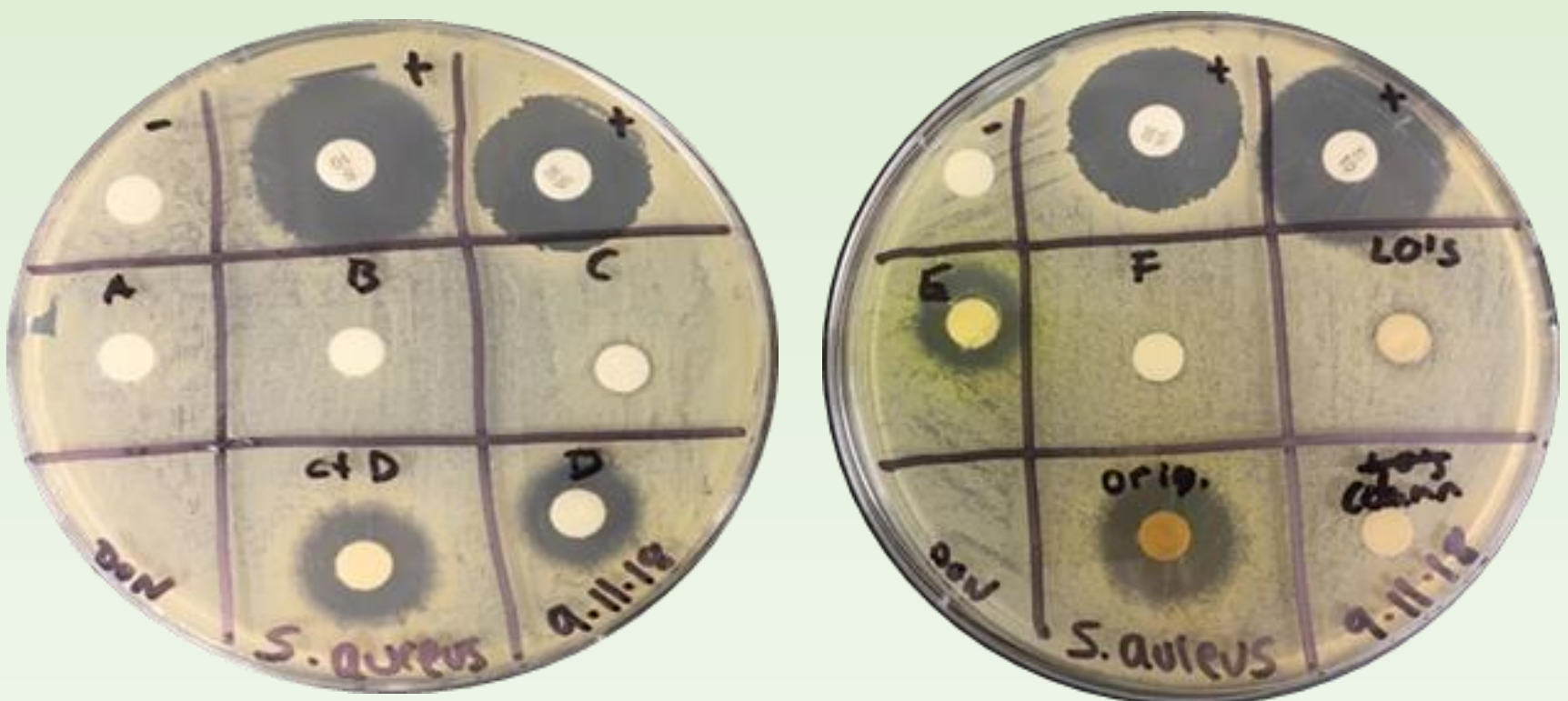


Figure 3: *S. aureus* spread over agar. Diffusion disks impregnated with samples were placed on *S. aureus*, and after two days, zones of inhibition were measured.

To confirm the successful purification of fractions D and E, these were compared by TLC to the original root extract (Figure 4). These isolated components were then analyzed by Mass-spectrometry and H-NMR in an effort to identify the molecules responsible for the antibacterial activity.

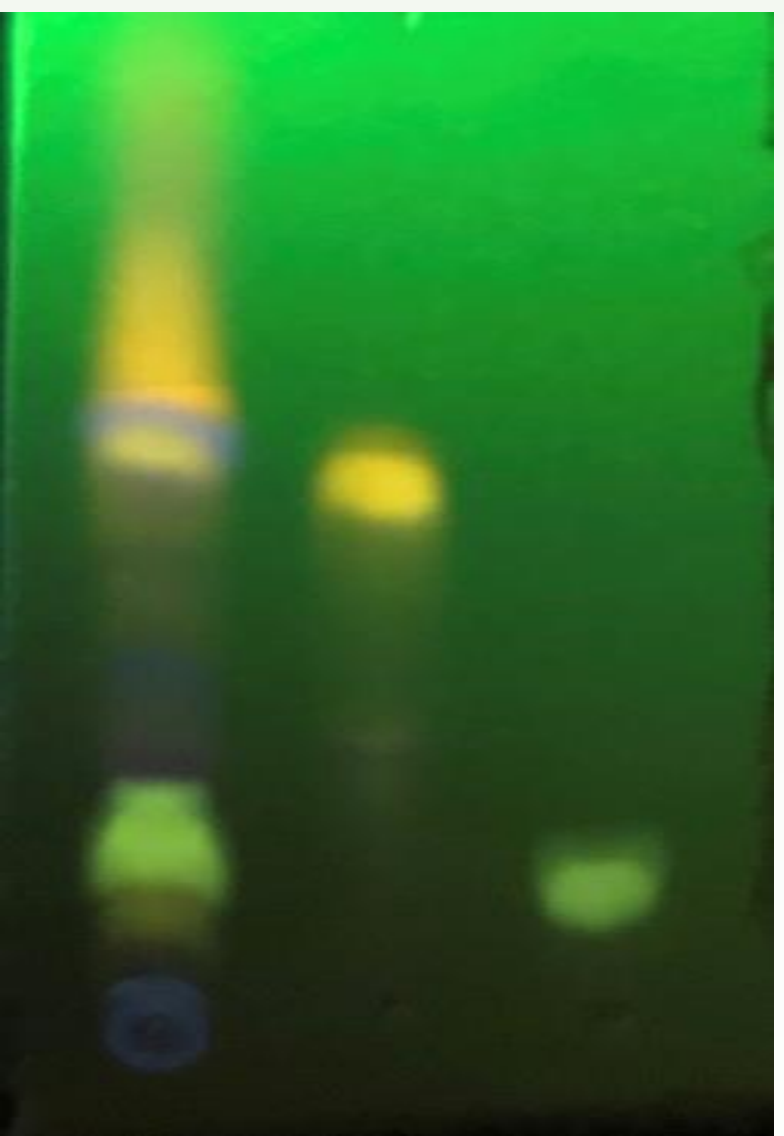


Figure 4: Purity of the separations were evaluated using a TLC plate

## Results

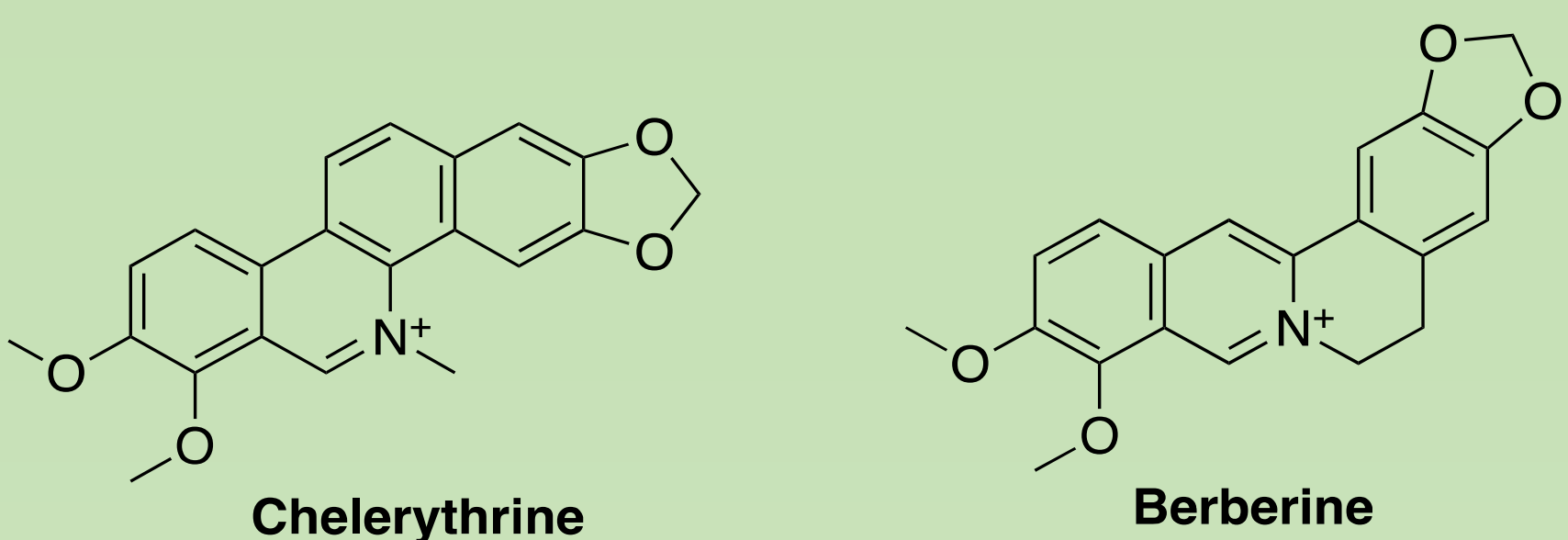


Figure 5: Molecular structures for Chelerythrine (root-D) and Berberine (root-E)

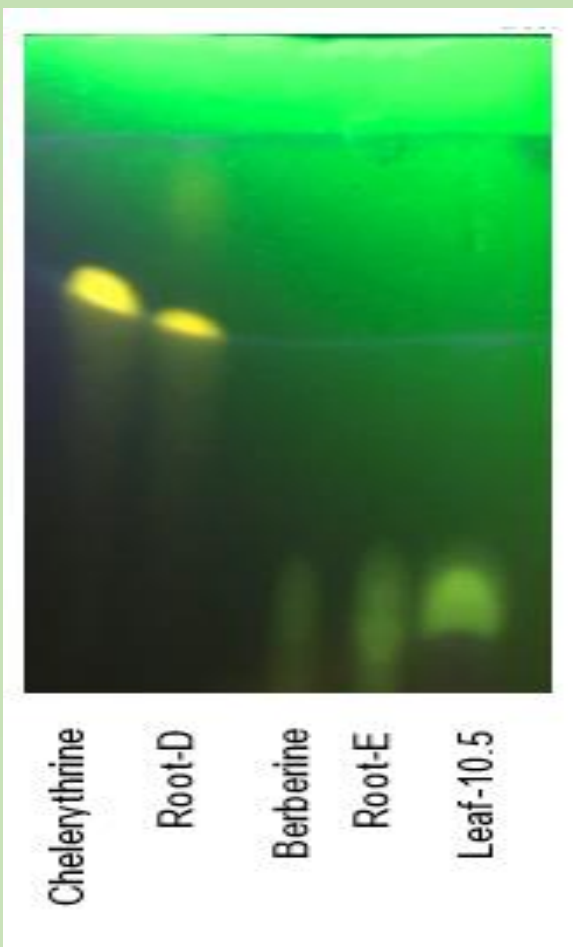


Figure 6: TLC plate for conformation of compounds

A commercial sample of Berberine was then chemically modified into three derivatives, and these synthetic-analogs were tested to see how these chemical modifications changed the bioactivity (Figure 7).

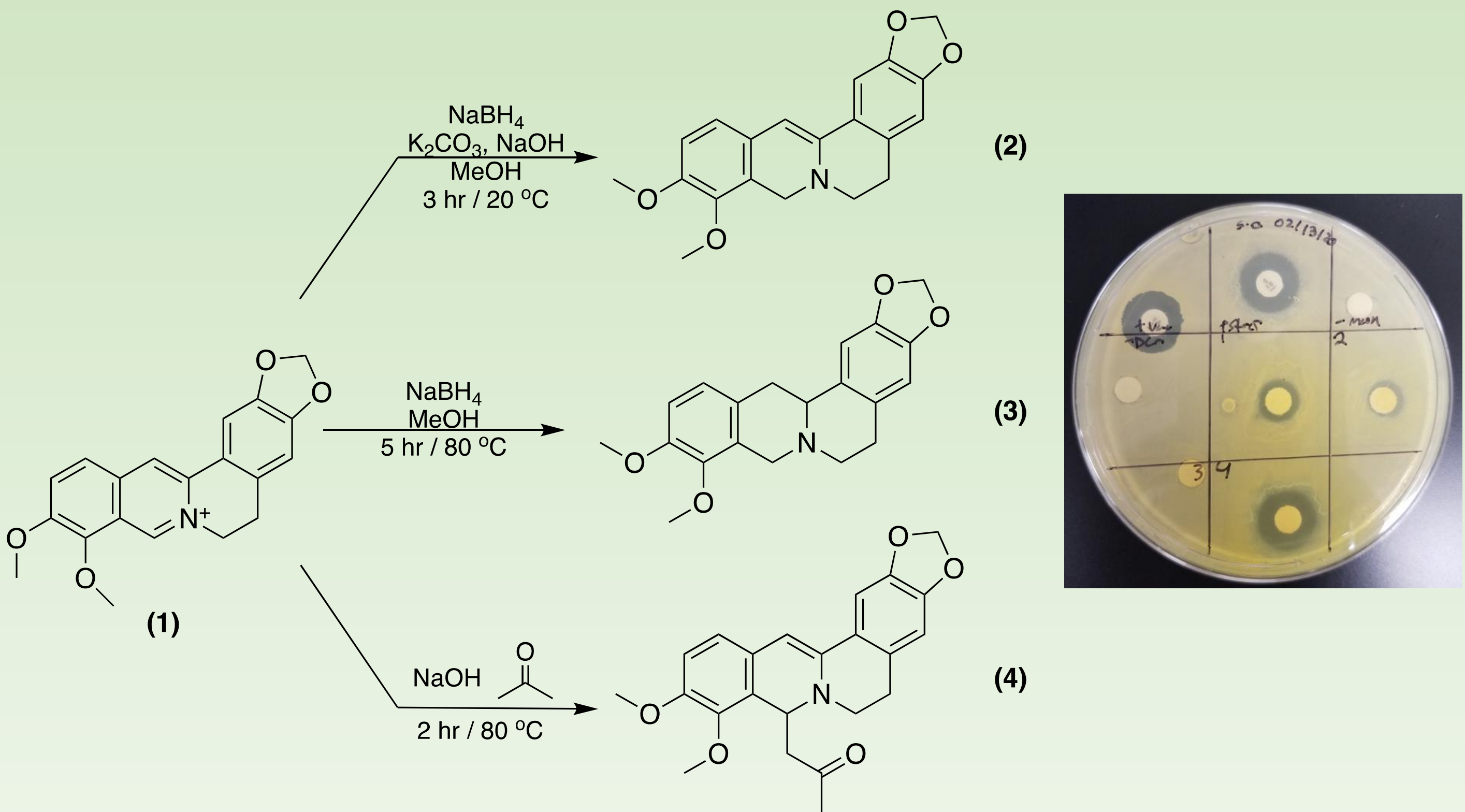


Figure 7: Berberine (1) was partially reduced to 2, fully reduced to 3, and reacted with an acetone enolate to give 4. *S. aureus* was treated with samples of 1-4, with 4 showing improved anti-microbial activity compared to the initial berberine.

## Conclusions

Bioactive extracts from the *A. mexicana* plant were separated into individual components to identify the source of activity. This showed Chelerythrine and Berberine to be responsible. We have also modified berberine to determine ways to improve these properties.

## References

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