Antimicrobial activities of several *Argemone mexicana*-inspired phytocompounds

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INTRODUCTION & HYPOTHESIS

The isolation, or creation, of novel antimicrobial agents is currently at the forefront of modern healthcare due to the stark decrease in antimicrobial drug development in recent years [1] (Figure 1) and due to the increasing rise of superbugs, or microorganisms that are resistant to more than one type of antimicrobial treatment, which are predicted by 2050 to cause 10 million deaths per year [2].

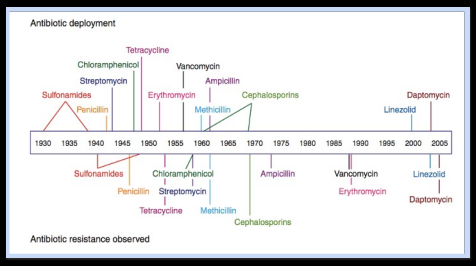


Figure 1. Timeline of antibiotic deployment and the evolution of antibiotic resistance (taken from [1]).

Plants produce many antimicrobial agents, which include a wide variety of natural defense compounds, such as phenolics, terpenoids, alkaloids, polyacetylenes, lectins and polypeptides [3]. Based on previous research done by our group on *Argemone mexicana*, several antimicrobial compounds were isolated from the roots and leaves of the *A. mexicana* plant, including berberine, chelerythrine, and sanguinarine (work published in [4]).

Since then, we synthesized multiple rationally-designed variants of these original phytocompounds, and in 2023, we published an article summarizing the biological activities of fourteen berberine and four chelerythrine variants, where several of these variant compounds show increased antibacterial effects against gram-positive bacteria, yet reduced toxicity against the eukaryotic fungal cell lines tested (berberine variants shown in Figure 2) [5].

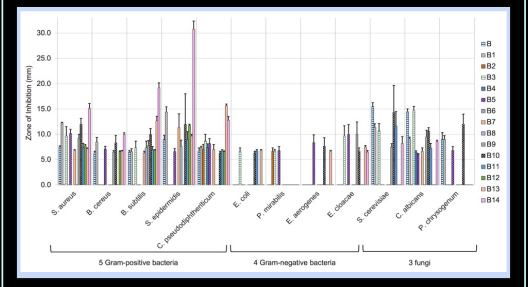


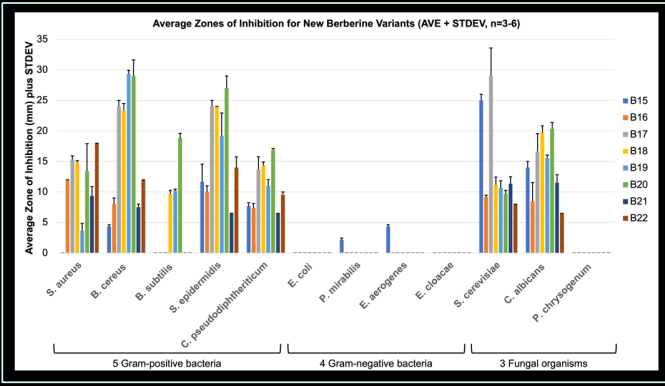
Figure 2. Antimicrobial activities of Berberine variants, as published by our group in 2023 [5].

From this work, we noticed several prominent structure-function trends, such as abolishment of activity when a polar hydroxy group is added to a specific central position within the compound, as well as an increase in activity when a specific methoxy group is lacking (possibly due to steric interactions). Additionally, we noticed an increase in activity against Gram-positive bacteria when the isoquinone ring was partially reduced. Therefore, we decided to explore this trend further via the creation of 8 new berberine variants (B15-B22).

HYPOTHESIS => The partial reduction of the isoquinone ring will result in increased antibacterial activity of Gram-positive microbes.

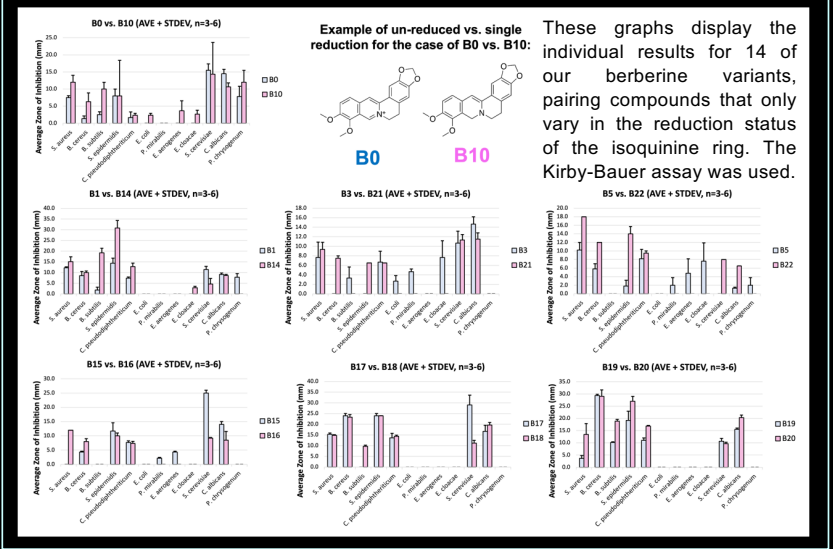
METHODS & RESULTS

Figure 3. Berberine Variant Activity



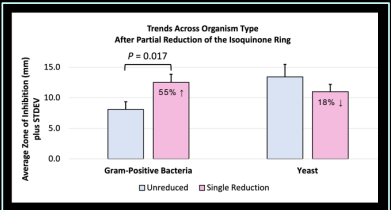
This graph shows the antimicrobial activity of our eight newest berberine variants (B15-B22) against twelve unique microbes. The method used was the Kirby-Bauer disc diffusion assay.

Figure 4. Effect of partial reduction of the isoquinone ring for seven unique berberine variant pairings (un-reduced vs. partial reduction comparisons)



These graphs display the individual results for 14 of our berberine variants, pairing compounds that only vary in the reduction status of the isoquinine ring. The Kirby-Bauer assay was used.

Figure 5. Organismal Trends



All 7 unreduced *vs*. reduced berberine compounds were averaged for all Gram-positive or yeast organisms, and a two-tailed T-test was used to determine statistical significance ($P < 0.05$ cutoff).

CONCLUSIONS & ON-GOING WORK

- Eight new berberine variant compounds were synthesized, and several showed even greater antimicrobial activity than the previous variants (Fig. 3 *vs.* Fig. 2).
- Comparing seven pairings (Fig. 4), our hypothesis that a partial reduction of the isoquinone ring increases Gram-positive antibacterial activity was supported (Fig 5).
- We are exploring potential mechanisms of action, as well as anticancer activities.
- A manuscript is currently being prepared to publish these findings.

REFERENCES

1. Clatworthy *et al.* (2007). Targeting virulence: a new paradigm for antimicrobial therapy. *Nature Chemical Biology*. 3: 541-548.
2. Global Health Estimates 2016: Disease burden by Cause, Age, Sex, by Country and by Region, 2000–2016. Geneva, World Health Organization; 2018.
3. Cowan (1999). Plant Products as Antimicrobial Agents. *Clin. Microbiol. Rev*. 12(4): 564-582.
4. Orozco-Nunnelly, Pruet, *et al.* (2021). Characterizing the cytotoxic effects and several antimicrobial phytocompounds of *Argemone mexicana*. *PLOS ONE*. 16(4): e0249704.
5. Villegas *et al.* (2023). Synthesis and biological evaluation of *Argemone mexicana*-inspired antimicrobials. *Beilstein J. Org. Chem.*. 19: 1511–1524.

This work was supported by a NASA Indiana Space Grant to Drs. Orozco-Nunnelly (Biology) and Pruet (Chemistry)