Antibacterial Properties of *Argemone glauca* Extracts against Oral Bacteria *Streptococcus mutans* and *Fusobacterium nucleatum*

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Abstract

The sap and seed of *Argemone glauca*, a flowering plant in the family Papaveraceae, is endemic to the Hawaiian Islands and has traditionally been used to alleviate toothaches and to prepare for oral surgery. The objective of this study was to investigate the antibacterial properties of *A. glauca* extracts against oral bacteria *Streptococcus mutans* and *Fusobacterium nucleatum* using a Kirby-Bauer disk diffusion susceptibility test. Hexane, methanol, and water were used as solvents during the extraction process of the seeds, stems, and leaves. *Fusobacterium nucleatum* was inhibited by hexane seed and methanol stem extracts, and inhibition was observed in *S. mutans* by hexane seed and stem extracts, and methanol seed extracts. Significantly greater inhibition of *S. mutans* was achieved by the methanol seed extracts (*p = 0.013*) when compared to vancomycin. *Streptococcus mutans* exhibited greater sensitivity to *A. glauca* extracts than that of *F. nucleatum*.

Keywords: *Argemone glauca*, Pua Kala, antibacterial activity, *Streptococcus mutans*, *Fusobacterium nucleatum*, tooth decay, Kirby-Bauer disk diffusion

Introduction

*Argemone glauca*, the pua kala or prickly poppy plant, is endemic to the Hawaiian Islands (Merlin 1995). *Argemone glauca* grows on all main Hawaiian Islands in dry, sunny areas below 1,000 feet in elevation, and rapidly germinates after fires due to heat-resistant seeds (Whistler 1992). Ancient Hawaiians valued *A. glauca* for its medicinal properties (Lincoln 2009). The bright yellow sap applied to warts, cuts and teeth to aid healing and alleviate pain (Scott and Thomas 2000). Brews made from the sap and seeds were used to produce narcotics and analgesics, particularly for oral surgeries (Wood 2006, Lincoln 2009).

Tooth decay is a problem that affects 2.43 billion people worldwide, and if left untreated can lead to pain, infection and tooth loss (Vos et al. 2012, Laudenbach and Simon 2014). Tooth decay is exacerbated through the actions of oral bacteria such as *Streptococcus mutans* and *Fusobacterium nucleatum*, among others. *Streptococcus mutans* adheres to enamel salivary pellicle and produce strong lactic acids which lower the pH and demineralize tooth enamel (Forssten et al. 2010), and *F. nucleatum* invades gingival epithelial cells and induces high levels
of IL-8 secretion, a pro-inflammatory cytokine which destroys tissue and breaks down fibers of
the periodontal ligament (Signat et al. 2011).

Studies performed on extracts of other poppy species, including *Argemone mexicana*, were found to possess antibacterial activities (Rosas-Piñón et al. 2012), as well as anthelmintic, anti-inflammatory, wound healing, antifungal, and anti-HIV activities (Rajvaidhya et al. 2012, Chang et al. 2003). There are no published reports of antimicrobial activities in *A. glauca*. The purpose of this study was to investigate antimicrobial activity in *A. glauca* extracts against the oral bacteria *S. mutans* and *F. nucleatum*.

**Materials and Methods**

The seeds, leaves, and stems of *A. glauca* were collected on the southeast side of Oahu, Hawaii. Plant identification was visually confirmed by Alex Lau, a Research Affiliate with the Bishop Museum in Honolulu, HI. Plant materials were frozen in liquid nitrogen and ground into powder using a mortar and pestle. Hexane, methanol, and water were used as solvents during the extraction process. The extracts were passed through Whatman 1 filter paper using vacuum filtration, and dried with a rotary evaporator (BUSCHI Switzerland). The extracts were re-dissolved in dimethyl sulfoxide (DMSO) to 125 µg/µL and further diluted to 0.05, 0.5, 5, 50 and 100 µg/µL.

*Streptococcus mutans* (ATCC 25175) was grown on brain heart infusion agar at 37°C under aerobic conditions. *Fusobacterium nucleatum* (ATCC 25586) was grown on brucella agar with 5% defibrinated sheep blood at 37°C in an anaerobic gas chamber with a GasPak™ EZ (BD Biosciences) to create an atmosphere of 80% N₂, 10% CO₂, and 10% H₂. The ability of the extracts to inhibit growth of *S. mutans* and *F. nucleatum* was evaluated by a modified Kirby-
Bauer disk diffusion susceptibility test with multiple disks of varying concentrations (Bauer et al. 1966). Extracts (20 µL) were applied to each of six 6 mm paper disks (Microxpress) and allowed to dry at room temperature.

Bacteria colonies were suspended in phosphate buffered saline, adjusted to approximately $1 \times 10^8$ CFU/mL using a 0.5 McFarland turbidity standard and applied to agar plates using a sterile applicator swab to generate a bacterial lawn on their respective agars. Extract and control disks were applied to the agar surface with pure DMSO used for the negative control and vancomycin (30 µg/µL) and kanamycin (50 µg/µL) used as the positive controls for S. mutans and F. nucleatum, respectively. Plates were incubated at 37°C for 24 h and the diameters of the resulting zones of inhibition were measured. Each assay was performed in triplicate and data was analyzed using a one-way ANOVA and post-hoc Tukey’s tests.

**Results**

*Fusobacterium nucleatum* and *S. mutans* were inhibited by *A. glauca* extracts in a dose-dependent manner (Limsong et al. 2004). *Fusobacterium nucleatum* exhibited sensitivity to the hexane seed extract and methanol stem extracts at 125 µg/µL (Figure 1), with zones of inhibition measuring 11 ± 1.2 mm and 12 ± 1.7 mm respectively. Kanamycin exhibited a zone of inhibition against *F. nucleatum* measuring 22 ± 1 mm. *Streptococcus mutans* exhibited sensitivity to hexane seed and stem extracts, and methanol seed extracts at 125 µg/µL (Figure 2), with zones of inhibition measuring 23 ± 3.2 mm, 16 ± 3.5 mm and 30 ± 0.6 mm respectively. Significantly greater inhibition of *S. mutans* was achieved by the methanol seed extracts ($p = 0.013$) when compared to vancomycin’s zone of inhibition of 28 ± 0.6 mm.
**Figure 1.** The *A. glauca* extracts and antibiotic inhibition against *F. nucleatum* with standard deviation.

**Figure 2.** The *A. glauca* extracts and control inhibition against *S. mutans* with standard deviations. * Significantly greater inhibition of the positive control ($p < 0.05$).

**Discussion**

*Argemone glauca* extracts exhibited antibacterial activity against both *S. mutans* and *F. nucleatum*. *Fusobacterium nucleatum* exhibited less sensitivity to *A. glauca* extracts than *S. mutans* which may likely be due to its outer membrane rich in lipopolysaccharides, which act as
a barrier to the penetration of antibacterial molecules (Stevens et al. 1992). The greater
sensitivity in *S. mutans* may suggest that the causative agent affects peptidoglycan, which is
much thicker in gram positive bacteria. The lack of activity in the leaf and water extracts may be
due to the low amounts of sap in the leaves or waters high polarity wasn’t able to produce active
constituents responsible for antimicrobial activity during the extractions (Bharracharjee et al.
2006). *Argemone glauca*’s methanol seed extract showed statistically significant activity by
exhibiting a zone of inhibition greater than that of vancomycin against *S. mutans*.

This study suggests that *A. glauca* contains significant antimicrobial activity and supports
its medicinal use by ancient Hawaiians. Further research should be conducted focusing on the
isolation and identification of the active agents that may be useful in the development of new
pharmaceuticals for the prevention and treatment of tooth decay.

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References


