Immunomodulatory Activity of Sambucus mexicana and Trichostema lanatum on LPS Stimulated RAW 264.7 Macrophage Cells

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Chumash medicinal plants *Sambucus mexicana* (Mexican elderberry) and *Trichostema lanatum* (woolly blue curls) were tested for immunomodulatory activity. Anti-inflammatory effects were determined by treating LPS induced RAW 264.7 macrophage cells with plant extracts and measuring the concentration of cytokines: tumor necrosis factor alpha (TNF-alpha) and interleukin 10 (IL-10). We hypothesized that both plants would exert immunomodulatory activity by reducing the pro-inflammatory production of TNF-alpha or by promoting M2 polarization with a concurrent increase in IL-10 production. At concentration 0.01 mg/mL woolly blue curls and Mexican elderberry demonstrated anti-inflammatory activity by reducing the concentration of TNF-alpha in vitro, while levels of IL-10 were indistinguishable.

The Chumash people, natives of Southern California, used a variety of medicinal flora to treat ailments. Plants, Mexican elderberry and woolly blue curls were recorded to be used to treat infections, cuts, and injuries. We were interested in the immunomodulatory capabilities of these plants given that medicinal plants have a prominent position in drug discovery. The term “immunomodulatory” refers to the ability of a substance to activate, suppress, or regulate one or more functions of the immune system. Anti-inflammatory immunomodulators can be identified by measuring TNF-alpha, a dynamic pro-inflammatory cytokine produced by stimulated classically activated (M1) macrophages. Healing immunomodulators can be identified by measuring the concentration of IL-10, an anti-inflammatory cytokine released by alternatively activated (M2) macrophages. IL-10 down regulates the pro-inflammatory response induced by M1 macrophages, which is essential for angiogenesis and wound repair.

All assays were preceded by a 16 hour incubation and used controls: DMSO at 0.01% and LPS at 0.001 μg/μL. All components were added during the same time period; no pre-treatments. 

**Cell Line:** RAW 264.7 Macrophage cells (ATCC), cultured in DMEM complete with 10% FBS and 1% Antibiotic-Antimycotic. Incubated at 37°C with 5% CO₂. 

**Plant Extraction:** Plants collected from the Santa Monica Mountains were extracted and dried thrice with 100% methanol, rotary evaporation, and centrifugal evaporation. Variables were Mexican elderberry and woolly blue curl extract dissolved in DMSO and added to cells at 0.001 mg/μL and 0.01 mg/μL concentrations. 

**Macrophage Proliferation:** Cell proliferation was measured using CCK-8 (Dojindo Laboratories).

**Cytokine Production:** TNF-alpha and IL-10 levels were measured by collecting supernatants at t=12 and t=24 and performing an ELISA according to the manufacturer (ebioscience.) 

**References**

1. Timbrook, J. *Chumash Ethnobotany: Plant Knowledge Among the Chumash People of Southern California.* (Santa Barbara Museum of Natural History, 2007).