Identification, Pharmacological Screening, and Antivirulent Mechanistic Determination of Fractions and Compounds Isolated from Chondrus crispus and Laminaria digitata Extracts

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The increasing prevalence of drug-resistant bacteria constitutes a multinational health, biosecurity, and economic crisis. As a result, the discovery of new antimicrobial chemotherapeutic agents is a critical scientific endeavor. Squalene, a biosynthetic precursor, was previously structurally characterized with mass spectrometry from a methanolic fraction of Chondrus crispus algal extract eliciting antimicrobial activity on six human pathogens ($p \leq 0.05$ on a student-modified biofilm formation assay and two planktonic cell assays). Notably, squalene was identified as a downregulator of the production of staphyloxanthin, a membrane-bound virulence factor by which S. aureus evades death via reactive oxygen species. Downregulation was demonstrated using a student-created assay that was chemically verified with UV-visible spectroscopic analysis. Oxidant susceptibility assays, at $p \leqq 0.05$, likewise evinced squalene's dose-dependent augmentation of S. aureus free radical susceptibility and microbial death. To elucidate the molecular mechanism of staphyloxanthin downregulation, squalene was docked with staphyloxanthin biosynthesis operon proteins using SwissDock. Squalene was predicted to preferentially bind to crtM (Gibbs Free Energy = -10.50 kJ/mol), a key enzyme in staphyloxanthin biosynthesis. The crtM enzyme was overexpressed in E. coli BH21(DE3) with pET-21a+ and purified via Ni-NTA affinity chromatography, and a specialized crtM assay was utilized to assess the kinetics and potency of squalene's inhibition of crtM activity (inhibition of $p \leqq 0.05$ at non-toxic concentrations). Therefore, squalene, due to these data and its inexpensive production and druglikeness, is suggested to have notable potential for use in an antivirulent therapy for S. aureus.

Awards Won:

Second Award of $2,000