Title: PepSAVI-MS reveals proline-rich antimicrobial peptide in *Amaranthus tricolor*

Abstract:
Traditional medicinal plants are a rich source of antimicrobials; however, the bioactive peptide constituents of most ethnobotanical species remain largely unexplored. Herein, PepSAVI-MS, a mass spectrometry-based peptidomics pipeline, was implemented for antimicrobial peptide (AMP) discovery in the medicinal plant *Amaranthus tricolor*. This investigation revealed a novel 1.7 kDa AMP with strong activity against *Escherichia coli* ATCC 25922, deemed Atr-AMP1. Initial efforts to determine the sequence of Atr-AMP1 utilized chemical derivatization and enzymatic digestion to provide information about specific residues and post-translational modifications. EThcD (electron-transfer/higher-energy collision dissociation) produced extensive backbone fragmentation and facilitated *de novo* sequencing, the results of which were consistent with orthogonal characterization experiments. Additionally, multistage HCD (higher-energy collisional dissociation) facilitated discrimination between isobaric leucine and isoleucine. These results revealed a positively-charged proline-rich peptide present in a heterogeneous population of multiple peptidoforms, possessing several post-translational modifications including a disulfide bond, methionine oxidation, and proline hydroxylation. Additional bioactivity screening of a simplified fraction containing Atr-AMP1 revealed activity against *Staphylococcus aureus* LAC*,* demonstrating activity against both Gram-negative and Gram-positive bacterial species unlike many known short chain proline-rich antimicrobial peptides.