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Quantitative Proteome Analysis and Bioactivity Testing of New Zealand Honeybee Venom

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Abstract : Bee venom, a complex mixture of peptides, proteins, enzymes, and other bioactive compounds, has been widely studied for its therapeutic application. This study investigated the proteins present in New Zealand (NZ) honeybee venom (BV) using bottom-up proteomics. Two sample digestion techniques, in-solution digestion and filter-aided sample preparation (FASP), were employed to obtain the optimal method for protein digestion. Sequential Window Acquisition of All Theoretical Mass Spectra (SWATH–MS) analysis was conducted to quantify the protein compositions of NZ BV and investigate variations in collection years. Our results revealed high protein content (158.12 µg/mL), with the FASP method yielding a larger number of identified proteins (125) than in-solution digestion (95). SWATH–MS indicated melittin and phospholipase A2 as the most abundant proteins. Significant variations in protein compositions across samples from different years (2018, 2019, 2021) were observed, with implications for venom's bioactivity. In vitro testing demonstrated immunomodulatory and antioxidant activities, with a viable range for cell growth established at 1.5-5 µg/mL. The study underscores the value of proteomic tools in characterizing bioactive compounds in bee venom, paving the way for deeper exploration into their therapeutic potentials. Further research is needed to fractionate the venom and elucidate the mechanisms of action for the identified bioactive components.

Keywords : honeybee venom, proteomics, bioactivity, fractionation, swath-ms, melittin, phospholipase a2, new zealand, immunomodulatory, antioxidant
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