Bioactive peptides from some medicinal plants by differential expression using proteomics approach and heterologous expression of corresponding genes

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ABSTRACT
Search for novel antifungal and antibacterial compounds has increased lately due to a remarkable increase in resistance in the microorganisms against the available antimicrobial compounds. Pakistan is the country having altitude range of 0-8611 m, hosting a large number of medicinal plant species. We screened 110 medicinal plants from different regions ranging from Northern area to Cholistan desert for antimicrobial peptides. Plant species were identified by DNA barcoding analysis. Antimicrobial activities of the plants were determined with and without heat/protease treatment to determine which extracts yielded the activities due to peptides. Eleven medicinal plants with peptides having high antimicrobial activities, both antibacterial and antifungal, were selected for differential expression studies using proteomics approach. Proteomes of the plants were characterized by MS. Fractionation of phenol-extracted proteins ensured reproducibility that helped label-free feature-by-feature comparison followed by covariance analysis. LC-MS/MS workup enabled the comparison of observed peptide spectra with known plant genomes. Scaffold analysis revealed several proteins while Progenesis analysis was conducted to resolve some issues that could not be resolved with the scaffold analysis. Conditions were also optimized for differential gel electrophoresis (DIGE) leading to identification of many differentially regulated spots. Generally top-down approach was employed, the peaks correlating with bioactivities were subjected to de novo sequencing. Several AMPs were isolated and sequenced. Based on the results, peptide genes were isolated from RNA isolated from three medicinal plants viz Psoralea corylifolia, Ricinus communis and Silybum marianum using degenerate primers. The genes were cloned and expressed in E. coli and recombinant peptides were characterized for antimicrobial activities against bacterial (Escherichia coli, Staphylococcus aureus, Bacillus subtilis and Pasteurella multocida) and fungal (Fusarium solani, Trichoderma harzianum, Guanoderma lucidum and Alternaria alternate) strains. The recombinant peptide from S. marianum also exhibited strong anticancer activity whereas the other two had moderate activities. Conclusively, the peptides have promise for development of novel antimicrobial and anticancer agents in agriculture and medicine.

Keywords: Proteomics, Bioactive peptides, Mass spectrometry, Medicinal plants, Antimicrobial, Anticancer