

Gel free proteomics and classical isolation of antimicrobial compounds from tropical flowers

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Hospital infection is a health problem of global concern. Due to pathogen resistance to usual therapies, different antimicrobial compounds have been screened in different plants. In the present studied the antimicrobial potential of *Grevillea banksii*, *Tecoma stans* and *Catharanthus roseus* were evaluated. Flowers were macerated with a solution containing 0.6 M NaCl and 0.1% HCl and further precipitated with (NH₄)₂SO₄ (100%) following dialysis 3.5 kDa cut off. The subsequent steps analyses followed two different and complementary directions. Firstly crude extract from three flowers were previously separated in Phenyl-Sepharose CL-4B column and further applied onto a reversed phase semi-preparative HPLC column (Vydac C-18 TP510), in which retained fraction was eluted using a linear acetonitrile gradient (0-100%) at a flow rate of 1.0ml.min⁻¹. These fractions were evaluated against *Klebsiella* sp., *Proteus* sp., *Escherichia coli* and *Staphylococcus aureus*. *C. roseus* exhibited inhibitory activity against *Klebsiella* sp, *Proteus* sp. and *S. aureus*. Moreover, *T. stans* and *G. banksii* diminished *Proteus* sp. and *S. aureus* development, respectively. Furthermore, the crude extract from *C. roseus*, which showed inhibitory activity against microorganism previously reported was subjected to ZOOM® IEF Fractionator. Samples were separated by isoelectric focused and applied in HPLC showing the presence of one major protein fraction between pI 3-4 and further eluted in 27% of acetonitrile. Indeed pI 4-5, 5-6 and 6-7 showed the similar default chromatogram with two fractions eluted in 52% of acetonitrile. Further MS analyses will help us to find protein and peptide functions. In summary, results here reported indicate that two methodologies could be utilized for isolation of peptides and proteins from *C. roseus* flowers with activity toward human pathogens.

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