
Activity of protein-based trypsin inhibitors present in local variety of *Canavalia ensiformis*

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Protein-based trypsin inhibitors isolated from different legumes have been recognized as effective agents for protease targeted therapy against certain diseases including cancers and neurodegenerative disorders. The current study was designed to evaluate trypsin inhibitory activity (TIA) in seeds of local cultivar of *Canavalia ensiformis* (Awara), released from Horticultural Crop Research and Development Institute, Sri Lanka. A concentration gradient (1.25%, 2.5%, 5%, 10% and 20%) of the aqueous seed extract was assessed for TIA using casein as the substrate and the total protein content was estimated by Bradford assay. The protein-based trypsin inhibitors contain in concentration with maximum TIA were fractionated by ammonium sulphate precipitation. It was observed that the 20% seed extract showed the highest TIA ($60.76 \pm 1.30\%$), while 10% and 5% extracts exerted TIA of $40.64 \pm 0.85\%$ and $11.96 \pm 2.76\%$ respectively. The 2.5% and 1.25% concentrations did not show considerable activity. Total protein content of crude extract was 1.45 ± 0.04 mg/ml and accordingly the specific TIA in 20%, 10% and 5% extracts were 41.90, 28.02 and 8.24 % per gram of total protein, respectively. The protein-based trypsin inhibitors fractionated using different ammonium sulphate saturations showed that the highest trypsin inhibitory activity was exhibited by the proteins precipitated using 60% ammonium sulphate saturation ($56.62 \pm 0.81\%$). The protein fractions obtained by 30% and 90% ammonium sulphate saturations exhibited inhibitory activity of $48.51 \pm 2.11\%$ and $36.81 \pm 3.11\%$ respectively. The observed trypsin inhibitory activity in screening and partial purification assays suggest that the seeds of the local cultivar of *C. ensiformis* may contain therapeutically potential protein-based trypsin inhibitors.

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