# STUDIES ON SERINE PROTEASE INHIBITORS IN THE BARK EXTRACT OF *Derris parviflora*

**N.P.A. Hasna**

Postgraduate Institute of Science, University of Peradeinya, Peradeniya, Sri Lanka
Department of Molecular Biology and Biotechnology, University of Peradeinya, Peradeniya, Sri Lanka

Proteases are enzymes that conduct proteolysis by the hydrolytic cleavage of specific peptide bonds in the polypeptide chain of target proteins. Serine proteases are one of the best-characterized families of proteases. Protease inhibitors (PI) are the compounds that inhibit the activities of proteases thus exerting dramatic biological effects. Plant originated protease inhibitors are widely used in research, therapeutic and biotechnological applications. Plant PIs (PPIs) are small molecules, ranged from 8 kDa- 25 kDa in size. *Derris parviflora* is a climbing leguminous plant in which the roots contain rotenone, a strong insecticide and fish poison which is used in fishing. The present study was conducted to investigate the occurrence of serine protease inhibitors in the bark extract of *Derris parviflora* and their properties.

Bark of *Derris parviflora* was homogenized to prepare 5%, 10% and 20% extracts. Serine protease inhibitory activity of the crude bark extract was determined using trypsin as the enzyme and casein as the substrate at pH 7.6 in 0.05 M phosphate buffer. The optimized conditions were used to modify the assay by introducing an additional step to determine serine protease inhibitory activity.

The molecular weight of the inhibitory substance was estimated by dialyzing the crude extract in phosphate buffer (pH 7.6) using a dialysis bag with a molecular weight cut off point of 8 kDa. Partial purification of serine protease inhibitor/s in the bark extract was carried out using DEAE cellulose chromatography.

Assay procedures used to determine the trypsin inhibitory activities for 5%, 10% and 20% crude bark extracts. Among them, 5% crude bark extract showed a significant inhibition, it was optimized for further studies. The optimum volume of the crude extract which exhibited the highest inhibition was 30 µl. There is no reduction in the inhibitory activity when dialyzed, suggesting that the inhibitor/s is a macromolecule greater than 8 kDa. Eluted fractions from DEAE-cellulose column showed a significant inhibitory activity suggesting that inhibitory substance binds to DEAE-cellulose. All saturated fractions obtained from ammonium sulphate precipitations showed significant protease inhibitory activities. This inhibitor seems to be stable at 4 °C.

**Keywords:** *Derris parviflora*, bark extract, serine protease, protease inhibitors, serine protease inhibitory assay, thermal stability