STUDIES ON SERINE PROTEASE INHIBITORS IN THE BARK EXTRACT OF *DERRIS PARVIFLORA*

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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 (PIs) are the compounds that inhibit or modulate 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 generally small molecules, ranged from 8 kDa – 25 kDa in size. Derrisparviflorais 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 Derrisparviflora and their properties. Bark of Derrisparviflora was crushed using liquid N_2 and powdered samples were homogenized using ice cold distilled water to prepare 5%, 10% and 20% extracts. Serine protease inhibitory activity of the crude bark extract was determined using trypsin as the enzyme. Trypsin activity was evaluated using 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 the additional step to determine the 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 the 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 extracts were able to give 51.56%, 58.36% and 60.75% respectively. As 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 30µl. There is no reduction in the inhibitory activity when dialyzed suggesting that the inhibitor/s is a macromolecule with a molecular mass greater than 8 kDa. Eluted fractions from DEAE-cellulose column showed a significant inhibitory activity suggesting that inhibitory substance binds to DEAE-cellulose. Bark of Derris parviflora contains significant serine protease inhibitory activity and the inhibitory substance could probably be a protein or a high molecular weight substance. Further studies on the characterization of this protease inhibitory substance of Derris parviflora is in progress.

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