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Partial purification and characterization of a serine protease inhibitor from the seed extract of *Macroptilium lathyroides*

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Serine proteases carryout a diverse array of physiological functions. They have been shown to associate with many disease processes including cancer and neurodegenerative disorders. Some of the serine proteases inhibitors are targeted as key therapeutic agents in the treatment of various cancers and neurodegenerative diseases. Therefore investigation of natural inhibitors of serine proteases is essential. In this study, a potential inhibitor of serine proteases was isolated from seeds of *Macroptilium lathyroides.*

Mature seeds of *M. lathyroides* were grounded and homogenized in distilled water to obtain the seed extract. An assay procedure was developed to determine serine protease inhibitory activity of the seed extract using trypsin as the serine protease and casein as the substrate. Partial purification of the inhibitor present in the crude seed extract was performed by using DEAE cellulose-52 anion exchange chromatography at 4 ˚C. Thermal stability and the stability at different pHs of the crude extract and partially purified inhibitor were analyzed by incubating at different temperatures (0 - 37 ^{0}C) and over a broad pH range (2 - 10) for 14 days. The percentage remaining inhibitory activity was determined.

A significant inhibitory activity (79 %) was detected in water extracts of the mature seeds. Inhibitory activity of the crude extract was recovered completely during dialysis using a membrane with 12 kDa molecular mass cut off point suggesting that the inhibitor is a macromolecule, presumably a protein. Trypsin inhibitory activity was detected in a single peak in ion exchange chromatography suggesting the presence of a single inhibitor in the seeds of *M.lathyroides*. Activity of the inhibitor was significantly reduced at 25 ^{0}C and 37 ^{0}C with time, i.e. a common feature of most of the serine protease inhibitors. About 80 – 85 % of inhibitory activity remained after incubating inhibitor for 2 weeks at pH 4.0 and 6.0 and 4 ^{0}C. Further studies are in progress to purify, characterize and elucidate the structure of the inhibitor.