

E2-23: Screening and isolation of aspartic proteinase inhibitor from *Calophyllum cordato oblongum*

Senarath B P Athauda¹, W M A P Wanigasekera^{1,2}, Gishanthi P K Marasinghe^{1,2}, H R W Dharmaratne², Kenji Takahashi³

(¹Dept of Biochemistry, Faculty of Medicine, University of Peradeniya

²Institute of Fundamental Studies, Kandy ³Laboratory of Molecular Biochemistry, Molecular Science Division, School of Life Science, Tokyo University of Pharmacy and Life Science, Japan)

Studying of natural inhibitors of aspartic proteinase became important, because it might offer a novel therapeutic approach to control AIDS, malaria, hypertension and other pathological conditions. In this study potential inhibitor of aspartic proteinases was isolated from *Calophyllum cordato oblongum* (Kalukeena) and characterized.

Fresh plant materials were collected from Kanneliya forest. Inhibitory assay procedure was developed by pre-incubating the enzyme (porcine pepsin) with crude extract and determining the remaining activity. Percentage inhibitory activities of crude extracts prepared from powder of root bark, root stem, stem bark, leaves, twigs and buds were 76, 53, 40, 0, 53 and 44 respectively. This suggests the abundance of the inhibitor in root bark.

Inhibitory activity was not lost during dialysis against membrane with molecular cut off point 12,000 kd. Slight loss of activity (30%) was observed during freeze drying of crude extract. $(\text{NH}_4)_2\text{SO}_4$ was not useful to precipitate the inhibitor, because 60 - 70% of inhibitory activity was found in the supernatant even at higher percentages of salts. Inhibitor of crude extract bind to the DEAE-52 anion exchanger at pH 4.5 suggesting that inhibitor molecule has negative charge at the acidic pH. Inhibitor bound to the gel was eluted with linear gradient of pH 4.5 - 7.5 and 0 - 1.0 M NaCl and inhibitory activity was detected in a major peak with a shoulder. Fractions of major peaks were pooled

and concentrated by freeze drying. Concentrated sample was gel filtered on Sephacryl S-200. Inhibitory activity was detected in a peak eluted at the approximate molecular weight of 800,000 kd.

The inhibitor isolated from root bark of *Calophyllum cordato oblongum* was partially purified by successive chromatography on DEAE-52 and Sephacryl S-200. Approximate molecular weight of the inhibitor was 800,000 kd.

Financial assistance by NARESA (Research grant RG/98/C/02) is acknowledged.