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PURIFICATION AND CHARACTERIZATION OF A MAJOR ACID PROTEINASE FROM *NEPENTHES DISTILLATORIA* (BANDURA)

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Plant Aspartic proteinases have little attention in contrast to well-characterized mammalian Aspartic proteinases. Insectivorous plants have evolved the capability to nourish directly on insects and other small invertebrates captured and there by supplement their nutrition. The insectivorous plant *Nepenthes distillatoria* is available plenty in Sri Lanka and will be a good source of proteolytic enzymes. Preliminary studies done with crude *Nepenthes* juice suggest that these enzymes have unique properties. Two acid proteinases (a major and a minor) were identified from *Nepenthes* juice. In this study the major acid proteinase from crude juice was purified and characterized.

The proteinase was purified by elution through successive columns of DEAE cellulose, sephacryl S- 200,Pepstatin Sepharose and Mono Q to a homogenous form or single band at the SDS PAGE. The purified enzyme had a specific activity of 744 units/mg of enzyme towards substrate hemoglobin. The molecular mass of the purified enzyme was estimated to be 45 K Da by SDS PAGE under reducing conditions and 50 K Da by gel filtration. The maximum activity of the enzyme towards denatured hemoglobin was observed at pH 2.0-3.0 at temperatures 50^0-60^0°C. The proteinase activity at the acidic pH was completely inhibited by 0.1mM pepstatin suggesting that it belongs to the family of aspartic proteinases.

The purified enzyme and the crude juice showed a remarkable stability when they were incubated at pH 3.0 and different temperatures (4,25,37,50,60)°C for a period of one month compared to pig pepsin, which is a well-characterized member of the Aspartic proteinase family. Percentage remaining proteolytic activities of crude *Nepenthes* juice incubated for 7 and 30 days at 40°C were 99 & 95, at 25°C were 99 & 92, at 37°C were 99 & 92 and 50°C were 90 & 55 respectively. Percentage remaining activities of purified major proteinase incubated for 7 and 30 days at 40°C were 99 & 96, at 25^0C were 97 & 96, at 37°C were 96 & 90 respectively. Percentage remaining activities of pig pepsin incubated for 7and 30 days at 37°C were 10 and 0 and 50°C were 5 and 0 respectively. These results suggest the relative thermal stability of *Nepenthes* acid proteinase.

Thermally stable acid proteinases have not been reported so far. Observed thermal stability of *Nepenthes* acid proteinase may due to the absence of autocatalytic degradation of proteinase/s in the crude juice(acidic pH) as well as the purified enzyme and it can be stored at room temperature. Further studies are in progress to identify specific applications of this unique thermal stable acid proteinase.