PARTIAL PURIFICATION AND MOLECULAR MASS DETERMINATION OF DEOXYRIBONUCLEAESES FROM BANDURA (*Nepenthes distillatoria)*

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Introduction

There are several carnivorous plants of different genera in nature, which catch prey, mainly insects and digest them by their hydrolytic enzymes and absorb the digested products as nutrients. *Nepenthes distillatoria* is one such carnivorous plant endemic to Sri Lanka, which grows, in South West and Southern parts of the island. Recently Athauda *et al.* (2004) has successfully isolated and structurally characterized two acid proteinases from the pitcher juice of *N. distillatoria.* They have reported the unusual stability of the enzymes as the striking property. Both enzymes were remarkably stable at high temperatures and over a wide range of pHs. In addition to proteinase activity, preliminary investigations by Chin *et al.* (2007) indicated the presence of deoxyribonucleolytic (DNase) activity in the crude pitcher juice of *N. distillatoria.* DNases are important analytical enzymes and have found extensive applications in molecular biology and biotechnology. Hence, further purification of DNases was carried out to determine the molecular weight of each partially purified enzymes.

Materials and Methods

The assay procedure developed by Chin *et al* (2007) for analyzing DNase activity in *N. distillatoria* was used for purification and further studies. Partial purification of DNases present in the crude juice was performed using anion exchange chromatography at 4°C. First, a 100 ml DEAE-cellulose column was equilibrated using 10 mM sodium phosphate buffer at pH 7.0. Then 1.5L of *Nepenthes* crude juice previously equilibrated with the same buffer at pH 7.0 was injected into the column. The column was washed extensively with the same buffer and the bound proteins were eluted with a linear gradient of (0 -1M) NaCl in 0.01M sodium phosphate buffer at pH 7.0. Ninety 10 ml fractions were collected using a fraction collector and assays were performed to determine the DNase activity. Fractions with identical DNase activity were pooled together. In order to determine the pH stability, enzymes were separately incubated in buffers at different pH (from pH 2.0 - 8.0) over a period of two weeks at 4°C. Aliquots were removed at 0, 1, 3, 7, 11 and 14, days intervals and remaining DNase activity of each aliquot was determined. To determine the stability at different temperature, the enzymes were