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PARTIAL PURIFICATION AND CHARACTERIZATION OF AN ACID PHOSPHATASE FROM THE PITCHER FLUID OF *NEPENTHES DISTILLATORIA*

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Introduction

Carnivorous pitcher plants are known to secrete hydrolytic enzymes to digest trapped animals in pitchers, mainly insects, for uptake of nitrogen and phosphorous which are essential elements for plant growth and survival. *Nepenthes distillatoria,* locally known as "*bandura*" is a carnivorous plant endemic to Sri Lanka, and it inhabits areas with high rainfall and sunlight and containing soils that are water-saturated, acidic and deficient in nitrates or phosphates. A recent study on two aspartic proteases in *N. distillatoria* revealed that the proteases possess a remarkable stability at higher temperatures and over a wide range of pH[1]. Preliminary investigations on hydrolytic enzymes in the pitcher fluid showed the presence of acid phosphatases. Acid phosphatases act at an acidic pH optimum by hydrolyzing phosphoric acid monoesters into phosphate ions and molecules with free hydroxyl groups. In this study, we partially purified and characterized an acid phosphatase in the pitcher fluid of *N. distillatoria*.

Materials and Methods

Crude pitcher fluid was collected from open and unopened pitchers of *N. distillatoria* from Hakurugala forest patch at Ruwanwella. For the detection of acid phosphatase activity, the substrate p-nitrophenyl phosphate (pNPP) was used in sodium acetate buffer (pH 4.0). The reaction mixture contained 100μl of 0.5 mM pNPP, 100μl of 0.5 M sodium acetate at pH 4 .0 and 100μl of crude juice. After incubation at 37°c for 1.5 hours, 1 ml of sodium hydroxide was added to terminate the reaction and for the color development. Absorbance of paranitrophenolate released upon phosphatase action on pNPP was measured against a blank, at 405 nm. Control was treated in a similar manner except the crude juice was added after color development and just before measuring absorbance. All purification procedures were performed at 4°C. The collected pitcher fluid was filtered to remove the insoluble material and dialyzed against 0.01 M Tris-HCl at pH 7.5. Dialyzed fluid collected from open and unopened pitchers were fractionated on a DEAE cellulose column (2 cm x 55 cm) equilibrated with 0.01 M Tris-HCl buffer at pH 7.5. The column was eluted using a linear NaCl gradient from 0-1 M NaCl. Fractions with highest phosphatase activity were combined and the enzyme was further purified using a Sephacryl S-200 gel filtration column (3 cm x 65 cm). The purity of the enzyme was determined using SDS-PAGE. To characterize the