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Detection and preliminary characterization of glycosidase/s from *Nepenthes distillatoria*

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The principal nutritional requirements of carnivorous plants are largely held in the prey in organic form. In order to break complex molecules down to their simpler, absorbable components most carnivorous plants secrete hydrolytic enzymes. *Nepenthes distillatoria* is one such carnivorous plant endemic to Sri Lanka. In this study, we partially characterized glycosidase activity detected in the crude pitcher fluid of *N. distillatoria.*

Crude juice of pitchers of *N. distillatoria* was collected from Hakurugala forest patch at Ruwanwella, filtered and stored at -20˚C until use. Preliminary investigation for glycosidase activity was conducted by incubating a 2.5% glycogen solution with 100 µl of pitcher juice in sodium acetate buffer at pH 5.2. The reaction mixture was incubated at 37˚C for 90 minutes. The reaction was terminated by the addition of 1% 2, 4 - dinitrosalicylic acid followed by heating up to 100˚C for 10 minutes. Then a solution 40% K-Na tartarate was added to stabilize the products. The amount of glucose produced by the digestion of glycogen substrate was determined by measuring the absorbance at 540 nm. Optimum pH and temperature for glycosidase activity were determined by incubating the reaction mixture at different pHs and at different temperatures. Crude pitcher fluid was incubated at different pHs and temperatures separately over a period of two weeks. Aliquots were removed at different time intervals and the remaining glycosidase activity of each aliquot was determined by carrying out the standard assay procedure.

A low, but detectable glycosidase activity was observed in the crude *Nepenthes* pitcher juice. The optimum pH for glycosidase activity was pH 5.5. The optimum reaction temperature was obtained as approximately 40°C. The glycosidase activity in the pitcher juice is not stable for more than a week even at 4˚C. Moreover, glycosidase activity in crude juice was not stable over a broad pH range as anticipated. However, they are stable when stored in pH 2.0 at 4˚C. At basic pHs beyond pH 8.0, complete loss of activity was observed within a day. This observation is in accordance with the pH of the crude juice, which is extremely acidic in nature. Further studies are necessary to precisely determine the nature of these enzymes.

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