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CHARACTERIZATION OF ASPARTIC PROTEINASE INHIBITOR FROM *SPONDIAS PINNATA*

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Study of natural inhibitors of aspartic proteinases have become very important and essential. They were identified as very important therapeutic target points in the control of AIDS, Malaria and Hypertension. In this study potential inhibitor/s of aspartic proteinases were isolated and partially characterized from *Spondias pinnata.*

Fresh stem bark of *Spondias pinnata* was ground at room temperature and at -70 °C by using liquid Nitrogen. Crude extract of powder was prepared in H_2O. Assay procedure to determine inhibitory activity of aspartic proteinase was developed by using porcine pepsin as the enzyme and denatured hemoglobin as the substrate. Percentage inhibitory activity of crude extract prepared by grinding at different temperature did not differ significantly. Inhibitory activity of crude extract was not changed significantly during incubation at 37°C and at cold temperatures. This suggests the relative stability of inhibitory constituents in crude extracts at room temperature and subsequent studies were done at room temperature. But extract prepared from oven drying lowered activity compared to the fresh sample. This suggests the less stability of constituents in crude extract at higher temperatures.

60% of the inhibitory activity of crude extract was lost during dialysis against a membrane with molecular cut off point of 12,000 Da. But 100% of inhibitory activity was recovered by using a dialysis tube with cut off point of 3000. This result suggests that the inhibitory molecules have molecular weights between 3000-12.000. The inhibitor was partially purified using Diethyl amino ethyl cellulose-52 chromatography, Ammonium sulfate precipitation and Q sepharose chromatography. In DEAE-52 chromatography, inhibitor molecules were eluted with linear gradient of 0.1 M NaCl in 0.02 M phosphate buffer, pH 7.0. Inhibitory activity was detected in 2 peaks eluted at 0.2 M, 0.4 M NaCl suggesting the presence of two inhibitor molecules in the crude extract with different charges.

pH stability of inhibitor was analyzed by incubating the crude extract at different 'pH's (pH 2.0,3.5,4.0 and 5.0) and determining remaining inhibitory activity. Relatively higher stability of inhibitor at acidic pH was observed. Further highest pepsin inhibitory activity was observed at pH 2.0. Purification and further characterization of the two inhibitory molecules are in progress to elucidate the structure of the inhibitors.