E2-05 Isolation of aspartic proteinase inhibitor from *Spondias pinnata*

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Recently proteinases belonging to the family of aspartic proteinase were identified as therapeutic target points in the control of AIDS, malaria and hypertension. Therefore investigation of natural inhibitors of aspartic proteinase is very important. In this study, one potential inhibitor of aspartic proteinase was isolated and partially characterized from *Spondias pinnata*.

Fresh stem bark of *Spondias pinnata* was ground at room temperature (25°C) and under liquid nitrogen (-70°C) and extracted in 0.02M phosphate buffer, pH 7.0. Assay procedure to determine inhibitory activity of aspartc proteinase was developed by using porcine pepsin as the enzyme and denatured haemoglobin as the substrate. Percentage inhibitory activities of crude extract prepared by grinding at 25°C and -70°C were 30 and 76, respectively. Inhibitory activity of crude extract was not changed significantly during incubation at room temperature or at 37°C. This suggests the relative stability of inhibitor at room temperature and subsequent studies were done at room temperature.

Inhibitory activity of crude extract was lost completely during dialysis against membrane with molecular cutoff point 12,000 kd suggesting that molecular size of the inhibitor is less than 12,000 kd. Inhibitory activity was not lost during freeze drying. Crude extract was applied to a column of anion exchanger DEAE-52 cellulose equilibrated in 0.01M phosphate buffer, pH 7.0. Inhibitor was eluted with linear gradient of 0-1M NaCl in the same buffer. Inhibitory activity was detected in a major and a minor peak suggesting the possibility of the presence of two inhibitor molecules in the crude extract. Pooled fractions of two peaks were concentrated by freeze drying and gel filtered on Sephadex G-10, separately. Volume of elution of major and minor peaks from Sephadex G-10 column differ significantly.

These results suggest presence of two aspartic proteinase inhibitors in the crude extract of *Spondias pinnata* with different charge and molecular size.

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