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Partial purification and characterization of inhibitors of aspartic proteinase from stem bark of *Spondias plannata*

Natural inhibitors of aspartic proteinases are very important as they are identified as likely therapeutic target point in the control of ADIS and malaria. Isolation of inhibitory activity from stem bark of *S. pinnata* was reported earlier. In this study potential inhibitors of aspartic proteinases were partially purified and characterized.

Fresh stem bark samples wee ground and extracts were prepared in distilled water at 4 ^{o} C it shows 76/mL inhibitory activity for 60 mg/mL of crude protein extract. A crude extract was applied to a column of DEAE-52, equilibrated with 0.02M phosphate buffer at pH 7, and the bound material was eluted with a linear gradient of 0-1M NaCl in the same buffer. Inhibitory activity was detected in two peaks eluted at 0.02 M (a minor, 45% inhibitory activity). Fractions of the two peaks were collected separately and concentrated with ammonium sulphate at 80% saturation. Seventy- seven percentages and sixty-five percentage inhibitory activities were recovered in the pellets of the major and minor constituents respectively. The resulting pellets were dissolved in 0.02M phosphate buffer at pH 7, separately applied into Sephadex G-75. Major and minor inhibitory activities were eluted at 230 and 180 mL from Sephadex G-75 column and

their approximate molecular weights were 10 KD and 18 KD respectively. Gel filtered sample was further purified with Q-Sepharose chromatography at ph 7. Purified major inhibitor was analyzed on SDS polyacrylamide gel elctrophpresis (15%) with silver staining and a single band appeared at 10kD. Inhibitory activity of the major peak (partially purified, protein concentration of 0.2 mg/mL) is 30.5/ mL. Inhibitory activity for the partially purified minor inhibitor is 33.5/ mL for the same protein concentration.

pH stability of the inhibitor was investigated by incubating the crude exteract at different pH values (pH2-12) at 4 ^{o}C, 26 ^{o}C and 37 ^{o}C for two months and determining the remaining inhibitory activity. Inhibitor was stable from pH 2 to 7at temperatures 4 ^{o}C to 37 ^{o} C, but at alkaline pH the inhibitory activity decreases. When the crude extracts made in pH 2, 5, 7 buffers were incubated at -20 to 80 ^{o} C. inhibitory activity didn't change significantly, suggesting higher thermal stability of inhibitors.