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SUM

ISOLATION, PARTIAL PURIFICATION AND CHARACTERIZATION OF INHIBITORS FOR ASPARTIC PROTEINASES FROM *SPONDIAS PINNATA*

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ISOLATION, PARTIAL PURIFICATION AND CHARACTERIZATION OF INHIBITORS FOR ASPARTIC PROTEINASES FROM *SPONDIAS PINNATA*

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Proteinases are involved in a wide variety of physiological and biological processes. Inhibitors of serine, cystein and metallo proteinases have been isolated from animal, plant and bacterial organisms and have been well characterized. But relatively, few studies have been reported on inhibitors of aspartic proteinases. Aspartic proteinases participate in a variety of physiological processes and pathological conditions such as hypertension, gastric ulcers, gastric cancers and inflammation.

Recently aspartic proteinases were identified from human immunodeficiency virus, malaria and filarial parasites. Aspartic proteinases participate in the processing of viral precursor protein to form mature structural and functional protein in AIDS, hemoglobin breakdown in malaria and as functional enzyme in filarisis and hence identified as therapeutic target points in the control of the above disease conditions. Therefore investigation of natural inhibitors of aspartic proteinases is very important and essential. The objective of this study was isolation, characterization and purification of inhibitors for aspartic proteinases.

Assay procedure to screen inhibitors of aspartic proteinases was developed by using 0.02 mg/ml porcine pepsin as the aspartic proteinase and 2.5 % denatured hemoglobin as the substrate. In this study 50 medicinal plants used in Ayurvedic medicine were collected from available places in Sri Lanka and screened for inhibitors of aspartic proteinases. Highest inhibitory activity was detected in water extracts of *Spondias pinnata* and it was selected for further investigations. Extracts prepared from fresh stem bark of *Spondias pinnata* showed highest inhibitory activity than other plant parts.

Fresh stem bark of *Spondias pinnata* was ground at 4^0C and water extracts were prepared. Purification of the extract revealed presence of two types of DEAE cellulose bound aspartic proteinase inhibitors in crude extract of stem bark. The major inhibitor compound contributes 55% inhibitory activity and minor inhibitory compound contributes 45 % inhibitory activity to the total inhibitory activity. Both inhibitor compounds were purified to near homogeneity using anion exchange chromatography with DEAE cellulose at pH 7, ammonium sulphate precipitation with 70 % saturation, gel filtration with Sephadex G- 75 at pH 7 and anion exchange with Q Sepharose at pH 7. Purification fold and yield obtained after the affinity chromatography step, were 1100 times and 10.5 % respectively for major inhibitor compound and 1505 times 8.5% respectively for minor inhibitory compounds.

Inhibitors obtained at each step of purification were analyzed using biomolecular identification tests such as Molisch's test, Sudan III test, SDS PAGE with coomassie blue staining and silver staining, trypsin digestion, HCl digestion and UV spectrum. Major and minor inhibitory compounds were identified as short peptides according to trypsin and acid digestions, UV spectrum and SDS PAGE banding pattern. Molecular weights of major and minor inhibitors were found as 10 and 14 KD according to gel filtration chromatography. Study on pepstatin sepharose chromatography of major and minor inhibitors and incubation with pepsin at pH 4 revealed binding of inhibitor to the active site of pepsin. Increase in K_m with increasing inhibitor concentrations suggested competitive inhibition. K_i values of the major and minor inhibitors were 0.53 μM and 0.6 μM respectively.

Temperature dependency and pH dependency of inhibitor reactions and inhibitor stability were studied. The inhibitors were thermally stable between 0 ^{0}C - 97 ^{0}C in crude extract and for purified inhibitors stability was observed between 0 ^{0}C - 60 ^{0}C. The inhibitors functioned optimally between pH 2 to 7.