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PARTIAL PURIFICATION OF ASPARTIC PROTEINASE INHIBITOR/S FROM *TARENA ASIATICA*

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Aspartic proteinases are an important family of enzymes associated with pathological conditions such as hypertension (renin), gastric ulcers (pepsin), neoplastic disease (cathepsin D and E), AIDS (HIV proteinase) and malaria (plasma pepsins). At present there is a profound interest in aspartic proteinases, since the human immuno deficiency virus proteinase and malarial parasite proteinases are targeted as the key therapeutic intervention points in the treatment of AIDS and malaria respectively. In this respect investigation of natural inhibitors of aspartic proteinases is of extreme importance. Yet few studies have been reported on natural inhibitors of aspartic proteinases compared to the other three classes of proteinases, serine, cysteine and metalo proteinases. This study deals with the purification of inhibitor/s of aspartic proteinases from the fruit of *Tarena asiatica.*

The crude extract of fruit of *Tarena asiatica* was prepared by homogenizing 10g of powder obtained by grinding the air dried fruit of *Tarena asiatica* in 50ml of distilled water and then centrifuging for 30 minutes at 6000 rpm. The resulting pellet was re-extracted five times in water and the resulting supernatants were combined. Inhibitory activity was determined by carrying out developed inhibitory assay procedure using denatured haemoglobin as substrate and pig pepsin as enzyme. The inhibitor/s was incubated with the pig pepsin for 10 minutes at 37°C and the remaining pepsin activity was determined. There was significant inhibitory activity in all five extracts. (69,74,16,15,and 25 units per milliliter respectively.)

Inhibitory activity did not change on incubation of crude extract at room temperature or at 37°C for two weeks. This indicates that the inhibitor/s is relatively stable at these temperatures. Therefore subsequent studies were carried out at room temperature. Inhibitory activity of crude extract was completely recovered on dialysis using a membrane with a molecular cut off point of 12kDa. This suggests that the molecular weight of the inhibitor/s is more than 12000Da. The inhibitor was partially purified using TCA precipitation, ammonium sulfate precipitation, and CM cellulose chromatography.

The inhibitor/s binds to cation exchanger CM cellulose 52 column at pH 7.5. This indicates that the inhibitor/s bears a positive charge at neutral pH. The bound inhibitor/s was eluted with a linear gradient of 0 to 1M NaCl in 0.02M phosphate buffer at pH 7.5. Three major peaks with inhibitory activity were detected. This suggests the presence of three inhibitor molecules in the crude extract Further studies are in progress to purify, characterize and to elucidate the structure of the inhibitors using chromatographic and spectroscopic methods.