ISOLATION AND CHARACTERIZATION OF A TRYPSIN INHIBITOR FROM SEEDS OF *Tamarindus indica*

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ISOLATION AND CHARACTERIZATION OF A TRYPSIN INHIBITOR FROM SEEDS OF *Tamarindus indica*

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Tamarindus indica is native to Africa and Asia. This study was carried out to purify trypsin inhibitors present in tamarind seeds and to characterize them in terms of their molecular masses, pH and thermal stability and the effects of metal ions on their activity.

First, the assay procedure was developed to determine trypsin inhibitory activity using *Tamarindus indica* crude seed extract. This developed assay procedure was used throughout the study, to investigate the trypsin inhibitory activity. Significant trypsin inhibitory activity (75%) was detected in aqueous seed extract of *Tamarindus*, and an inhibitor of trypsin was partially purified by CM-cellulose ion exchange chromotograpy followed by Gel filtration chromotograpy using sephacryl S-200. The purity of the inhibitor was assessed by SDS-PAGE.

The molecular mass of the purified inhibitor was determined by SDS PAGE to be 23 kDa or 31 kDa. It was found to be 25 kDa using gel filtration chromotograpy.

Thermal stability studies on inhibitor indicated that it is not stable for a long time above 4 ^{o}C. The remaining inhibitory activity of the trypsin inhibitor at room temperature and 37 ^{o}C were 56% and 53% after ten days of incubation, respectively. More than 83% of activity remained after 10 days at 4 ^{o}C in pH 7.6.

At pH 7.6, the isolated trypsin inhibitor showed highest activity. But even at pH 2.0 the inhibitor showed a significant activity toward trypsin.

The effect of metal ions on the activity of partially purified inhibitor was analyzed using the same assay procedure. Its activity was enhanced by the presence of Zn^{2+}. However, the activity seem to be unaffected by the presence of Ca^{2+} and Mg^{2+} ions. Results indicated that for crude juice, the optimum concentration of Zn^{2+} was 5 mM and it resulted 42 % enhancement of activity. This inhibitor seem to be stable at 4°C and at neutral pHs. Further investigations are necessary to determine the activity of this inhibitor on other serine proteases.