

## Exploiting the Biological Roles of the Trypsin Inhibitor from *Inga Vera* Seeds: A Multifunctional Kunitz Inhibitor

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**INTRODUCTION:** Plant peptidase inhibitors exhibit many biological activities that have attracted much interest, especially in agricultural and health sciences. **OBJECTIVES:** In this work, we described the purification, biochemical and biological properties of a trypsin inhibitor from *Inga vera* seeds (IVTI). MATERIAL AND **METHODS:** The IVTI was purified by ion-exchanged chromatography. The Inhibitory activity of IVTI was determined against bovine trypsin and chymotrypsin by measuring hydrolytic activity against BApNA and BTpNA, respectively. The stability studies were performed over a range of temperature, pH and concentration of the reducing agent DTT. The IVTI amino acid sequence was obtained by MALDI-TOF-MS/MS. The biological activity of inhibitor was investigated against insect pests, pathogenic microorganisms and cell lines. RESULTS AND DISCUSSION: Based in amino acid sequence, IVTI showed homology to Kunitz inhibitor family. Moreover, biochemical analysis revealed that IVTI is composed of a single 20 kDa polypeptide chain with one disulfide bridge, inhibited bovine trypsin at 1:1 molar ratio displaying a dissociation constant of 1.15 nM. The inhibitor was stable over a wide range of temperature, pH and DTT. The IVTI also inhibited the trypsin-like enzymes from lepidopteran pests midgut, such as Anagasta kuehniella (89%), Spodoptera frugiperda (83%), Corcyra cephalonica (80%), Heliothis virescens (70%) and Helicoverpa zea (64%). Furthermore, bioassays against A. kuehniella demonstrated that IVTI affected the larval development by impairing the weight gain and the survival rate, as well extending the larval stage. The microbiological assays demonstrated that IVTI was fungicidal to Candida buinensis and bacteriostatic agent against Escherichia coli. Further assays revealed that IVTI was a chemopreventive agent against human epithelial colorectal adenocarcinoma cells, reducing cell viability by 70% at 200 µg/mL. **CONCLUSION:** Based in these findings, we hope to contribute with a better understanding regards the potential of Pls, as well their application in pest control and therapeutic agents development.

Keywords: Antimicrobial; Anticancer and Bioinsecticidal.

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