Isolation, Purification and Properties of a New Kunitz-type Tripsin Inhibitor from *Piptadenia moniliformis* and its Effects Over Hemostasis.


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Coagulation is the main phenomenon in the maintenance of hemostasis. Functionally, it is mainly composed by serine proteases and their inhibitors. Distribution of proteinase inhibitors in living beings is ubiquitous. In plants they're represented by a multifunctional arsenal whose performance concerns defense, nutritional reserves and regulation. Common in legumes, serine protease inhibitors, are the most studied group and its main representative members are the Kunitz-type inhibitors. This study aimed to purify an inhibitor from seeds of Catanduva tree (*Piptadenia moniliformis*), determinate its physicochemical properties and enzymatic specificity and evaluate its interference over hemostasis. The Kunitz-type trypsin inhibitor was purified by TCA precipitation, Trypsin-Sepharose chromatography and reversed-phase HPLC. The inhibitor, termed PmTKI, showed a Mr of 19.2 kDa as determined by SDS-PAGE. PmTKI was a competitive inhibitor with Ki value of $1.5 \times 10^{-11}$ and $3.0 \times 10^{-4}$ mM against trypsin and chymotrypsin, respectively. Trypsin inhibition was stable in the pH range 2-12, and had an 80% residual activity at a temperature of 100 ºC. Also, PmTKI presented variable inhibition to human neutrophil elastase, pancreatic elastase, papain and pancreatic kallikrein. N-terminal sequence had identity with members of the Kunitz-type inhibitors from Mimosoideae and Caesalpinoideae subfamilies. The Cephalin-Kaolin Time was prolonged (dose-dependent), while no change in Quick Time (aPTT and PT tests, respectively) or the activity of FXa was showed. PmTKI showed no cytotoxicity to human red blood cells. These results indicate PmTKI as a potential candidate for drug development in the therapy of coagulation disorders and hemostasis.


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