Identification of protease inhibitors in seeds extracts of *Crotalaria spectabilis*

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**Introduction.** Legumes of the genus *Crotalaria* contain about 550 species dispersed in tropical regions of the world. Many their compounds have been isolated had deleterious effect against bacteria, insects and parasites, among then protease inhibitors. **Objectives:** Characterization of protease inhibitor activity in seed extracts of *Crotalaria spectabilis*. **Material and Methods.** Fresh seeds were homogenized in distilled water using a blender, and the supernatants obtained after centrifugation were lyophilized for the production of different extracts: CS-ST and CS-SP extracts were obtained using Tris-HCl and phosphate buffers respectively. Protein content was estimated by Bradford method and SDS-PAGE analysis was carried out to analyze protein profile. The activity on trypsin, papain and pepsin were determined with 10µg of protein of the extracts. Both extracts were fractionated by molecular exclusion chromatography using Sephadex G-75. Fraction II obtained from CS-SP was submitted to a trypsin-agarose affinity chromatography. Structural studies of the active fractions were performed using mass spectroscopy. **Results and Discussion.** The inhibitory activity on trysin and pepsin was higher for CS-ST than CS-SP, however both extracts inhibited completely the papain activity. Chromatograms of Sephadex G-75 were very similar but the inhibitory activity had distinct molecular weights: A 47kDa fraction of CS-SP preserved important inhibitory activity against trypsin and fractions of about 59-50kDa and 3-2kDa exhibited papain inhibitory activity. For CS-ST, fractions below 10 kDa were able to inhibit trypsin, papain and slightly pepsin activity. Analysis of the trysinized fractions CS-ST FII and CS-SP FI by mass spectrometry (LC/MS-Orbitrap) revealed the presence of homologous proteins of phylogenetically closely related plants, for example, *Medicago truncatula*. **Conclusions.** These results suggested that seed extracts of *C. spectabilis* are valuable sources of serine, cystein and aspartic protease inhibitors. Purification and characterization of new inhibitors is paramount for understanding the basic molecular mechanisms of plant natural defense against the action of proteases activity.

**Key words:** protease inhibitor, *Crotalaria spectabilis*, seeds extracts

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