

PURIFICATION AND CHARACTERIZATION OF A BIFUNCIONAL KUNITZ TYPE INHIBITOR FROM *PITHECOLOBIUM DUMOSUM* AND ITS EFFECT *IN VITRO*. ON INSECT PESTS

Oliveira, A.S.^{1,2}, Pitanga, J.C.M.¹, Ribeiro, J.K.C.¹, Macedo, L.L.P.¹, Migliolo, L.¹, Aquino, R.O.¹, Coelho, F.P.³, Sanches, A.P.C.³, Bemquerer, M.P.⁴, Santos, E.A.¹; Kiyota, S.³, Sales, M.P.¹

1. Laboratório de Química e Função de Proteínas Bioativas, Depto de Bioquímica, UFRN, Natal-RN; 2. Depto de Bioquímica e Biologia Molecular, Centro de Ciências, UFC, Fortaleza-CE; 3. Laboratório de Bioquímica de Proteínas e Peptídeos, CPDS, Instituto Biológico, São Paulo, SP; 4. Depto de Bioquímica e Imunologia, ICB, UFMG, Belo Horizonte-MG

A serine-papain proteinase inhibitor was purified from Jurema Branca (*Pithecolobium dumosum*) seeds. The purification procedure involved precipitation with trichloroacetic acid, affinity chromatography on trypsin-Sepharose, and reversed-phase HPLC. The inhibitor was called JBI and analysis by SDS-PAGE showed that it is a protein with a single polypeptide chain of 20 kDa. JBI had a competitive inhibitory activity against trypsin with K_i 1.65×10^{-8} M and non-competitive activity against papain with a 5.07×10^{-7} M. The inhibitory activity *in vitro* against *Callosobruchus maculatus*, *Zabrotes subfasciatus*, *Plodia interpunctella*, *Alabama argillacea* and *Ceratitis capitata* enzymes was 70%, 74%, 48%, 14% and 70%, respectively. In conclusion the inhibitor purified JBI showed properties of serine proteinase inhibitors from Kunitz family with additional inhibitory activity on papain and its insecticidal properties indicate its role in plant defense against insect pests.

Supported by: CAPES, CNPq, FINEP and FUNDECIBNB