

PURIFICATION OF A NOVEL CYSTEINE PROTEASE INHIBITOR FROM THE
Crotalaria pallida ROOTS.

Andrade, L.B.S.^{1,2}, Oliveira, J.T.A.², Kiyota, S.³, Oliveira, A.S.^{4,2}, Cunha, A.G.², Sales,
M.P.⁴

¹Universidade Estadual Vale do Acaraú; ²Depto de Bioquímica e Biologia Molecular,
Centro de Ciências, UFC, Fortaleza, CE; ³Laboratório de Bioquímica de Proteínas e
Peptídeos, CPDSA, Instituto Biológico, São Paulo, SP; ⁴LQFPB, Depto de
Bioquímica, UFRN, Natal, RN;

In plants, cysteine protease inhibitors (CPI) are involved in the regulation of protein turnover and play an important role in resistance against insects and pathogens. Several plants have been screened to isolate and characterize such proteinases inhibitors, among them the genus *Crotalaria*, which belongs to the Fabaceae family. *Crotalaria* species have high agronomical and agricultural potentials. These leguminous plants develop a high resistance to the main species of root-knot nematodes. Extracts of the *Crotalaria pallida* leaves and roots have been shown to contain CPI activity, but the CPI has not been isolated or characterized. In this work, our objective was to isolate a CPI from *C. pallida* roots. The isolation scheme included extraction, heat treatment, ammonium sulfate precipitation and C₁₈ reversed-phase HPLC. The M_r of this inhibitor is estimated to be ~15 kDa by SDS-PAGE, with high inhibitory activity against papain. The purified inhibitor was found to be heat-stable when subjected to boiling (100 °C) at neutral pH. This is the first CPI purified from the root of a leguminous and could indicate the potential of control to root-knot nematode.

Supported by CNPq, CAPES, FINEP and BNB-FUNDECI

Keywords: cysteine protease inhibitors, *Crotalaria pallida*, root-knot nematode