Identification and partial purification of a cysteine proteinase inhibitor from *Calotropis procera* latex.

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Latex is remarkably common in plants. Nearly 8% of all plant species have pressurized canal systems from which latex is exuded upon damage. However, few laticifer plants are studied in details, mainly biochemical aspects of their latex. The role played by latex in plants is poorly understood. The hypothesis more accept is its involvement in plant defense against insects and phytopathogens. In this study, *Calotropis procera* latex showed insecticidal activity at concentrations as low as 0.1% on *Callosobruchus maculatus* (Coleoptera: Bruchidae). Proteomic approaches of 2-D electrophoresis and analysis by MALDI-TOF-TOF allowed identification of nine Pathogenesis-Related proteins in latex: peroxidase (1), chitinases (2), cysteine proteinases (2), proteinase inhibitor (1) and osmotin (3). Laticifer proteins of *C. procera* were active on *C. maculatus* larvae even after heat treatment at 98 °C for 30 min. This fraction was able to inhibit papain and gut proteases of *C. maculatus*, confirming the presence of a cysteine proteinase inhibitor identified by mass spectrometry. Two peaks were obtained when laticifer proteins were subjected to affinity chromatography on a papain-sepharose column. The retained proteins (PII) by the matrix were eluted after adding 50 mM Glycine buffer pH 10.0 and presented strong inhibitory activity to papain. PII was represented by two protein bands with insecticidal activity. These results support the hypothesis of the involvement of cysteine proteinase inhibitors in plant defense against insects.

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