

THE FIRST OSMOTIN PURIFIED FROM LATEX: BIOCHEMICAL CHARACTERIZATION; BIOLOGICAL ACTIVITY AND ROLE IN PLANT DEFENSE.

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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. Nonetheless, 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 accepted is its involvement in plant defense against insects and phytopathogens. In this study, *Calotropis procera* latex inhibited both, fungi germination and growth at concentrations as low as 100 and 20 µg/mL to *Rhizoctonia solani*, respectively. 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). The latex proteins were fractionated and a highly purified protein was obtained. The N-terminal sequence (40 AA) exhibited similarity to osmotin (87 %) and thaumatin-like (82 %) proteins. Its isolation procedures entailed two ion exchange chromatography steps. *C. procera* osmotin (CpOsm) appeared as a single band (20.1KDa) in SDS-PAGE and as two spots in 2-D electrophoresis (pI 8.9 and 9.2) identified by mass spectrometry as two osmotins. The two isoforms were glycoproteins as indicated by Schiff's reagent. The CpOsm exerted antifungal activity against *Fusarium solani*, *F. oxysporium*, *Rhizoctonia solani* and *Colletotrichum gloeosporioides* and values of IC<sub>50</sub> were within the 9.8-28.8 µg/mL. The deleterious effects of latex on these phytopathogens reinforce the hypothesis of the involvement of laticifer fluids in plant defense against fungi and indicate *C. procera* latex to be a source of promising fungicidal proteins.

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