ISOLATION AND BIOCHEMICAL CHARACTERIZATION OF A SERINE PROTEASE FROM THE LATEX OF *EUPHORBIA MILII*

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A wide range of important applications have been reported for *Euphorbia milii*, a flowering plant of the family Euphorbiaceae. The latex of this plant has been used to control mollusks and is also frequently used in traditional medicine against liver fluke, schistosomiasis in sheep, cattle, and even humans. The latex contains many valuable alkaloids such as β-sitosterol, euphol, euphorbol, euphorbol hexacosanoate, and a potent antileukaemic macrolide-lasiodiplodin. However, the proteins and other biochemical constituents of the latex have not been investigated in detail. The present work intended to isolate the peptidases from the latex of E. Milii. We collected latex by cutting the stem, following several purification steps: 1.Precipitation in organic solvent (Acetone), 2.Gel filtration of Sephacryl S-100, 3.Cation exchange chromatography. Three fractions were obtained and analyzed by SDS-PAGE in a 12,5% gel, using silver nitrate for staining. The proteolytic assays were performed using 1% casein as a substrate, measuring the absorbance at 280nm after 20 minutes. Preliminary data showed that the latex from *E. milii* has an active serine peptidase with a molecular mass (estimated by SDS – PAGE), around 60 kDa. Optimum pH and temperature of the enzyme were pH 7.5 and 55ºC, respectively. This enzyme retains full proteolytic activity over a wide range of pH (5.0-12) and temperature (up to 86ºC). The activity is inhibited by serine and cysteine protease inhibitors like PMSF, PCMB, DTNB, but not by EDTA. The data indicate that it is a serine protease like proteinase K.
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