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Partial Purification of Multiple Proteases from the Latex of *Ficus heterophylla* L.f. using Hydrophobic Interaction Chromatography

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Abstract

Proteases are biologically important hydrolytic enzymes that catalyze the cleavage of peptide bonds in proteins and have wide physiological and biotechnological significance. Plant latex, a milky secretion produced by laticifer cells, represents a rich natural source of diverse proteolytic enzymes. In this study, hydrophobic interaction chromatography (HIC) was employed for the resolution and partial purification of multiple proteases present in the latex of *Ficus heterophylla* L.f. Crude enzyme extract was prepared from latex collected from the plant fruits and subjected to ammonium sulfate fractionation up to 1.5 M saturation. The resulting supernatant was applied onto a Phenyl Sepharose 6 Fast Flow (high sub) column pre-equilibrated with 0.05 M sodium acetate buffer (pH 5.0) containing 1.5 M ammonium sulfate. Bound proteins were eluted using a stepwise decreasing ammonium sulfate gradient (1.0, 0.8, 0.7, 0.6 and 0 M). Multiple chromatographic fractions exhibiting proteolytic activity were obtained. The presence of multiple proteases in the crude latex extract was confirmed by casein zymography, while SDS-PAGE and zymographic analyses of the HIC fractions demonstrated effective separation and partial purification of the enzymes. Protease activity was quantified using azocasein as the substrate, and protein concentration was determined by the modified Lowry method. Overall, the study demonstrates that step-gradient HIC is an efficient strategy for resolving latex proteases from *Ficus heterophylla* L.f., providing a basis for further biochemical characterization.

Keywords: Proteases, Latex, Ammonium sulfate, Phenyl Sepharose, SDS-PAGE, Zymography

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