

INVESTIGATION OF THE OCCURRENCE OF PROTEASES IN THE LATEX OF *Calotropis gigantea*

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Proteases are ubiquitous biomolecules that play a crucial role in regulating protein activity in all living organisms. Dysregulation of protease functions gives rise to various pathological conditions, including cancer, neurodegenerative diseases, and cardiometabolic diseases. There is a growing interest in identifying proteases from latex-bearing plants, as they can be used in therapeutic and biotechnological applications. Giant milkweed, a medicinally important plant native to Sri Lanka, is utilised in Ayurvedic medicine for its antivenomous, antibacterial, and antifungal properties. Although previous studies suggest that giant milkweed latex contains proteases, detailed investigation into their occurrence and protease activity (PA) remains limited. This study aimed to investigate the presence of naturally occurring proteases in the latex of giant milkweed. Latex samples were collected from mature, flowering-stage plants, and a crude extract was obtained. A PA assay was successfully developed and optimised to achieve the maximum PA by varying different parameters, including temperature, pH, incubation time, substrate concentration, and crude concentration, using a one-factor-at-a-time approach. Subsequently, three different protease inhibitors were used to evaluate their inhibitory effects on the crude proteases. Inhibitors E-64, Pepstatin, and Leupeptin showed minimal, moderate, and strong inhibition, respectively. This suggests the presence of cysteine, aspartic, and serine proteases in the latex, with serine protease contributing more to the overall protease activity. Furthermore, the crude proteases were fractionated using ammonium sulfate precipitation, and a caseinolytic activity assay was performed. PA progressively increased from fraction 1 to 3 (0 – 60)%, followed by a decline from fraction 4 to 5 (60 – 90)%. Fraction 3 (45 – 60)% showed a peak activity, indicating that there is a significant protease enrichment in this fraction compared to other fractions. Further studies are required to determine the overall protease profile of the latex and the variation of the protease profile under different growth stages and climatic conditions.

Keywords: Ammonium sulfate precipitation, *Calotropis gigantea*, Latex proteases, Protease activity