10th Brazilian Conference on Natural Products
XXXVI RESEM
4-7 November 2025, Belo Horizonte, MG, Brazil

Section: 01

PRODUCTION AND CHARACTERIZATION OF PROTEASE INHIBITORS OBTAINED FROM CALLUS CULTURE OF Bauhinia holophylla

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Preservation processes are essential for the conservation of plant species. Among these preservation techniques, *in vitro* cell and tissue culture stands out, as it enables the production of significant amounts of bioactive compounds in a short period of time, avoiding impacts of plant extractivism. For instance, callus and cell suspension cultures can be employed to produce a wide variety of bioactive compounds, including flavonoids, cardenolides, lectins, and protease inhibitors (PIs). PIs are particularly sought after for their broad biological potential against bacteria, viruses, insects, and tumor cells. In legume seeds, these compounds are abundantly expressed, representing about 1–10% of the total soluble protein (TSP) content. *Bauhinia holophylla* (Fabaceae: Cercidoideae) is a native plant from Cerrado and to date, the *in vitro* production of PIs from this species has not been reported in the literature. Therefore, this project aimed to produce and characterize protease inhibitors from callus cultures of *B. holophylla*. Callus cultures from leaf explants were established using MS (Murashige & Skoog) and WPM (Wood Plant Medium) medium. The influence of the plant growth regulators 2,4-dichlorophenoxyacetic acid (2,4-D) and 6-benzylaminopurine (BAP), at different concentrations on callus induction, TSP and PI content were also assessed. The obtained inhibitors were purified by crude protein precipitation, followed by clarification and molecular exclusion chromatography (SEC). Protease inhibitory activity was evaluated using trypsin inhibition assay with the substrate BAPNA (α-N-benzoyl-DL-arginine-p-nitroanilide hydrochloride). The TSP content was determined using the Bradford method. For characterization, denaturing gel electrophoresis was performed using Biorad® system. In crude protein precipitate, Kunitz-type inhibitors (approximately 20-21 kDa) were identified. Combinations of 2,4-D and BAP in WPM medium appear to promote higher total soluble protein (TSP) content, more efficient callus induction, and increased production of protease inhibitors (PIs) in *B. holophylla* callus. The authors thank the support from UFSJ and FAPEMIG. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001.

Keywords: *Protease inhibitors, Callus culture, Bauhinia holophylla, In vitro plant preservation, Cerrado.*