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Optimisation of enzyme-assisted extraction of camptothecin from Nothapodytes nimmoniana and its characterisation

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Abstract

Introduction: Efficient extraction of camptothecin (CPT), an anticancer agent from the commercial source Nothapodytes nimmoniana (J. Graham) Mabb in India, is of paramount importance. CPT is present in the highest concentration in the stem portion, and the stem can be readily harvested without uprooting the plant. The fluorescence microscopy mapping of the bark matrix for CPT revealed its presence in a free form within both the outer (epidermal and cortical tissues) and inner (xylem and phloem tissues) sections. The bark matrix primarily consists of cellulose, hemicellulose, and lignin, rendering it woody, rigid, and resistant to efficient solvent penetration for CPT extraction. We proposed a hypothesis that subjecting it to disruption through treatment with hydrolytic enzymes like cellulase and xylanase could enhance solvent diffusion, thereby enabling a swift and effective extraction of CPT.

Objective: The present study was aimed at enzyme-assisted extraction, using cellulase and xylanase for hydrolytic disruption of the cells to readily access CPT from the stem of the plant N. nimmoniana (J. Graham) Mabb.

Methodology: The hydrolytic cell disruption of ground powder from the stem bark was studied using cellulase and xylanase enzymes. The enzymatically pretreated stem bark powder was subsequently recovered by filtration, dried, and subjected to extraction with methanol to isolate CPT. This process was optimised through a Box-Behnken design, employing a one-factor-at-a-time approach to assess parameters such as enzyme concentration (2-10% w/w), pH (3-7), incubation time (6-24 h), and solid-to-solvent ratio (1:30-1:70 g/mL). CPT was characterised using proton nuclear magnetic resonance (^{1}H-NMR) and Fourier transform infrared (FTIR) spectra, and a high-performance liquid chromatography (HPLC) method was developed for quantification.

Results: The cellulase and xylanase treatment resulted in the highest yields of 0.285% w/w and 0.343% w/w, with efficiencies of 67% and 81%, respectively, achieved in a significantly shorter time compared to the untreated material, which yielded 0.18% with an efficiency of only 42%. Extraction by utilising the predicted optimised process parameters, a nearly two-fold increase in the yield, was observed for xylanase, with incubation and solvent extraction times set at 16 and 2 h, respectively. Scanning electron microscopy (SEM) images of the spent material indicated perforations attributed to enzymatic action, suggesting that this could be a primary factor contributing to the enhanced extraction.

Conclusion: Enzyme-mediated hydrolytic cell disruption could be a potential approach for efficient and rapid isolation of CPT from the bark of N. nimmoniana.

Keywords: Nothapodytes nimmoniana; camptothecin; cellulase; enzyme-assisted extraction; response surface methodology; xylanase.