

Optimization of CTAB DNA Extraction Protocols for Downstream Applications of *Salacia Sp.*

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Abstract

Salacia spp. belong to the family Celastraceae, with some species native to Sri Lanka and listed as endangered or near threatened on the National Red List 2012 of Sri Lanka. The higher content of secondary metabolites in this plant hindered the extraction of sufficient quantities of good-quality DNA for downstream processes like PCR and DNA sequencing. Therefore, this research aimed to optimize the DNA extraction protocol to obtain a high amount of high-quality DNA from *Salacia sp.* in the absence of liquid Nitrogen. DNA was extracted using sixteen different methods fewer than two main protocols based on the CTAB miniprep procedure. Three replicates were maintained for each method. The quality and quantity of extracted DNA were measured using a nano spectrophotometer and verified by electrophoresis. The best protocol for obtaining high-quality DNA from *Salacia sp.* involves the following steps: Immature, healthy leaf samples were collected and stored at -80°C. The frozen samples were ground using a pre-cooled mortar and pestle. The ground material was then mixed with a preheated (60°C) CTAB buffer containing PVP and incubated for 30 minutes. After incubation, the mixture was centrifuged at 13,000 rpm for 10 minutes, and the supernatant was treated with 500 µL of chloroform, followed by another centrifugation at 13,000 rpm for 10 minutes. This extraction step was repeated. The resulting supernatant was mixed with absolute ethanol and ammonium acetate and stored at -20°C overnight. The next day, the mixture was centrifuged at 13,000 rpm for 15 minutes. The pellet was washed with 70% ethanol, dried, and dissolved in ultra-pure water. This protocol effectively yielded the highest DNA concentration ($0.636 \pm 0.339 \mu\text{g}/\mu\text{L}$), with an A260/A280 ratio of 2.008 ± 0.041 , indicating minimal contamination among the sixteen different samples. A distinct DNA band was observed on a 0.8% agarose gel using that method, confirming the quality of DNA. Therefore, this modified method successfully enabled the isolation of good-quality DNA from *Salacia sp.* with a considerable yield.

Keywords: CTAB, DNA extraction, downstream applications, *Salacia sp.*, secondary metabolites