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Optimization of a cost-effective DNA extraction protocol and PCR conditions to amplify *rbc*L marker in *Rhinacanthus* species

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Abstract
Rhinacanthus is an important genus that belongs to the family Acanthaceae. Species in this genus are used in traditional medicine to treat a variety of disease conditions. *Rhinacanthus flavovirens*, a recently discovered endemic herb in Sri Lanka known as *Maha Anitta* in traditional medicine, has lately been brought up for its medicinal properties and molecular level identification. For molecular biological investigations of an organism, it is necessary to extract pure, high molecular weight genomic DNA. However, the presence of high levels of polysaccharides and polyphenols in plants of this genus interferes with pure DNA isolation and downstream reactions such as PCR amplification. The primary aim of this study was to optimize the cost-efficient protocols for DNA isolation and PCR, to attain optimal amplification of selected primers using R. flavovirens. A comparison was made between the CTAB-based DNA extraction method, with some modifications, and the SDS-based approach. The DNA extraction using a modified CTAB method, which included 2M NaCl, 2.5% CTAB, 3.0% β-mercaptoethanol, and 4.0% PVP, resulted in a DNA yield of 79.43ng/L. The A260/A280 value was 1.940, indicating minimal levels of contamination. The PCR program was optimized to produce robust and reproducible amplification products using universal plant barcoding primers from the *rbc*L region by modifying the temperature and time intervals during denaturation, annealing, and elongation. An initial denaturation at 94°C for 3 min followed by 30 cycles at 94°C for 30 sec, annealing at 50°C for 30 sec and extension at 72°C for 1 min, followed by one final extension at 72°C for 10 min produced optimal amplification. PCR results showing high intensities of amplification also indicate that the extracted genomic DNA was of good quality and uncontaminated by interfering substances. The findings of this study demonstrate that the improved protocol for DNA isolation and PCR can be used to facilitate future research on molecular identification, genetic diversity analysis, and phylogenetic studies of *R. flavovirens*, as well as the development of conservation strategies for this rare and endemic species in Sri Lanka and other *Rhinacanthus* species.

Keywords: Cetyltrimethylammonium Bromide (CTAB), Polymerase Chain Reaction (PCR), *rbc*L, Rhinacanthus

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