

Molecular Identification of Endemic Medicinal Herb *Rhinacanthus flavovirens* based on Chloroplast DNA Barcodes

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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. However, the presence of similar morphological characters makes identification and recognition of endemic *R. flavovirens* from *R. nasutus* difficult at an early stage. Therefore, the objective of this study was to construct an efficient DNA barcode that could discriminate *R. flavovirens* from the closely related species, *R. nasutus*. In this study, we used the chloroplast DNA marker *rbcL* to distinguish these two species. As genomic DNA from *Rhinacanthus* leaves indicated contamination of secondary metabolites that prevented PCR, this study attempted to optimize CTAB-based and SDS-based DNA isolation protocols. The CTAB method with few modifications yielded high-quality DNA in sufficient quantities for PCR. The PCR program was optimized to produce robust and reproducible amplicons using specific primers from the *rbcL* region and subsequently the PCR products were sequenced through Sanger sequencing. The *rbcL* sequence of *R. flavovirens* was compared with the *rbcL* sequences of *R. nasutus* retrieved from NCBI GenBank, and the efficacy of barcoding was evaluated. The results revealed only 2 variable sites between *R. flavovirens* and *R. nasutus* at *rbcL* marker. The smallest pairwise distances (0.0095) were found between *R. flavovirens* and *R. nasutus* voucher PS0730MT01 and *R. nasutus* CHULA-065. Phylogenetic analysis showed a sister relationship between *R. flavovirens* and *R. nasutus* vouchers, however the complete phylogeny of *Rhinacanthus* species could not be resolved. Overall, our results indicated that the universal barcoding region *rbcL* does not discriminate *R. flavovirens* from its close relative *R. nasutus*.

Keywords: Cetyltrimethylammonium Bromide (CTAB), DNA barcoding, Polymerase Chain Reaction (PCR), *rbcL*, *Rhinacanthus*

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