

## MOLECULAR DISCRIMINATION OF *Rhinacanthus* SPECIES USING *rbcL* MARKER

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### Abstract

*Rhinacanthus* is an important genus belonging to the family Acanthaceae and possesses valuable medicine properties used in traditional medicine for diverse therapeutic purposes. Some species of this genus are endemic to Sri Lanka, emphasizing the urgency of accurate identification for their conservation and future utilization. Therefore, this study aimed to categorize *R. flavovirens* from *R. nasutus* at the molecular level using the *rbcL* gene in the chloroplast. The genomic DNA from *R. flavovirens* leaves was extracted using optimized CTAB protocol and quality DNA was obtained for polymeric chain reaction (PCR). The PCR was performed using a universal primer targeting the chloroplast *rbcL* region (*rbcL*-BF and *rbcL*-724R) and the PCR product was subsequently subjected to Sanger sequencing. The obtained *rbcL* sequence for the *R. flavovirens* was deposited in GenBank under accession no. OQ181218. A comparative analysis was conducted by aligning the *rbcL* sequence of *R. flavovirens* with sequences of *R. nasutus* retrieved from GenBank (accessions GQ436493.1, LC461831.1, and KF381120.1). Results revealed only two variable sites differentiating these two species for *rbcL* marker. The smallest pairwise distance (0.0095) was observed between *R. flavovirens* and *R. nasutus* accessions GQ436493.1 and LC461831.1. While phylogenetic analysis indicated a sister relationship between *R. flavovirens* and *R. nasutus* vouchers, the complete phylogeny of *Rhinacanthus* species could not be fully resolved. Overall, our results indicated that the universal *rbcL* primer we used to be not enough to categorize *R. flavovirens* from its close relative *R. nasutus*. Future studies should explore additional molecular markers to achieve a more accurate delineation of *Rhinacanthus* species. These findings will contribute to understanding of the genetic diversity within this genus, aiding in effective conservation and sustainable utilization of these medicinally significant plant species.

**Keywords:** Endemic, CTAB, phylogenetic relationship, *rbcL*, *Rhinacanthus flavovirens*, *Rhinacanthus nasutus*

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