Unexplored Plant Family (Violaceae) in Sri Lanka; Potential for Bioactive Cyclic Peptides

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Cyclotides are a unique class of ribosomally synthesized cysteine-rich mini proteins. Their compact structure consists of a head-to-tail cyclized backbone stabilized by the knotted arrangement of three conserved disulfide bonds, in the combination known as cyclic cysteine knot (CCK) motif. Due to these unique features that imparts ultra -stability to its structure, cyclotide has attracted interest as peptide-based templates for drug design applications. Although cyclotides have a wide range of biological activities such as antimicrobial, anti-HIV, antineurotensin, cytotoxic and hemolytic activities, their natural bioactivity in plants is thought to be as insecticidal with a role in plant defense. To date, the plant families Rubiaceae, Solanaceae, Fabaceae, Cucurbitaceae and Violaceae have been shown to contain cyclotides. However, until the current study was initiated, the cyclotides distribution in Sri Lankan plant species remained unexplored. This investigation was conducted according to a systematic screening of plants belonging to the family Violaceae for the presence of cyclotides. Gel filtration followed by Liquid Chromatography Mass Spectroscopy (LC-MS) techniques were used to identify the molecular weights of cyclotides in the crude fractions of *Viola* species. Further confirmation of cyclic peptides was carried out by reduction and alkylation reactions. Out of the four plants screened for the discovery of cyclotide masses, presence of cyclotides was confirmed in three *Viola* species. In the LC-MS chromatogram scan range of 400 to 2000 Da, the cyclotides retention times were in the range from 25 min to 40 min with molecular weights detected at $(M+2H)^{2+}$. Our study reinforces that investigating of cyclotides in unexplored geographical locations by a systematic approach guided by ethnomedical evidence is a promising strategy to expand our knowledge on the biodiversity and bioactivities of cyclotides.

Keywords: Medicinal plants, Cyclotides, Violaceae family, LC-MS