



**Bioactive cyclotides and other
secondary metabolites isolated from
selected medicinal plants of
Rubiaceae, Solanaceae and Violaceae
in Sri Lanka**

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Rajendran Sanjeevan
Faculty of Science
University of Colombo
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Abstract

Sri Lanka is an island which possesses high degree of biodiversity. There are about 3,154 indigenous flowering plant species of flora and about 894 of that is endemic to the country. Natural product researchers are interested in bioactive compounds isolated from plant species to treat infectious diseases caused by microorganisms. Several researches have reported that medicinal plants contain bioactive compounds, peptides, alkaloids, terpenes, polyketides, phenols, saponins and flavonoids. Among those peptides, cyclotides have a wide range of biological activities such as antimicrobial, anti-HIV, antineurotensin, cytotoxic, hemolytic and insecticide activity. The plant families Rubiaceae, Solanaceae, Fabaceae, Cucurbitaceae and Violaceae are known to contain bioactive cyclotides. Cyclotides are a unique class of ribosomally synthesized cysteine-rich mini proteins. The structure is formed by a head-to-tail cyclized backbone and three conserved disulfide bonds in a knotted arrangement. The main focus of this study was to identify cyclotides and other bioactive compounds from the plant families Rubiaceae, Solanaceae and Violaceae. Organic and aqueous extracts of the plants were investigated for the presence of bioactive secondary metabolites, with a focus on cyclotides that exhibit anti-microbial and cytotoxic activities.

Plant species were selected based on their ethnopharmacology uses from the families Rubiaceae, Solanaceae and Violaceae were screened for the presence of cyclotides using LC-MS techniques. The late eluting peaks with molecular weights ranging from 2500 to 3300 Da were considered for the presence of cyclotides. Among all plants screened *Geophila repens*, *Viola pilosa*, *Viola betonicifolia* and *Viola hamiltoniana* were positive for the presence of cyclotides. *G. repens* was selected for further study. Aerial dried parts of *G. repens* (140 g) was extracted with 60% aqueous methanol to yield 22 g of extract. LC-MS analysis of this extract showed the presence of about 15 different types of putative cyclotides. Five of the cyclotides present in the mass range 2955.6⁺ Da to 3278.4⁺ Da were purified and sequenced using LC-MS-MS and were tested against microorganisms *Escherichia Coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Candida albicans*. Out of the 5 cyclotides, the cyclotide with the mass 3140⁺ (gere A) was active against all microorganisms tested at 100 µg/mL or concentrations below 100 µg/mL. All five cyclotides were screened for cytotoxicity in the Fluorometric Microculture Cytotoxic Assay (FMCA) using Lymphoma U937 cells. All tested cyclotides showed activity of 100% cell death in the range of 12.5-50 µg/mL. Taken together all these results showed that *G. repens* is a good source of bioactive cyclotides. Furthermore,

distribution of cyclotides presence in the leaves and petiole were analyzed using immunohistochemistry. Antibodies were raised in rabbits using cycloviolacin O2 (cyO2), and their specificity was determined by Western and dot blot experiments. The results showed that cyclotides are distributed mainly in the epidermis region of the leaf of *G. repens*. Endophytic fungi associated in the plant tissues of *G. repens* were also evaluated for their anti-bacterial activities. Four endophytic fungi were isolated and identified. Crude extracts of fungi showed potent activity against Gram positive and Gram negative bacteria.

The presence of cyclotides in the Violaceae family was previously reported. However, it was not studied in the Sri Lanka. To identify the diversity of cyclotide sequences in *Viola betonicifolia*, next generation sequencing (Transcriptomic analysis) was carried out. Interestingly, three known (kalata S, viba 17, viba 11) and 28 new sequences of cyclotides/acyclotides were discovered.

Not only cyclotides, small molecules produce in plants also showed bioactivities. The plants from Rubiaceae (15) and Solanaceae (7) were subjected to micro fractionation protocol followed by antimicrobial and cytotoxicity screening to identify small molecules related to respective activity. Out of all plants screened *Wendlandia bicuspidata* was selected to further studies. The plant (leaf) was extracted with methanol and large scale fractionation carried out using preparative HPLC. The target molecule was purified using bio active guided screening and analytical purification. Structure elucidation using NMR techniques (¹H, ¹³C, COSY, HMBC, HSQC) identified the molecule as the iridoid glycoside class of ixoside and molecular mass was identified using QTOF/mass spectrometry. Although ixoside was identified from other plants, this is the first time of reporting presence of ixoside in *W. bicuspidata*, the plant that is endemic to Sri Lanka.