Antimicrobial secondary metabolites produced in culture by endophytic fungi of Sri Lankan flora

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Abstract

Escalating numbers of multidrug resistant bacterial strains are presently a global issue that needs urgent attention. In addition, the numbers of immuno-compromised individuals who are susceptible to fatal systemic fungal infections are on the rise. Thus there is a compelling reason for the discovery of new and more effective clinically useful antibacterial and antifungal agents. Endophytic fungi are plant symbiotic microbes and they are presently emerging as a valuable and an almost inexhaustible source for the discovery of new antimicrobial substances. Sri Lanka is considered as a biodiversity hotspot with a high degree of endemism. Endophytic fungi inhabiting the Sri Lankan flora represent a unique and almost completely uninvestigated resource for the discovery of new bioactive natural products.

In this study endophytic fungi were isolated using surface sterilized aerial parts and/or roots of 2 endemic plants (*Nymphaea nouchali* and *Dipterocarpus zeylanicus*), an endemic Pteridophyte (*Cyathea walkerae*) and two native plants (*Calamus thwaitesii* and *Munronia pinnata*). The isolated fungi were identified using molecular and morphological data. The ethyl acetate extracts of laboratory cultures of each isolated fungus were screened for antibacterial and antifungal activities by disc diffusion assay. Fungi/fungus with most prominent activity for the antimicrobial bioassay was/were selected for the isolation of active metabolites. Each selected fungus was grown on large scale and bioassay guided fractionation of the crude extracts led to the isolation of the active compound(s). The chemical structure of each active compound was elucidated using high resolution nuclear magnetic resonance and mass spectroscopic data. The minimum inhibitory concentration's of the active compounds were also determined.

From the five plants, 72 endophytic fungal strains were isolated and were identified. Out of these, 34 showed activity against at least one microorganism tested, with many of them showing potent activities against several microorganisms. From the seven endophytic fungi selected (*Mycoleptodiscus* sp. from *C. thwaitesii*, *C. globosum* from *N. nouchali*, *Aspergillus terreus* and *Fusarium langsethiae* from *D. zeylanicus*, *Paecilomyces inflatus* and *Bipolaris* sp. from *M. pinnata* and *Diaporthe helianthi* from *C. walkerae*) for the bioassay guided fractionation, 8 active compounds were isolated. Spectroscopic characterization of these compounds showed that all of them were known. The identities of the active compounds are: mycoleptodiscin B from *Mycoleptodiscus* sp., chaetoglobosin A and C from *C. globosum*, austdiol from *Aspergillus terreus*, zearalenone from *Fusarium langsethiae*, terphynellin from *Paecilomyces inflatus*, cynodontin from *Bipolaris* sp. and dicerandrol B from *Diaporthe helianthi*. However, this is the first record of antimicrobial properties of mycoleptodiscin B, terphynellin and cynodontin.

In conclusion, this research has been proved that endophytic fungi inhabiting on Sri Lankan flora are a valuable source for metabolites with potent antimicrobial activities