

Endolichenic Fungi in Sri Lanka; A Treasure Trove for New Bioactive Molecules

Professor Priyani A. Paranagama

Director, Institute of Indigenous Medicine, University of Colombo
and Chair of Chemistry, Department of Chemistry, University of Kelaniya



The demand for new and safe bioactive compounds from natural resources is increasing due to emergence of new diseases, development of drug resistance to pathogenic bacteria and development of toxic viruses, etc. This situation has forced researchers to explore new natural sources with potent novel bioactive compounds as they continue to deliver a great variety of structural templates for drug discovery and encounter the challenges of the twenty first century. Fungi are an important resource of novel natural bioactive compounds with potential applications in agriculture, pharmaceutical industry and food industry. The first milestone in the field of drug innovation research from fungi was started with the discovery of penicillin, the first natural antibiotic discovered from a fungus, *Penicillium notatum*. Thereafter, exploration of microbial diversity has been encouraged by the fact that microbes are essential for sustainable development of bioactive compounds. Importance in the discovery of new bioactive compounds from fungi has garnered greater attention than that of plants by the fact that fungi can be grown using fermentation methods or solid cultures and need insignificant amounts of raw material indicating that it does not affect the biodiversity of the country. Since over 60% of the approved drugs available in the market are of natural origin, there is a great demand for bioactive secondary metabolites isolated from fungi with unique structural diversity in order to strengthen the drug discovery programs. Examples for isolation of biologically active molecules from fungal extracts with anticancer, antifungal and antibacterial activities are monocillin, radicicole and curvularin. Subsequently, there is a growing interest to identify new fungal species and isolate their secondary metabolites in order to obtain naturally occurring structurally diverse new bioactive compounds.

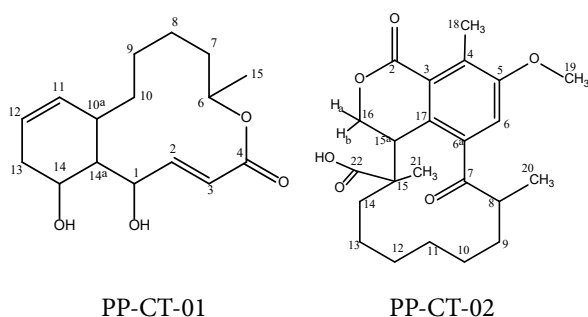
The endolichenic fungi (ELF) are the fungi that

inhabit lichen tissues asymptotically without causing disease symptoms, and their occurrence is similar to the endophytic fungi live within healthy plant tissues. It is evident that ELF are important in producing chemically diverse secondary metabolites with novel anti-inflammatory, antioxidants and anticancer compounds. In the course of the last 11 years, we have isolated ~1500 endolichenic fungi from lichens in tropical forest and mangroves and screened them for their biological activities. Many bioactive compounds have been isolated and structures of the biologically active compounds have been determined. The substances isolated originate from different biosynthetic pathways: isoprenoid, polyketide, amino acid derivatives, and belong to diverse structural groups: terpenoids, steroids, xanthenes, chinones, phenols, isocumarines, benzopyranones, tetralones, cytochalasines and enniatines. The first report of isolation of bioactive compounds from ELF has been published in 2007 stating dehydroherbarin was isolated from an endolichenic fungal strain, *Corynespora sp.* occurring in the cavern beard lichen, *Usnea cavernosa* with significant inhibition of migration of human metastatic breast and prostate cancer cell lines, MDA-MB- 231 and PC-3M, respectively.

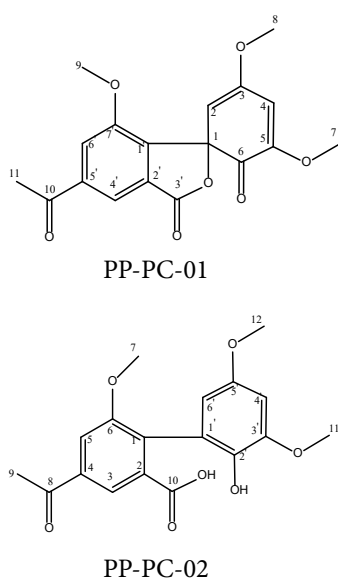
In Sri Lanka, a new research program on searching bioactive compounds from ELF was initiated by myself at Department of Chemistry, University of Kelaniya in 2009. Sri Lankan forests contain higher diversities of many varieties of lichen species such as fruticose, foliose and crustose. *Usnea sp.*, *Parmotrema sp.* and *Pseudocypellaria sp.* are some of the dominant lichen species in the lower elevation (1750 m) of Knuckles forest and Hakgala botanical garden in Sri Lanka were used in the present study to isolate ELF. In our investigations, it was revealed that isolated ELF can utilize substrates such as cellulose, starch, pectin and lignin, and it is indicated that all the fungal strains utilized starch except *Cladosporium sp.* and *Curvularia sp.* The cellulose utilization ability was demonstrated by *Phoma sp.*, *Penicillium sp.* and *Chrysosporium sp.* whereas *Chrysosporium sp.*, *Curvularia sp.* and *Penicillium sp.* showed the ability to produce both polygalacturonase and pectatylase

enzymes. *Broomella sp.* and *Nigrospora sp.* produced the polygalacturonase enzyme whereas *Cladosporium sp.*, *Chrysosporium sp.* and *Phoma sp.* were able to produce pectatelyase enzyme. Only *Broomella sp.* showed the ability to produce all the lignases. Antifungal activities of ELF have been evaluated and it was found that growth of *Fusarium sp.* was inhibited by *Cladosporium sp.*, whereas *Nigrospora sp.* inhibited the colony growth of *Botrytis sp.* and *Colletotrichum sp.* Furthermore, *Chrysosporium sp.* were able to inhibit the colony growth of *Colletotrichum sp.* Similarly, *Chrysosporium sp.* was also be able to inhibit the colony growth of *Fusarium sp.*

Isolation of two new biologically active compounds, PP-CT-01 and PP-CT-02, with anticancer and antioxidant activities were reported from *Curvularia trifolii*, an endolichenic fungal strain in *Usnea sp.* in Sri Lanka.



Ethyl acetate extract of *Penicillium citrinum*, an endolichenic fungus isolated from the lichen, *Parmotreama sp.*, produced two novel polyketides, PP-PC-01 and PP-PC-02 with radical scavenging activity in DPPH antioxidant assay.



Being an island, Sri Lanka has several extensive coastal wetlands rich in mangrove forests covering

approximately an area of 6000–7000 ha. The largest mangrove ecosystem in Sri Lanka is in Puttalam Lagoon covering 3385 ha. Although Sri Lanka is a biodiversity hot-spot, it is reported that prevalence and identification of lichens and microorganisms are understudied. Therefore, this research programme was extended to investigate bioprospecting of mangrove-associated endolichenic fungi. Considering the fact that harsh environmental conditions prevailing in mangrove ecosystems, it was hypothesized that ELF of lichens living in mangrove ecosystems are rich in secondary metabolites. Since Sri Lankan mangrove associated ELF are untouched and no information is available, a study was conducted to evaluate bioactive compounds in ELF and identify the lichen species and to report the fungal diversity, and determine antioxidant, anti-lipase and amylase inhibition activities of secondary metabolites produced by solid cultures of ELF. This was the first comprehensive research on identification, phylogenetic analysis and bioactivity of endolichenic fungi in mangrove ecosystem of Puttalam lagoon in Sri Lanka and the results obtained in this study will provide a starting point for discovering novel bioactive compounds from endolichenic fungi isolated from the lichens collected from mangrove and mangrove associated plants in Pullalam lagoon in Sri Lanka. In this study, identification of about 100 strains of ELF in lichens collected from mangroves was reported. The secondary metabolites produced by each ELF have been assessed for antioxidant activity using DPPH, anti-diabetic activity using amylase inhibition and anti-obesity using lipase inhibition assays. The ethyl acetate extracts of *D. eschscholtzii* and *Sordaria sp.* showed better radical scavenging activity. Hence it is suggested that the extracts with low IC_{50} for the antioxidant assay and the fungal extracts with high anti-lipase activities might be of therapeutic interest with respect to the treatment of obesity and other non-communicable diseases.

In conclusion, endolichenic fungi have become a promising source to isolate novel bioactive compounds and there is a dearth of information on endolichenic fungi. This is the first research programme on ELF in Sri Lanka and young researchers comprising of postgraduate and undergraduate students were trained on isolation and identification of endolichenic fungi and analytical techniques involved in the field of natural product chemistry to isolate bioactive compounds. Therefore, this research program has made a significant contribution to human resource development of the country and thereby provides an impetus to the study of bioactive compounds in endolichenic fungi in Sri Lanka.