Sri Lanka Association for the Advancement of Science

Proceedings of the 69th Annual Sessions

Part I
Abstracts

428/D

Isolation and molecular identification of endolichenic fungi inhabiting in the lichen *Pseudocypherllaria sp.* available in Sri Lanka

F Pary, C Kulasekera, S Wickramaarchchi, P Paranagama*
Department of Chemistry, University of Kelaniya, Kelaniya

Lichens formed by the symbiotic association of fungi and algae, are recently known as hosts for endolichenic fungi that live asymptomatically within the lichen thalli, similar to plant endophytes. Even though their diversity, composition and distribution within and between the host lichens were not comprehensively studied, their role is believed to be important for the succession and evolution of the host. *Pseudocyphellaria sp.* is one of the common foliose lichens found in tropical mountain forests in Sri Lanka. A preliminary investigation of the taxonomy of the endolichenic fungal community, that occur within the particular lichen, was carried out in a recent project. Thalli samples of *Pseudocyphellaria sp.* were collected from the Hakgala strict natural reserve. Endolichenic fungi were isolated from the segments of healthy thallus, according to the surface sterilization technique. Fungal DNA from pure cultures were extracted and the Internal Transcribed Spacer (ITS) region of rDNA was selectively amplified by polymerase chain reaction (PCR). Sequences of amplified DNA were aligned with already existing sequences in Genbank using Basic local alignment search tool (BLAST) algorithm of the National Center for Biotechnology Information (NCBI). The total number of fungal strains isolated from *Pseudocypherllaria sp.* was 18 and the identity of 14 were confirmed by molecular taxonomy. Pure cultures of these identified fungi were vouchered in sterile water, and deposited at the collection maintained by the Department of Chemistry, University of Kelaniya. The presence of endolichenic fungi within the particular lichen and their ability to succeed independently on synthetic growth media, were confirmed by this study. According to the study *Daldinia, Hypoxylon* and *Xylaria* species were the dominants, with two *Daldinia* species, three *Hypoxylon* species and two *Xylaria* species. In addition, *Sordariomycetes, Fusarium, Graphium, Penicillium, Giberella, Aspergillus* and *Paecilomyces* species were also identified. As reflected by this result the endolichenic fungal community that occurs within the *Pseudocyphellaria sp.* shows comparatively high diversity at the genus and species levels. The abundance of such diverse fungal populations within the same lichen species suggests that they may have a defined important ecological role that affects the successful colonization of the lichen.

Keywords: Endolichenic fungi, molecular taxonomy, *Pseudocyphellaria sp.*

Acknowledgements: Financial assistance by NRC (NRC/08/13)

priyani@kln.ac.lk

Tel: 011 2903262

614/E2

Isolation of bioactive metabolites in the endolichenic fungus, *Daldinia eschscholzii*, occurring in the lichen, Parmotrema sp. in Sri Lanka

U Samanthi, S Wickramaarchchi and P Paranagama*
Department of Chemistry, University of Kelaniya, Kelaniya

Since natural products are adapted to a specific function in nature, the search for novel secondary metabolites should concentrate on organisms that inhabit novel biotopes. The potential role of the endolichenic fungus (EF) and its biologically active metabolites in its association with its thalli has been investigated. The aim of this study is to isolate the biologically active compounds of the EF, *Daldinia eschscholzii,* occurring in the lichen, *Parmotrema sp.* at Hakgala montane forest in Sri Lanka. The EF, *Daldinia eschscholzii,* was grown in 64 large PDA plates and incubated at room temperature for two weeks and the secondary metabolites were extracted into EtOAc (6 L). The crude EtOAc extract was then subjected to antioxidant and antifungal bioassays to test the bioactivity. The concentration series of EtOAc extract (10 – 50 µg/mL) was tested with DPPH, $ABTS^+$ and NO radical scavenging assay. The standard synthetic antioxidant, BHT and ascorbic acid were used for the comparison of the results. The ferric reducing power of the crude EtOAc extract was compared with BHT. The antifungal bio assays against *Colletotrichum musae* (isolated from banana) and *Aspergillus flavus* (isolated from rice) were carried out according to the well diffusion method and were compared with the positive control, Bavistin and the negative control, DMSO:MeOH (1:1). The EtOAc extract (500 µg) did not show any significant inhibition of the growth or sporulation of both fungi. Since the crude extract showed high antioxidant activity, it was partitioned with hexane, chloroform ($CHCl_3$) and 60% MeOH and the bioactive fractions were identified using the above antioxidant assays. The antioxidant activity of $CHCl_3$ and MeOH fractions were confirmed and they were further fractionated using bioassay guided column chromatography (silica, sephadex) and preparative TLC to isolate pure compounds. A total of 05 pure compounds were isolated from the $CHCl_3$ fraction. NMR and HRMS spectra of the pure compounds have been obtained and characterization of these compounds is in progress.

Keywords: Antioxidant activity, bioactive, *Daldinia eschscholzii,* endolichenic.

Acknowledgements: Financial assistance by National Research Council (grant NRC-08-13). Prof A. A. Leslie Gunatilaka and Dr. E. M. Kithsiri Wijerathne (University of Arizona) for providing NMR data of the pure compounds in this study

priyani@kln.ac.lk Tel: 112903279

614/E2

Isolation of bioactive metabolites in the endolichenic fungus, *Daldinia eschscholzii*, occurring in the lichen, Parmotrema sp. in Sri Lanka

U Samanthi, S Wickramaarchchi and P Paranagama*
Department of Chemistry, University of Kelaniya, Kelaniya

Since natural products are adapted to a specific function in nature, the search for novel secondary metabolites should concentrate on organisms that inhabit novel biotopes. The potential role of the endolichenic fungus (EF) and its biologically active metabolites in its association with its thalli has been investigated. The aim of this study is to isolate the biologically active compounds of the EF, *Daldinia eschscholzii,* occurring in the lichen, *Parmotrema sp.* at Hakgala montane forest in Sri Lanka. The EF, *Daldinia eschscholzii,* was grown in 64 large PDA plates and incubated at room temperature for two weeks and the secondary metabolites were extracted into EtOAc (6 L). The crude EtOAc extract was then subjected to antioxidant and antifungal bioassays to test the bioactivity. The concentration series of EtOAc extract (10 – 50 µg/mL) was tested with DPPH, $ABTS^+$ and NO radical scavenging assay. The standard synthetic antioxidant, BHT and ascorbic acid were used for the comparison of the results. The ferric reducing power of the crude EtOAc extract was compared with BHT. The antifungal bio assays against *Colletotrichum musae* (isolated from banana) and *Aspergillus flavus* (isolated from rice) were carried out according to the well diffusion method and were compared with the positive control, Bavistin and the negative control, DMSO:MeOH (1:1). The EtOAc extract (500 µg) did not show any significant inhibition of the growth or sporulation of both fungi. Since the crude extract showed high antioxidant activity, it was partitioned with hexane, chloroform ($CHCl_3$) and 60% MeOH and the bioactive fractions were identified using the above antioxidant assays. The antioxidant activity of $CHCl_3$ and MeOH fractions were confirmed and they were further fractionated using bioassay guided column chromatography (silica, sephadex) and preparative TLC to isolate pure compounds. A total of 05 pure compounds were isolated from the $CHCl_3$ fraction. NMR and HRMS spectra of the pure compounds have been obtained and characterization of these compounds is in progress.

Keywords: Antioxidant activity, bioactive, *Daldinia eschscholzii,* endolichenic.

Acknowledgements: Financial assistance by National Research Council (grant NRC-08-13). Prof A. A. Leslie Gunatilaka and Dr. E. M. Kithsiri Wijerathne (University of Arizona) for providing NMR data of the pure compounds in this study

priyani@kln.ac.lk Tel: 112903279