
K.A.U. Samanthi¹, S. Wickramaarachchi¹, E.M.K. Wijeratne², P.A. Paranagama¹

Secondary metabolites of fungi have become an important point of study in the search for novel biologically active small molecules. Although many secondary metabolites are not involved directly in the growth or reproduction of the fungus, it is believed that they play key roles in regulation of vital metabolic activities in an organism. It has been reported that many fungal secondary metabolites exhibit wide range of biological activities with diverse novel chemical structures which can be developed as new pharmaceutical drugs or agrochemicals. Today, there is a great demand for new natural sources of novel bioactive secondary metabolites. Recently, endolichenic fungi (ELF) that live asymptotically within the thalli of lichens have been identified as a rich source of bioactive compounds. One of the ongoing research programs carried out in the University of Kelaniya is isolation and identification of novel bioactive metabolites of endolichenic fungi available in Sri Lankan lichen species. The objective of the present study was isolation and identification of secondary metabolites in the endolichenic fungus, *Penicillium citrinum* isolated from the lichen *Pamotrema sp.* In this research *P. citrinum* was cultured in 50 large potato dextrose agar (PDA) plates and incubated at room temperature for two weeks. The secondary metabolites were extracted into ethyl acetate and evaluated using DPPH antioxidant assay. They were further separated using bioassay guided fractionation in order to isolate active pure compounds. The results of this study led to the isolation of a novel bioactive compound, 5'-acetyl-3,5,7'-trimethoxy-3'H-spiro[cyclohexa[2,4]dione-1,1'-isobenzofuran]-3',6-dione with moderate antioxidant activity (IC₅₀ = 159.6 ± 22.3 µg/mL). This compound was identified using ¹H, ¹³C NMR, 2D NMR and MS data. Biosynthesis of this compound was evaluated and it was revealed that it is originated from a heptaketide via condensation, reduction, oxidation, decarboxylation and methoxylolation to form the final polyketide product.

*Key words: Antioxidant activity, endolichenic fungi, secondary metabolites, Penicillium citrinum*

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¹ Department of Chemistry, Faculty of Science, University of Kelaniya, Sri Lanka. priyani@kl.edu.lk

² SW Center for Natural Products Research and Commercialization, School of Natural Resources and the Environment, College of Agriculture and Life Sciences, University of Arizona, United States.