Antioxidant and antifungal activities of secondary metabolites of the endolichenic fungus, *Penicillum pinophilum* isolated from the lichen *Pseudocypherllaria sp.* available in Sri Lanka.

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Since the bioactivity directed exploration of secondary metabolites from endolichenic fungi is a most recent trail for the discovery of effective pharmaceuticals, the objective of this study is to isolate secondary metabolites from endolichenic fungus *Penicillum pinophilum* and to investigate their bioactivity. *Penicillum pinophilum* was isolated from the lichen genus; *Pseudocypherllaria,* available in Haggala mountain forest, Sri Lanka and its identity was confirmed using morphological characters and molecular identification (DNA sequencing). *In vitro* large scale cultures of the particular fungus were prepared, inoculating a spore suspension of it into Petri dishes containing potato dextrose agar medium and incubating at 30 ^{0}C (room temperature) for 14 days, for the extraction of secondary metabolites into ethyl acetate. The crude extract was then subjected to two types of bio assays, for the investigation of antifungal and antioxidant activities. Antifungal activity assays were carried out according to the well diffusion method, using 500 ì L of a 1 mg/mL crude EtOAc extract dissolved in DMSO/methanol 1:1 mixture, with a positive control of Bavistin and DMSO/methanol 1:1 mixture as a negative control. Both of the antifungal activity assays; one against a common banana pathogen *Colletotrichum musae* and the other against an aflatoxin producing fungus *Aspergillus flavus* , did not show any prevention or a significant inhibition of the growth of pathogens by the test extract . Antioxidant activity of the same extract was explored by carrying out DPPH free radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay, varying the concentration (100-500 ì g/ mL) of the extract and the results were compared with the activity of the standard antioxidant, BHT (Butylated Hydroxy Toluene) (figure 1).

As it was positive for both assays, the extract was partitioned into hexane, chloroform and aqueous methanol fractions and the antioxidant activity of each fraction was tested using above two assay methods (figure 02).

Antioxidant active methanol and chloroform fractions were subjected to further purification through chromatographic techniques and nine pure compounds were isolated. One of the pure compounds (CK/01/47/02) with strong antioxidant activity shows that the molecular weight 391 and indicates that it is a dimer. ^{1}H, ^{13}C NMR, HSQC, HMBC and DQF-COSY spectra of those compounds are being analyzed for elucidation of structure.

DPPH assay FRAP assay

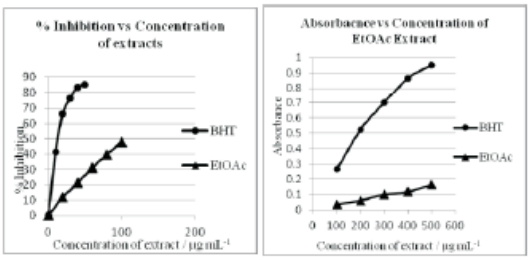

Figure 01. Antioxidant activity of ethyl acetate extract of *Penicillium pinopilum with* DPPH free radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay

DPPH assay FRAP assay

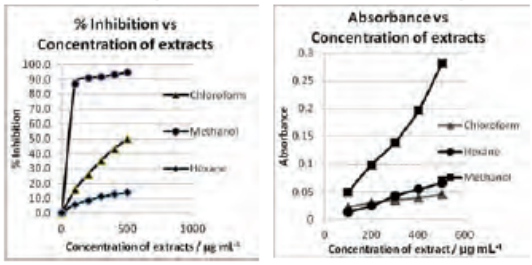

Figure 02. Antioxidant activity of hexane, chloroform and methanol extracts of *Penicillium pinopilum with* DPPH free radical scavenging assay and Ferric Reducing Antioxidant Power (FRAP) assay.

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