

ISOLATION, IDENTIFICATION AND BIOACTIVITY OF
ENDOLICHENIC FUNGI FROM *Usnea* SPECIES AVAILABLE IN
HAKGALA AND KNUCKLES MONTANE FORESTS



By

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ABSTRACT

It has been proved that endolichenic fungi are new sources for novel bioactive natural products with anticancer, antimicrobial, anti-inflammatory, antioxidant and insecticidal activities. Hence the present study was focused on searching for antifungal and insecticidal activity of endolichenic fungi isolated from lichen *Usnea* sp. at Hakgala and Knuckles montane forests.

Fungal isolations were carried out using surface sterilization method. Preliminary identification was performed using standard identification keys following the sticky tape method and the molecular identification was carried out using gene sequencing method. The mean percentage frequencies of occurrence were calculated using standard equations. Total of 21 endolichenic fungal species, were isolated from the lichen *Usnea* sp. from Hakgala and Knuckles forests. *Nodulisporium* sp. (19%) and *D. eschscholzi* (21 %) were isolated at highest frequency of occurrence from Hakgala and Knuckles forests respectively.

The secondary metabolites of the fifteen endolichenic fungi were extracted into EtOAc and antifungal activity of the EtOAc extract of each endolichenic fungi were tested against the pathogenic fungus *Colletotrichum musae* which causes anthracnose disease in banana using filter paper disk method. The insecticidal activity of the EtOAc extracts were also evaluated using the test insect, cowpea bruchid, *Callosobruchus maculatus* using Residual Film Bioassay and Treated Seed Bioassay. The bioactive EtOAc extracts were partitioned using hexane, CHCl_3 and 80% MeOH. In all cases, the CHCl_3 fraction showed the bioactivity against antifungal assay or insecticidal assay, therefore the active fractions were further

separated using silica gel column chromatography or reverse phase column chromatography or preparative TLC.

The bioactive fractions isolated from the endolichenic fungi, *C. trifoli*, *N. sphaerica*, *S. fimicola* and dark sterile sp. 3 exhibited the highest antifungal and insecticidal activity. The extract of *A. fumigatus* showed only a higher antifungal effect and *D. eschscholzi* showed only a higher insecticidal effect. Hence results of the present study revealed that endolichenic fungi isolated from the lichen, *Usnea* sp. are potential sources of bioactive compounds and this study can be extended to isolate and identify pure bioactive compounds present in the endolichenic fungi.

Key words

Lichens, Endolichenic fungi, Antifungal, Insecticidal, Secondary metabolites