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## Bioactivities of ethyl acetate extracts isolated from endolichenic fungi *Daldinia eschscholzii* and *Nigrospora sphaerica*

Although numerous bioactive natural products occur in endolichenic fungi, only a limited number of secondary metabolites with a variety of bio-activities have been isolated during the past few years. The objective of this study was to evaluate the potential of ethyl acetate extracts of endolichenic fungi *Daldinia eschscholzii* and *Nigrospora sphaerica* inhabiting the lichen, *Usnea* sp. from Hakgala montane forest using antifungal and insecticidal properties.

Endolichenic fungi *D. eschscholzii* and *N. sphaerica* were extracted with ethyl acetate (EtOAc) to obtain the secondary metabolites. Antifungal effects of these extracts were tested against *Colletotrichum musae*, which cause anthracnose disease in banana. Residual Film Bioassay (RFB), and Treated Seed Bioassay (TSB) were carried out to evaluate the insecticidal activity of the fungal extracts containing secondary metabolites against cowpea bruchid, *Callosobruchus maculatus*. Since the EtOAc extracts of *D. eschscholzii* (671 mg) and *N. sphaerica* (503 mg) were bioactive against *Colletotrichum musae* and *Callosobruchus maculatus*, bioassay guided fractionations of hexane, chloroform and aqueous were conducted for the EtOAc extracts of these fungal species. The results revealed that chloroform fractions of both extracts showed higher insecticidal and antifungal activities. Therefore they were further purified using silica gel column chromatography.

Three pure compounds CR/01/99/02, CR/01/100/01 and CR/01/100/06 were isolated from *N*. *sphaerica* and yielded 13 mg, 9 mg and 19 mg respectively. CR/01/94/06 (8 mg, 16%), CR/01/95/03 (11 mg, 16.4%) and CR/01/96/02 (21 mg, 48.8%) were the purified bioactive compounds isolated from *D. eschscholzii*. Antifungal activity against *C. musae* revealed that CR/01/100/06 showed higher inhibitory effects and the lowest MLC (80  $\mu$ g) and MIC (50  $\mu$ g) values. 100% mortality of *C. maculatus* was observed at the dose of 100  $\mu$ g for the compound of CR/01/96/02. LD<sub>50</sub> values for RFB and TSB for this compound were 37.4  $\mu$ g and 41  $\mu$ g respectively. These purified compounds will be identified using IR, NMR, mass spectroscopy, etc.