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Isolation of secondary metabolites from an endophytic fungus in Acalypha indica

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Endophytic fungi are a remarkable group to investigate bioactive secondary metabolites. They live inside plant cells at least for a one stage of their life cycle without causing any disease to the plant. This study is focused to isolate and identify secondary metabolites from an endophytic fungus associate with the inflorescence of Acalypha indica L. (Euphorbiaceae), commonly called as 'Kuppameniya'. Healthy A. indica plants were collected from the Central Province, Sri Lanka. Parts of the inflorescence were surface sterilized and segmented into small pieces. They were placed on Potato Dextrose Agar plates and incubated at room temperature in dark for 5-6 days. The fungus emerged from the piece of inflorescence were named as JK/AI/K and it was sub-cultured to obtain the pure culture. The external characteristics and the spores from the pure cultures were microscopically observed. Molecular level identification was carried out by amplifying the Internal Transcribed Spacers (ITS) of rDNA gene using universal eukaryotic primer ITS 1F and ITS 4. The fungus was identified as Diaporthe melonis. D. melonis was large scale cultured on Potato Dextrose Broth medium for 3-4 weeks. At the end of the fermentation, the broth was filtered and partitioned with EtOAc (1:1). The mycelium was crushed into pieces and extracted into EtOAc by sonicating for 30 minutes. The extracts were evaporated to dryness. The two crude extracts were combined due to the similarities observed in their TLC. Next, the combined crude was subjected to chromatographic separation (silica gel column followed by Sephadex LH 20 and PTLC). This resulted, two UV active compounds namely 8-methoxynaphthalene-1-ol (1) and 1,8-dimethoxynaphthalene (2). Their structures were elucidated using ¹H-NMR, ¹³C-NMR and Mass spectrometric data. Compound 1 and 2 will be further studied to determine their bioactivities. It can be concluded that, D. *melonis* is a promising endophytic fungal species to isolate natural naphthalene derivatives.

Keywords: Chromatography, Diaporthe melonis, naphthalene derivatives