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Secondary metabolites of an endophytic fungus from the leaves of *Citrus aurantiifolia*

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Natural products refer to secondary metabolites that naturally produced by microorganisms, plants, or animals. These compounds serve as an ongoing reservoir of unique bioactive metabolites. Endophytes possess substantial potential as undiscovered sources of natural products. The primary goal of this study is to isolate pure compounds from endophytic fungi associate with the leaves of Citrus aurantiifolia (Rutaceae,'lime') and to perform molecular identification of the isolated fungus. Fresh and healthy leaves of C. aurantiifolia were collected from Central Province of Sri Lanka. Small segments (5 mm \times 5 mm) of the tripled sterilized leaves were placed on the Potato Dextrose Agar and placed in the dark for seven days at room temperature (27 °C) for incubation until the appearance of fungal mycelium. The emerged fungus was sub cultured to obtain the pure culture. The isolated fungus was identified as Biscogniauxia capnodes by amplification of ITS regions of rDNA gene. It was cultured large scale and kept for 21 days in shakers at room temperature in dark. After that, the medium was filtered and the broth was extracted using EtOAc. Mycelium was crushed and extracted into EtOAc and MeOH sequentially. The dried EtOAc extract of the broth and the mycelium were combined based on similar TLC pattern. It was subjected to column chromatography over silica gel, then through Sephadex LH 20. The collected fractions were further purified by PTLC to obtain two pure compounds namely, 5-methyl mellein and 6-O-methyl reticulol using NMR and mass spectroscopic data. Therefore, a variety of secondary metabolites can be isolated from endophytic fungi.

Keywords: Biscogniauxia capnodes, endophytic fungi, column chromatography, reticulol