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Metabolites of endophytic fungus associated with Cardiospermum halicacabum

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Cardiospermum halicacabum of the family Sapindaceae, commonly known as Balloon vine is used in traditional medicine to treat various conditions such as rheumatism, nervous diseases, and skeletal fractures. Endophytic fungi live in the inter-cellular spaces of plant tissues, without causing any disease to the host, and these fungi produce bioactive substances that involve in a host-endophyte relationship. The primary function of most of the secondary metabolites is to increase the survival of organisms. This study was carried out to investigate the secondary metabolites produced by endophytic fungi associated with C. halicacabum. The leaves of the plant were triple sterilized. They were cut into pieces ($5mm \times 5mm$), and the leaf segments were placed on potato dextrose agar plates for incubation. After five days, the emerged fungus was repeatedly sub cultured to obtain a pure culture of the fungus. Molecular identification of the fungus by analysis of ITS 1F and ITS 4 regions of the rDNA gene identified the fungus as Xylaria feejeensis. The fungus was large scale cultured in potato dextrose broth medium in ninety Erlenmeyer flasks, and incubated for 21 days. After completion, the broth was filtered and extracted into ethyl acetate (EtOAc). The mycelium was also extracted into EtOAc using sonication. The solvents were evaporated using a rotary evaporator and the crude extracts were obtained. Both extracts were combined together by examining similarities in thin layer chromatography. The extract was subjected to chromatographic separations using gravity columns, sephadex LH-20 columns, high performance liquid chromatography using reverses phase C-18 columns, and preparative thin layer chromatography to obtain pure compounds. The compounds were identified using NMR, and Mass spectroscopy as, 2-chloro-5-methoxy-3-methylcyclohexa-2,5-diene-1,4-dione (1), 2-hydroxy-5-methoxy-3-methylcyclohexa-2,5diene-1,4-dione (2), cycloepoxytriol A (3), phomopsiketone B (4), and 8-hydroxy-5-methoxy-3-methylisochroman-1-one (5). Determination of bioactivities of the compounds 1-5 is in progress.

Keywords: Cardiospermum halicacabum, endophytic fungi, Xylaria feejeensis