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Bioactive secondary metabolites from an endophytic fungus associated with *Gymnema sylvestre*

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Gymnema sylvestre R.Br. (Masbedda) of Asclepiadaceae family is a medicinally important plant with many therapeutic effects, mainly antidiabetic property. Endophytic fungi live symbiotically within plants, produce secondary metabolites with a broad range of bioactivities and have emerged as alternative sources of bioactive compounds. This study was conducted to isolate and investigate the enzyme inhibitory activities of secondary metabolites of an endophytic fungus associated with G. sylvestre. Fresh leaves of G. sylvestre were collected from the Central Province, of Sri Lanka. Small segments (5×5 mm) were triple sterilized, placed on Potato Dextrose Agar and incubated at room temperature. Endophytic fungus obtained was tentatively identified as Colletotrichum sp. by amplification of ITS regions of fungal rDNA gene. Further identification by other gene regions is in progress. Pure culture of endophytic fungus was inoculated into Potato Dextrose Broth, and kept for 21 days with shaking at room temperature. Then medium was filtered and filtrate was partitioned with Ethyl acetate (EtOAc). Mycelium was separately extracted with EtOAc. Both EtOAc extracts were combined based on similar TLC patterns. Chromatographic separation (silica gel column, Sephadex LH-20, HPLC, PTLC) of EtOAc extract furnished three compounds cytidine (1), uridine (2) and acropyrone (3). Their structures were elucidated by Nuclear Magnetic Resonance. All compounds were screened for enzyme inhibitory activities. Cytidine (1) and acropyrone (3) showed high α -glucosidase inhibitory activity with IC₅₀ 8.75 mg 1^{-1} and 6.29 mg 1^{-1} respectively. α -amylase inhibitory activity was not detected in any of the compounds. Cytidine (1) showed high acetylcholinesterase inhibition with IC₅₀ 22.07 mg 1^{-1} . Uridine (2) and acropyrone (3) showed low inhibitory activity against acetylcholinesterase enzyme with 7015.87 mg l^{-1} and 1521.59 mg 1^{-1} IC₅₀ values respectively. Compounds **1** and **2** showed mild lipase inhibition with IC₅₀ of 894.49 mg 1^{-1} and 647.89 mg 1^{-1} respectively. These findings suggest that this endophytic fungus produces secondary metabolites with enzyme inhibitory activities which can be used as potential drug leads.

Keywords: Enzyme inhibitors, Gymnema sylvestre, secondary metabolites

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