Potential Antibacterial Secondary Metabolites from an Enadolichenic Fungus Inhabiting a Lichen Collected from Negombo Lagoon, Sri Lanka

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With the revelation of intriguing bioactive properties from natural sources, the thirst to investigate more into the mysteries of nature has immensely expanded. Endolichenic fungi (ELF) became an interesting source during the recent past in this search for novel bioactive compounds and literature sources provide evidence of many such bioactive compounds isolated from these interesting organisms. These ELF asymptomatically live inside the lichen thallus which is formed by the symbiotic relationship of fungi with an algae or a cyanobacteria. A total of 31 lichens were collected from mangrove plants in Negombo lagoon and their molecular identification revealed that they belonged 10 different species. Healthy lichen thalli were surface sterilized and were cut into small segments and plated on 2% Malt Extract Agar (MEA) medium supplemented with 0.01% streptomycin in order to obtain ELF. The obtained pure cultures of ELF were identified using molecular techniques. DNA was extracted using CTAB method and its quality and quantity were determined by agarose gel electrophoresis. DNA was diluted accordingly and was subjected to Polymerase Chain Reaction (PCR) to amplify fungal ITS rDNA region using universal primers. PCR amplification was tested using agarose gel electrophoresis and the full sequences were obtained. Ethyl acetate crude extracts of 18 such identified ELF strains were subjected to anti-bacterial assay against *Escherichia coli* and *Staphylococcus aureus* using agar well diffusion method. The species *Xylaria feejeensis* isolated from the lichen *Graphis librata* showed remarkable activity against the two bacterial strains on par with the positive control Azithromycin. The assay was carried out using 100 µl of the extract and the positive control (5 mg/ml). The inhibition zone diameters (in cm) against *E. coli* and *S. aureus* for the fungal crude were 1.9 and 2.2 respectively and for Azithromycin was 2.2 against both. In order to isolate the active compounds, a larger crude of the same was obtained and partitioned into Hexane, Chloroform and Methanol fractions based on polarity. The assay results for the three fractions revealed that only Hexane and Chloroform fractions possessed anti-bacterial potentiality. Subsequently, silica gel normal phase column chromatography was performed for further fractionation. Collected 6 fractions from the column for Chloroform fraction showed inhibition diameters of 1.9, 1.9, 1.5, 1.2, 1.3, 1.1 against *E. coli* comparable with 2.2 of Azithromycin and 2.4, 2.2, 1.4, 0.0, 0.7,0.0 against *S. aureus* comparable with 2.5 of Azithromycin. The fraction 1 and 2 showed highest activity against both bacterial strains and fraction 4 and 6 showed lowest activity against *E. coli* and none against *S. aureus*. Further isolation is being carried out for active fractions and the structures of obtaining active compounds will be elucidated using spectroscopic methods.

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