

Isolation, purification and structure elucidation of antimicrobial and bio-active compounds of soil fungi

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Soil fungi are considered as a rich source of bioactive compounds and antibiotics. They are able to produce a great variety of secondary metabolites characterized by a broad spectrum of properties of bioactive compounds including antiviral, antibacterial, antifungal, and anticancer. Therefore, the objective of the study was isolation, purification and structure elucidation of antimicrobial and bioactive compounds of soil fungi. In the present study, soil samples were collected from the Kelani River mouth, Sri Lanka and twenty nine fungi were isolated on potato dextrose agar. The antimicrobial activity of the methanol crude extract of fungi was tested against two human pathogenic Gram-positive bacteria (*Bacillus* sp., *Staphylococcus aureus*-ATCC 2593), two human pathogenic Gram-negative bacteria (*Escherichia coli*-ATCC 25922, 2785 and *Salmonella typhi*) and two human pathogenic fungi (*Candida albicans* and *Candida tropicalis*) using agar disc diffusion method. One fungal isolate was selected based on the diameter of inhibition zone to isolate and purify antimicrobial (antifungal and antibacterial) compound. The antioxidant property in the crude extract was evaluated using DPPH (2,2-diphenyl-1-picrylhydrazyl) scavenging test (Inhibitory Concentration, IC₅₀ = 200.42 ppm). Secondary phytochemical screening for major classes of antibacterial compounds were done using TLC visualization reagent specific for major classes of antibacterial compounds. Structural elucidation of isolated compound was carried out using Gas Chromatography Mass Spectrometry (GC-MS) and Fourier-Transform InfraRed spectroscopy (FTIR). TLC plates of the crude extract developed using different solvent system showed different number of band with antimicrobial compounds as it is revealed by bioautochromatography, solvent system 1 (Ethyl Acetate (EA): Methanol (Me): Water (Wa) (100:13.5:10) 6 bands against *Bacillus* sp. solvent system 2 (EA :Toluene (93:7)) 2 bands against *Bacillus* sp. and two bands with antifungal activity was recorded against *C. albicans*. Band with antimicrobial compounds on Preparative Thin Layer Chromatography (PTLC) was scraped and dissolved in methanol (HPLC) to further purify using Solid Phase Extraction (SPE) and High Performance Liquid Chromatography (HPLC). Antibacterial activity of each purified compounds, was verified using bioautochromatography. According to the secondary chemical screening, the compound with the highest antimicrobial activity was found to have contain Arbutin and Anthraquinone glycoside. The results of the present study revealed that the crude extracts of soil fungi had antibacterial activity against some human pathogenic bacteria and the soil fungi is a potential candidate to utilize as a source to produce antibiotics.

Keywords: Antibacterial activity, HPLC, Phytochemicals, PTLC, TLC

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