

Isolation of Antibacterial Compounds from an Entomopathogenic Fungus Isolated from a Beetle, a *Harmonia* sp.

S. Abivarna, S.W.P.N.H. Patabedi and P.B. Ratnaweera*

Department of Science and Technology, Uva Wellassa University, Badulla, Sri Lanka

**Corresponding Author E-mail: pamoda@uwu.ac.lk, TP: +94552226676*

Antibiotic resistance of bacteria is a major human health concern in the world which requires research priorities. Therefore discovering new and effective antibacterial drug leads has become an urgent necessity. Entomopathogenic fungi act as parasites of insects which kill or disable them. However these fungi are known to synthesize various bioactive secondary metabolites. Thus, the objective of the current study was to isolate the antibacterial compounds from an entomopathogenic fungus isolated from a beetle (*Harmonia* sp.) in Sri Lanka. The fungus BET 05, which was microscopically identified as an *Aspergillus* sp. was grown in potato dextrose agar (PDA) and broth (PDB) media. After an incubation period of 06 days, both solid and liquid fungal cultures were extracted into ethyl acetate (EtOAc). The solid culture (30 large and 13 medium PDA dishes) yielded 804.8 mg of crude while the liquid culture (400 mL x 3) gave 436.7 mg. The solid and liquid crude extracts were subjected to an agar disc diffusion bioassay to determine the antibacterial activity and the inhibition zones diameters were 11.5 and 11.3 mm respectively, against the Gram positive *Staphylococcus aureus* (ATCC 25923) at 400 µg/disc concentration. The positive control used was Gentamycin, while the negative control was methanol. The main active compound in the extract was isolated using bioassay guided chromatographic techniques. The crude extract (436.7 mg) was first fractionated by solvent-solvent partitioning, using hexane and methanol/water (9:1), chloroform and methanol/water (6:4) and, EtOAc and water. The active chloroform fraction was purified by Sephadex LH20 size exclusion chromatography using methanol as the eluent. Fractions collected were grouped according to their TLC profiles and the combined fractions (A-F) were tested for antibacterial activity using a bioautography. The active fraction B was further purified by normal phase silica chromatography using 60:40 hexane: EtOAc to methanol solvent systems. A bioautography revealed, the combined fraction E contains main active compound while fraction C and G are also having some active compounds. Finally a preparative TLC was conducted using EtOAc as the mobile phase to get the main active compound purified (4.1 mg). This study reveals that entomopathogenic *Aspergillus* sp. (BET 05) is a potential producer of antibacterial compounds.

Keywords: Antibacterial; Antibiotic resistance; Entomopathogenic; *Aspergillus*