ABSTRACT

The expeditious rise of multidrug-resistance in common human pathogens have created a worldwide crisis for novel antibiotics and therapeutic substitutes. The emerging demand for antimicrobial drugs is leading towards extensive research on discovering natural sources including, microscopic-organisms particularly bacteria. Phylum Actinobacteria, especially members of genus *Streptomyces* are renowned for producing a diversity of secondary metabolites including tetracyclines, aminoglycosides, macrolides with their own definite structure and functions. Among these secondary metabolites *Streptomyces* are also documented to be a source of proteinaceous toxins with potential of biomedical applications, are still to be untapped. In this study, native *Streptomyces* isolates are screened and evaluated for umbrella toxin production, which are extracellular polymorphic protein complexes secreted during stationary phase of growth cycle, to test antimicrobial and antifungal activities. Six morphologically variable strains are subjected through bioassays including co-culture of all six *Streptomyces* strains against expected most potent participant between them, spot assay on selected *Streptomyces* strain to check aerial hyphae growth inhibition. Microscopy, morphological examination, biochemical tests analysis (Gram staining, catalase test with 3% H_2O_2, and casein hydrolysis) confirmed their taxonomic alignment.

Ammonium sulfate fractionation method was employed to extract the protein precipitates from production media (SCA broth) from all six strains respectively. Protein pallet dissolved in Tris-EDTA buffer was subjected to dialysis for 24 hours with 2-3 buffer (Tris-EDTA, pH 7.5) changes. Total protein yields were evaluated by 280nm spectrophotometric analysis. The dialyzed extracts were without further dilutions subjected through agar well diffusion method for checking antimicrobial activity against some potent common human pathogen strains

including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* spp., *Enterococcus* spp., *Enterobacter* spp., *Pseudomonas aeruginosa*, *Klebsiella* spp.. Similar concentration and quantity of protein extract was tested against three different pathogenic fungal strains including *Trichoderma viride*, *Mucor muceda*, *Penicilium digitatum* on PDA plates under fungal specific growth conditions. Hemolysis assays conducted with fresh erythrocytes which provided different peaks under detectable range at 540nm UV spectrophotometric analysis that showed membrane disruptive or pore forming activity in dialyzed extract.

The isolates then projected to the protein profiling such as SDS-PAGE analysis rectify the distinct protein band resembling to presumed umbrella toxin subunits. To achieve molecular confirmation PCR amplification of umbrella toxin coding gene was carried out in one representative isolate, which provided strong genetic evidence of biosynthesis of toxin.

This work provides the preliminary molecular characterization of umbrella toxins and overarching screening from indigenous *Streptomyces*. The combination of functional antimicrobial and antifungal testing, co-culture and spot assays, hemolysis activity and biophysical characterization emphasize the therapeutic potential of these toxins. These outcomes establish a base for future studies targeting towards purification and mode of action analysis of umbrella toxins as nominee for novel antimicrobial drug development.