

## PREFACE

The genus *Streptomyces*, a member of the phylum Actinomycetota, is notable for its high GC content (70%) in its genetic material. This filamentous, aerobic, gram-positive, saprophytic, soil-dwelling bacterium has the ability to synthesize a wide range of secondary metabolites, including antibiotics. *Streptomyces* is a significant source of antibiotics, responsible for synthesizing about 80% of the antibiotics used to combat various diseases. Synthetic drugs often have numerous side effects, so there is a pressing need to discover alternative drugs based on natural sources with fewer side effects and lower costs. *Streptomyces*, with its bioavailability, abundant secondary metabolites, biotic friendliness, low toxicity, and high effectiveness, can serve as a perfect alternative for producing bioactive compounds.

Furthermore, *Streptomyces* possesses both eukaryotic signaling systems and prokaryotic two-component regulatory systems, making it a promising candidate for developing an assay for screening kinase inhibitors in eukaryotic signaling pathways. *Streptomyces 85E* strain has been identified as a suitable strain for testing kinase inhibitors using agar diffusion methods due to its growth and development characteristics on solid media and its ability to reveal the cytotoxicity of tested inhibitors. With known eukaryotic kinase inhibitors, such as tyrphostin and genistein, inhibition of hyphae formation has been observed, leading to the formation of "bald" colonies. The *Streptomyces 85E* assay exhibits three distinct phenotypes that can be utilized to deduce the persistence of eukaryotic protein kinase inhibitors (PKIs). These phenotypes encompass the absence of a zone, indicating the drug's inactivity; a transparent zone, signifying the cytotoxicity toward the cell, inhibiting both growth and sporulation; and a turbid zone, characterized by the creation of "bald" colonies that inhibit sporulation and aerial hyphae formation. *Streptomyces clavuligerus* and *Streptomyces fragilis* are gram-positive, filamentous

actinobacteria that comprise aerial mycelium with branched hyphae and spores. *Streptomyces clavuligerus* is known for its production of  $\beta$ -lactam antibiotics, such as clavulanic acid, cephamycin C, penicillin N, O-carbamoyl derivative deacetylcephalosporin C, and deacetoxycephalosporin C. Clavulanic acid is a novel  $\beta$ -lactamase inhibitor with broad-spectrum antibiotic properties effective against both gram-positive and gram-negative bacteria. Apart from its antibiotic activity, *Streptomyces clavuligerus* is also capable of producing anticancer metabolites, including bleomycin, Clavulactones, and Tunicamycin. Furthermore, this species produces kinase and phosphatase inhibitors, showing potential as lead molecules in cancer treatment targeting specific cellular processes. *Streptomyces fragilis*, although less studied within the *Streptomyces* genus, is also notable for its production of Azaserine, an antibiotic with tumor-inhibiting properties effective against a range of organisms, including gram-positive and gram-negative bacteria, protozoa, and fungi. Further exploration of *Streptomyces fragilis* could uncover novel bioactive compounds, potentially including new anticancer agents and other valuable biochemical products, making it an exciting prospect for future scientific and industrial applications.

The research aimed to evaluate the antioxidant, protein kinase inhibitory (PKIs) potential and cytotoxicity activity of the extracts from *Streptomyces clavuligerus* and *Streptomyces fragilis*. The bioactive compounds were obtained through submerged fermentation of the strains at 30 degrees Celsius, 200 rpm, for 3 days. After the 3rd day, the broth was sonicated, centrifuged, and subjected to a two-phase separation technique to obtain glycosidic anthracycline. The purified extract obtained from four different media was tested for the presence of S. 85E kinase inhibitor in the fermented broth. The media showing the highest bald zone in the kinase inhibitory test was chosen for further studies. The DPPH assay demonstrated that the organic extract had a strong free radical

scavenging capacity, with an  $IC_{50}$  value of  $28.90 \pm 0.24 \mu\text{g/mL}$  for *Streptomyces clavuligerus* and  $38.76 \pm 0.24 \mu\text{g/mL}$  for *Streptomyces fragilis*. In addition, the PKIs test indicated that *S. clavuligerus* and *S. fragilis* extracts produced a white bald zone, suggesting the existence of PKIs. The MTT assay was used to evaluate the cytotoxic activity of the organic extract on MCF-7, Hop-62, SiHa, and PC-3 cell lines. It showed the lowest  $IC_{50}$  value against the MCF-7 cell line ( $128.93 \pm 3.1 \mu\text{g/mL}$ ), followed by the SiHa cell line for *S. clavuligerus*. Similarly, in the case of *S. fragilis* organic extract, SiHa cell lines ( $58.46 \pm 2.0 \mu\text{g/mL}$ ) showed the lowest  $IC_{50}$ , followed by the MCF-7 cell line ( $61.14 \pm 2.5 \mu\text{g/mL}$ ). This indicates potent growth inhibitory potential against human breast cancer and human cervical cancer cell lines. Multiple secondary metabolites from the organic and aqueous extracts of *S. clavuligerus* and *S. fragilis* were identified through HR-LCMS analysis after incubation at  $30^{\circ}\text{C}$  under 200 rpm for 3 days. The maximization of anthracycline compound production from *S. fragilis* was assessed through fermentation condition optimization. This included different carbon sources (dextrose, sucrose, fructose, corn flour, tapioca, lactose, and maltose) and nitrogen sources (beef extract, yeast extract, casein peptone, and ammonium sulfate) as well as varied ranges of temperatures ( $26^{\circ}\text{C}$ - $34^{\circ}\text{C}$ ), pH (6.5-7.5), C: N ratio (0.53-1.6) and agitation rates (150-250 rpm). The maximum production of anthracycline metabolites was achieved with molasses and yeast extract at a C: N ratio (1.15) at  $29.7^{\circ}\text{C}$  temperature, pH 7.09, and a 203-rpm agitation rate using batch fermentation. The RSM model predicted a maximum metabolite yield of 4.65 g/L, which closely matched the experimental yield of 4.59 g/L. In addition to RSM, machine learning (ML) models (LM, SVM, GRNN, BFGS, and SCG) were utilized to predict metabolite production, and the LM algorithm emerged as the most effective, demonstrating exceptional predictive accuracy. The RSM and ML

models predicted metabolite productions of 4.65 g/L and 4.58 g/L, respectively, with the experimental yield being 4.59 g/L.

Computational studies revealed that epirubicin demonstrated superior inhibitory activity against key kinase proteins such as CDK4, EGFR, PDGFR, and PI3K when compared to the FDA-approved drug doxorubicin. Additionally, the downstream process of the secondary metabolites and the isolation and structural characterization of epirubicin (anthracycline) were conducted using various analytical techniques such as HPLC, FTIR, and NMR. The thermal characteristics of epirubicin were elucidated by XRD, DSC, and TGA analysis. The DPPH assay revealed the potent free radical inhibitory capacity of epirubicin ( $IC_{50}$   $12.56 \pm 0.22$   $\mu\text{g/mL}$ ) compared to ascorbic acid ( $IC_{50}$   $14.38 \pm 0.14$   $\mu\text{g/mL}$ ). In MTT assays, epirubicin demonstrated effectiveness against human lung cancer cells (A549), with an  $IC_{50}$  of 5.435  $\mu\text{g/mL}$  and a 37% reduction in cancer cell viability at an 8  $\mu\text{g/mL}$  concentration. Subsequent transcriptomics analysis of A549 cells, using RNAseq analysis with a phred score of 35, revealed that 428 genes were significantly altered, with the highest number of downregulated genes primarily involved in cell cycle regulation. KEGG analysis indicated upregulation of the p53 pathway, with all other pathways containing genes that directly or indirectly enhance p53 pathway activation, leading to apoptosis and cell death. This research highlights the potential of compounds derived from *Streptomyces fragilis* and *Streptomyces Clavuligerus* in cancer treatment. It also demonstrates the feasibility of optimizing fermentation processes for large-scale production of metabolites using agricultural waste molasses and yeast extract.