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ISSN 1462 2815

BIOFILM INHIBITION OF ACTINOMYCETES EXTRACT ON *E. COLI* AND ITS ANTI PROLIFERATIVE EFFECT ON MG 63 CELLS

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DOI: 10.5281/zenodo.11076108

Abstract

Introduction: This research delves into the promising potential of Actinomycetes, filamentous bacteria renowned for their remarkable production of bioactive compounds. Focusing on their ability to combat antibiotic-resistant biofilm infections and influence cancer cell proliferation, this study represents a convergence of microbiology and oncology. By investigating the mechanisms through which Actinomycetes disrupt biofilms and exert cytotoxic effects on cancer cells, it offers valuable insights into versatile therapeutic strategies with significant implications for medical treatment.The findings presented in this study underscore the considerable potential of Actinomycetes as a promising therapeutic option for cancer treatment, as evidenced by their demonstrated efficacy in inhibiting cancer cell proliferation. Furthermore, the observed inhibitory effect on the bacterium E. coli highlights the multifaceted antimicrobial properties of Actinomycetes, suggesting their potential utility in combating antibiotic-resistant bacterial infections. These results emphasize the importance of further exploration and development of Actinomycetes-derived bioactive compounds for novel therapeutic interventions in both oncology and microbiology.

Keywords: Actinomycetes, Bioflim, Oncology, Antibiotic Resistance, MG63 cells.

INTRODUCTION

In the face of mounting concerns regarding antibiotic resistance and the persistence of microbial infections, the search for novel therapeutic approaches has become imperative.One of the major challenges in treating such infections is the formation of biofilms, intricate microbial communities encased in a self-produced matrix that bestows upon them a heightened resistance to conventional antibiotics. In this context, Actinomycetes, filamentous bacteria known for their prolific production of bioactive compounds, offer a promising avenue for the development of innovative anti-biofilm agents.(1) The capability of Actinomycetes-derived metabolites to inhibit biofilm formation presents a potential solution to combat microbial infections more effectively(2)(2,3).By disrupting biofilm integrity, these metabolites could pave the way for enhanced susceptibility of bacteria to existing antibiotics.Furthermore, the exploration of Actinomycetes extracts extends beyond combating infections, encompassing their potential impact on cell proliferation in diseases such as cancer (4) (5,6) (7). E. coli, often known as Escherichia coli, is a Gram-negative bacterium found in the lower gut of warm-blooded organisms(8) .It is used extensively in biotechnological research and is important as a model organism in microbiological investigations. E. coli plays a key role in many industrial processes, such as protein expression and recombinant DNA technology(9).Moreover, there is a noteworthy correlation between actinomycetes and E. Coli.Actinomycetes have the ability to suppress the development of E. coli and other bacteria by producing antibiotics, which highlights their influence on microbial dynamics and possible roles in maintaining ecological balance in a variety of settings.This study embarks on a journey to

investigate the biofilm inhibition potential of an Actinomycetes extract against *Escherichia coli* (E. coli), a renowned biofilm-forming bacterium.

The study's significance is not confined solely to the realm of biofilm inhibition. The evaluation of the Actinomycetes extract's impact on MG-63 cells, a human osteosarcoma cell line, adds a layer of complexity to its potential applications(10–12)(13)(14). Researchers were additionally recruited to evaluate the effectiveness of new medications for the treatment of osteosarcoma and other bone-related disorders(15)(16)(17)(18).Beyond its role in infection control, the extract's potential cytotoxic effects on cancer cells could hold the key to developing novel therapeutic strategies for malignancies(19). This study's interdisciplinary approach bridges the gap between microbiology and oncology, offering a comprehensive perspective on the extract's biological activities.(20)Understanding the intricate interactions within biofilms and their resistance mechanisms is crucial for devising effective strategies against persistent infections. By investigating the mechanisms through which Actinomycetes-derived compounds disrupt biofilm formation, this research contributes to the broader understanding of microbial communities behavior. Furthermore, the exploration of their effects on MG-63 cell proliferation sheds light on their potential as versatile therapeutic agents.

In summary, this manuscript delves into the unexplored potential of Actinomycetes extracts as biofilm inhibitors and their possible cytotoxic effects on cancer cells. Through a comprehensive investigation of their impacts on both microbial and human cell communities(21), this study bridges the gap between different scientific disciplines. The findings hold promise not only for addressing antibiotic resistance and infection control but also for advancing our understanding of complex diseases like cancer. By harnessing the bioactive potential of Actinomycetes, we endeavor to unlock innovative solutions for some of the most pressing challenges in modern medicine.The main objective of the study is to find the potential of Actinomycetes extract in preventing biofilm formation by Escherichia coli bacteria and to assess its impact on inhibiting the proliferation of MG 63 cells. The aim is to find the potential of Actinomycetes extract in preventing biofilm formation by Escherichia coli bacteria and to assess its impact on inhibiting the proliferation of MG 63 cells.

MATERIALS AND METHODS

Biofilm production: Single colony was taken from the MHA overnight bacterial culture, inoculated into 0.85% saline solution and vortexed to ensure that the bacterial suspension was homogeneous. Bacterial suspensions were analyzed using a densitometer and adjusted to 1 × 106 colony forming units (CFU/mL) by diluting with appropriate broth. The broths used were MHB, Tryptic Soy (TS, BD), Tryptic Soy supplemented with 1% glucose or 2% glucose, Brain Heart Infusion and Brain Heart Infusion supplemented with 1% glucose (BHIG). An aliquot of 200 µL of bacterial suspension per well was dispensed into a 96-well flat bottom microplate. Negative control wells were filled with 200 µL of media.

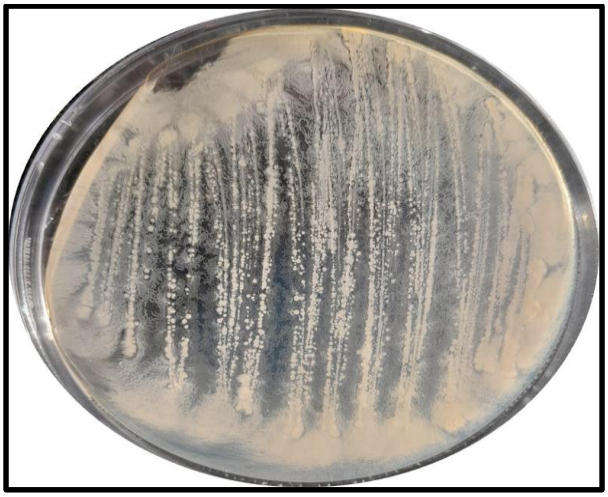

Fig.1: Isolation of actinomycetes from beach soil

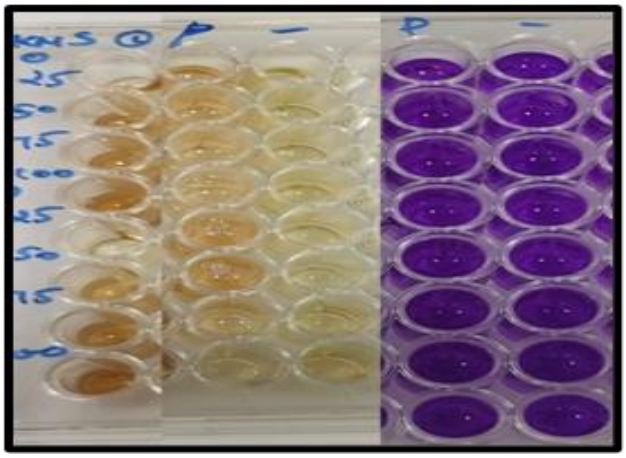

Fig.2: Biofilm inhibition of actinomycetes extract on E. coli.

Assessment of biofilm biomass by crystal violet staining: Biofilm biomass measurements by crystal violet (CV) staining were performed as previously described with some modifications. An aliquot of 190 µL of 0.01% CV (Sigma-Aldrich) aqueous solution was added to three wells of the 96-well flat bottom microplate containing biofilm, along with its respective control media (three wells), and incubated at room temperature for 30 min. Then, CV solution was removed and wells were washed three times with 200 µL of sterile water. During this wash step care was taken not to disturb the biofilm. The plate was left to dry for 30 min at 50 °C. Next, 200 µL of 96–99% ethanol was added to each well and biofilm was detached by vigorous pipetting. Absorbance measurement values at 570 nm were obtained.

Docking study: The structure of sitosterol ligand was derived from pubchem then LigPrep in Schrodinger software suite was used to prepare the epik states and to optimize the ligand. The protein structure (PDB:1Q8I) was downloaded from the PDB database, which has the structure of murine NF-kappaB including kinase bound imidazolebenzodiazepine compound. The docking was carried out using the extra precision method (XP). The Glide score (Gscore) was calculated using the following formula in kcal/mol.

G Score = a*vdW + b*Coul + Lipo+ Hbond +Metal + BuryP + RotB + Site (1)

where Van der Waals energy is represented by vdW, with coefficients a=0.065 and b=0.130. Coul is the symbol for Coulomb energy.Lipo is a symbol of lipophilic interaction

Agar well plate: Agar well diffusion method Agar well-diffusion method was followed to determine the antimicrobial activity.Wells (10mm diameter and about 2 cm a part) were made in each of these plates using sterile cork borer. Stock solution of each plant extract was prepared at a concentration of 1 mg/ml in different plant extracts viz. Methanol, Ethanol, Petroleum Ether, Water. About 100 µl of different concentrations of plant solvent extracts were added to the wells and allowed to diffuse at room temperature for 2 hrs. Control experiments comprising inoculums without plant extract were set up. The plates were incubated at 37°C for 18-24 h for bacterial pathogens and 28°C for 48 hours fungal pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated

RESULTS

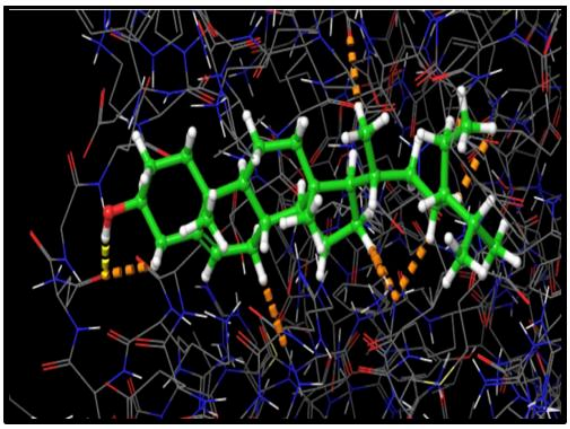

Fig.3: showing the site of interaction sitosterol with protein 1q8i.

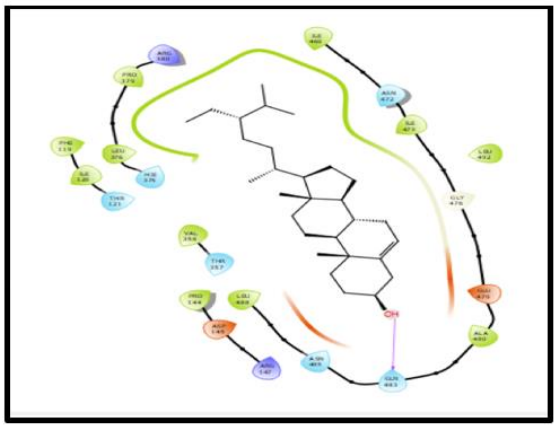

Fig.4: 2D Interaction Diagram of Sitosterol with protein 1q8i

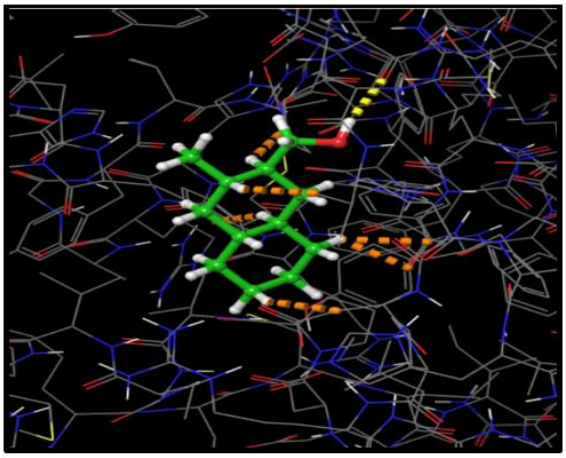

Fig.5: showing the site of interaction pentahydonapthalene with protein 1q8i

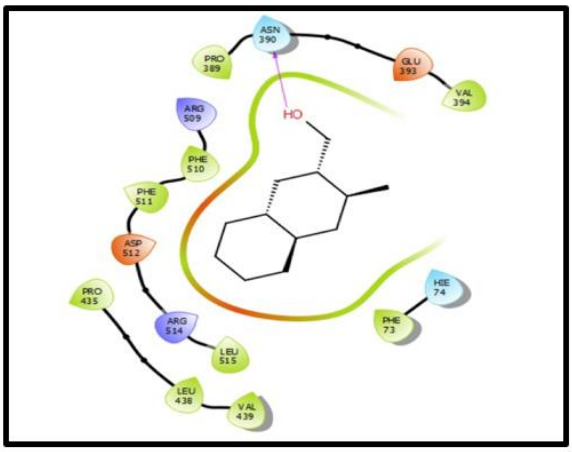

Fig.6: 2D Interaction Diagram of pentahydonapthalene with protein 1q8i.

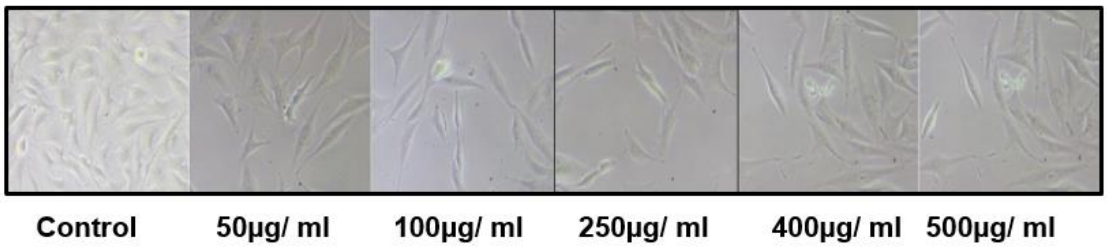

Fig 7: Anticancer activity of Actinomycetes extract on MG63 cells

In this study, bacterial suspensions were prepared and standardized. Biofilm biomass was assessed using crystal violet staining, revealing the biofilm's characteristics. Docking analysis with sitosterol ligand on murine NF-kappaB showed specific interactions. Antimicrobial activity was determined using the agar well diffusion method, indicating inhibition zones for various plant extracts. These findings provide valuable insights into biofilm formation and potential antimicrobial agents. Biofilm biomass assessment was performed using crystal violet staining, showing varying levels of biofilm formation under different growth conditions. The antimicrobial activity of plant extracts was determined using the agar well diffusion method, revealing inhibitory effects on both bacterial and fungal pathogens. Docking studies suggested potential interactions between sistosetrol ligand and murine NF-kappaB. These findings open avenues for innovative anti-biofilm agents and antimicrobial therapies.

DISCUSSION

The described methodology reveals a comprehensive approach to studying biofilm formation and the potential antimicrobial activities of different plant extracts. The initial steps involved preparing bacterial suspensions and adjusting their concentrations, ensuring accuracy in the subsequent analyses(5). The use of various broths allowed for a versatile examination of biofilm formation, considering factors like glucose supplementation. This method provides a sound foundation for assessing biofilm development in different environmental conditions.

The assessment of biofilm biomass through crystal violet staining is a well-established technique, and the modifications made in the described protocol align with standard practices(22). The use of crystal violet to stain the biofilms followed by careful washing and measurement at 570 nm allows for accurate quantification of the biofilm biomass. This method is crucial in understanding the impact of different variables on biofilm formation.

The docking study involving the sitosterol ligand is a significant component of this research (23). Utilizing the PDB structure of murine NF-kappaB with a bound imidazobenzodiazepine compound allows for insights into potential interactions between this ligand and the protein. The extra precision method (XP) used for the docking study is known for its reliability in predicting ligand-protein interactions. The calculation of Glide scores provides valuable data on the binding affinities between the sitosterol ligand and the target protein, offering insights into its potential as a biofilm inhibitor.

The agar well-diffusion method is a robust technique for evaluating the antimicrobial activity of plant extracts(24).The use of different solvent extracts, such as Methanol, Ethanol, Petroleum Ether, and Water, allows for a comprehensive assessment of the

plant's antimicrobial potential. The inhibition zones and activity index calculations are essential parameters for determining the efficacy of these plant extracts against both bacterial and fungal pathogens.

In summary, the methodology outlined in this research offers a well-structured approach to studying biofilm formation and assessing the antimicrobial properties of plant extracts(25) .The combination of biofilm quantification, molecular docking, and antimicrobial testing provides a holistic perspective on the potential applications of these extracts in combating biofilm-related infections. The outcome of these experiments will undoubtedly contribute to our understanding of biofilm dynamics and the development of novel antimicrobial strategies.

Future Scope

To bridge the gap between laboratory findings and real-world applications, future research could focus on in vivo studies using animal models. Assessing the safety, efficacy, and bioavailability of the Actinomycetes extract in vivo could pave the way for its translation into clinical trials. Additionally, exploring its potential as a topical treatment for biofilm-related infections or as an adjunct therapy for cancer treatment holds significant promise for clinical applications.

CONCLUSION

In conclusion, the research on the "Inhibition of Biofilm formation of Actinomycetes extract on Escherichia coli and its anti-proliferative effect on MG 63 cells" highlights the potential of Actinomycetes extract as a valuable therapeutic agent. It demonstrates its ability to prevent biofilm formation by E. coli and inhibit the growth of MG 63 cells. These findings pave the way for future studies and the development of innovative treatments for bacterial infections and cancer.

Acknowledgement

We extend our sincere gratitude to the Saveetha Dental College and Hospitals for their constant support and successful completion of this work.

Conflıct Of Interest

None to declare.

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