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BIOFILM INHIBITION AND GROWTH INHIBITORY ACTIVITY OF ACTINOMYCETES EXTRACT ON STAPHYLOCOCCUS MUTANTS

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ABSTRACT

Introduction:

The threat of contagious infectious diseases is consistently evolving as demographic explosion, travel globalization, and changes in human lifestyle increase the danger of spreading pathogens, resulting in accelerated changes in disease landscape. Of particular interest is the aftermath of superimposing viral epidemics (especially SARS-CoV-2) over long-standing diseases, such as Tuberculosis (TB), which remains a significant disease for public health worldwide and especially in emerging economies.

Aim:

To gather information about the problems faced by tuberculosis affected patients during corona pandemic.

Materials and Methods:

A self made questionnaire with 11 questions was circulated among the dental students through online platform "Google forms". The data was collected and analysed using SPSS software and the final results were produced.

Results:

The results clearly show us that there are many problems faced by Tuberculosis affected patients during the Corona pandemic. Lockdown, social distancing, isolation strategies and public health guidelines to prevent viral transmission impacted the delivery of all aspects of Tuberculosis care.

Conclusion:

Viral respiratory infections and TB impede the host's immune responses, it can be assumed that their lethal synergism may contribute to more severe clinical evolution. Hence more extensive management needs to be undertaken and there should be no break in the continuity of essential services for people affected with TB during the "COVID'19" pandemic.

Keywords: COVID-19, Pandemic, Tuberculosis, Impact, Viral epidemic

INTRODUCTION

The study investigates the biofilm inhibition and growth inhibitory activity of actinomycetes extracts on Staphylococcus mutans.Staphylococcus mutans refers to a specific strain or variant of the Staphylococcus bacteria that is associated with dental caries and oral health issues.Dental biofilms, particularly those formed by Staphylococcus mutans, pose a significant challenge in oral health.Actinomycetes, a group of bacteria, are known for their ability to produce bioactive compounds with antimicrobial properties.(1)

Due to their increased resistance to medicines and immunological responses, bacterial biofilms pose a serious obstacle to the effective treatment of illnesses. Staphylococcus mutants, which are renowned for their ability to build biofilms, are linked to a variety of human illnesses, further confounding therapeutic approaches. Actinomycetes, which are renowned for having a wide range of metabolic capacities, are also known for producing secondary metabolites that may have antibiotic effects. The goal of the current study is to investigate the ability of actinomycetes extracts to prevent biofilm formation and development in Staphylococcus mutants, providing insight into the strains' capacity to deal with biofilm-related problems.(2)

In the ever-evolving landscape of medical research, an enduring challenge persists – the formidable and enigmatic realm of bacterial biofilms. These intricate communities of microorganisms, encased in a self-produced matrix, stand as resilient fortresses, displaying heightened resistance to conventional antibiotics and immune responses. Among these biofilm architects, Staphylococcus mutants takes a prominent role, notorious for its adeptness in crafting robust biofilms that contribute to an array of human infections. As the medical community navigates the intricate maze of combatting entrenched biofilm formations, a promising beacon of hope emerges from an unexpected source – actinomycetes.(3)

Actinomycetes, a diverse group of filamentous bacteria, have captivated scientists for their ability to produce an array of bioactive compounds. These compounds, with their potent antimicrobial properties, present a tantalizing avenue for addressing the challenges posed by biofilms. At the heart of this study lies a compelling inquiry – the exploration of biofilm inhibition and growth inhibitory activity elicited by actinomycetes extracts against the unyielding adversary, Staphylococcus mutants.(4)

The significance of confronting biofilm-related complications resonates deeply within the realm of medical science. Bacterial biofilms have cast a shadow over treatment efficacy, contributing to chronic infections, medical device-associated woes, and therapeutic setbacks. In this context, the potential of actinomycetes extracts to disrupt biofilm formation and curtail bacterial proliferation emerges as a ray of optimism.(5)

The intricate interplay between actinomycetes extracts and Staphylococcus mutans biofilms forms the core of this investigation, promising insights that could redefine the landscape of biofilm inhibition. As researchers delve into the molecular intricacies of this interaction, the potential to uncover innovative strategies for combating biofilm-associated challenges stands as a tantalizing prospect. The unraveling of how actinomycetes compounds intricately disrupt the biofilm matrix and arrest bacterial growth could herald groundbreaking progress in the fight against infectious diseases.(6)

Beyond the confines of the laboratory, the implications of this research radiate outward, envisioning a paradigm shift in clinical approaches(7). The potential discovery of effective biofilm inhibitors derived from actinomycetes extracts holds the promise of reshaping medical practices, redefining treatment methodologies, and reinvigorating the prospects of patients grappling with biofilm-related ailments. As this exploration unfolds, we embark on a journey propelled by curiosity and guided by rigorous inquiry, seeking to illuminate the untapped potential of actinomycetes extracts as a potent adversary against the resolute biofilm formations of Staphylococcus mutants. Through meticulous experimentation and judicious analysis, we aspire to carve a path forward, one that pioneers innovative strategies to confront biofilm-associated hurdles and ushers in a new era of medical ingenuity.(8)

The actinomycetes, a fascinating group of filamentous bacteria known for producing copious quantities of bioactive substances with a wide range of pharmacological qualities. Researchers are interested in these microbes because they may open up new fronts in the struggle against microbial foes. Actinomycetes have become a promising source of chemicals that have the capacity to prevent the formation of biofilms and obstruct bacterial growth in the search for new antimicrobial drugs. This work explores the amazing ability of actinomycetes extracts to block the tenacious advances of Staphylococcus mutants, giving information on their power to suppress bacterial population growth and damage biofilm architecture. (9)

MATERIALS AND METHODS:

Biofilm production:

A 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 analysed using a densitometer (DEN-1, BioSan, Warren MI, USA) 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 (TSG, ICN Biomedicals, Irvine, CA, USA), or 2% glucose (TS2G), Brain Heart Infusion (BHI, Sigma-Aldrich, St Louis, MO, USA) 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 (Nunc, Roskilde, Denmark). Negative control wells were filled with 200 µL of media only. Microplates were then incubated at 37 °C for 24 h [Staphylococcus aureus and methicillin-resistant S. aureus (MRSA)] or 48 h [Enterococcus faecalis, vancomycin- resistant st.mutants (VRE), st.mutants and st.mutants VRE].

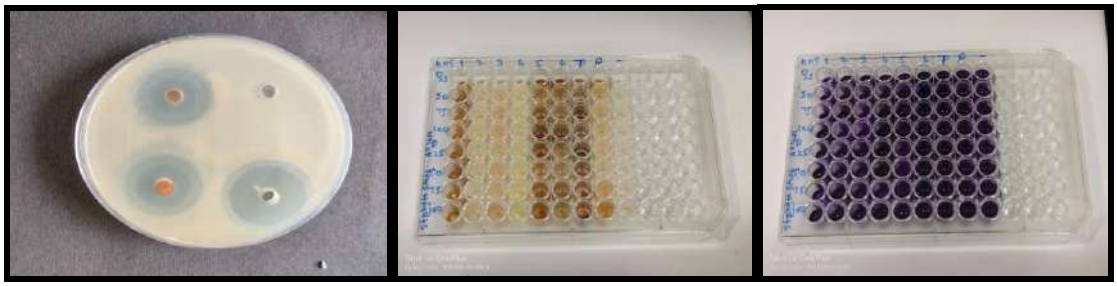

Fig1. growth inhibition activity of actinomycetes extract on st.mutans. **Fig 2 and 3.** Antimicrobial effect on actinomycetes extract on st.mutants.

Assessment of biofilm biomass by crystal violet staining

Biofilm biomass measurements by crystal violet (CV) staining were performed as previously described [12] 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 using the Multiskan GO (Thermo Fisher Scientific, Vantaa, Finland). If a negative value for optical density (OD) was obtained, it was presented as zero. The experiment was performed twice with three replicates.

Agar well plate

Agar well diffusion method Agar well-diffusion method was followed to determine the antimicrobial activity. Nutrient agar (NA) and Potato Dextrose Agar (PDA) plates were swabbed (sterile cotton swabs) with 8 hour old - broth culture of respective bacteria and fungi. Wells (10mm diameter and about 2 cm a part) were made in each of these plates using a 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 for fungal pathogens. The diameter of the inhibition zone (mm) was measured and the activity index was also calculated. Triplicates were maintained and the experiment was repeated thrice, for each replicates the readings were taken in three different fixed directions and the average values were recorded

RESULT:

The structure of dopamine ligand was derived from pubchem with CID 681, then LigPrep in Schrodinger software suite was used to prepare the epik states and to optimize the ligand. The protein structure (PDB: 3g7b) was downloaded from the PDB database, which has the structure Staphylococcus Aureus Gyrase B Co-complex With luteolin-7-O-glucoside. The protein wizard was used to refine the protein structure, then the binding site detector is used to find the binding pockets in the protein. The Receptor grid was used to create grids for docking. 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.

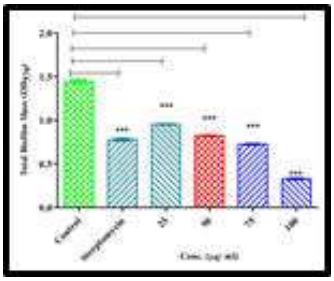

Fig 4. Biofilm inhibition using crystal violet

Fig 5. Electrostatic potential Map on ligand and protein at the binding site

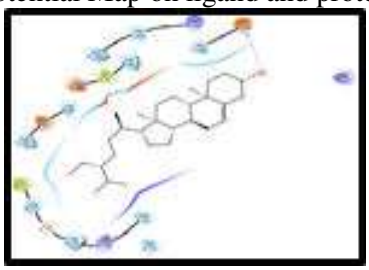

Fig 6. 2D Interaction Diagram Dopamine with protein 3g7b

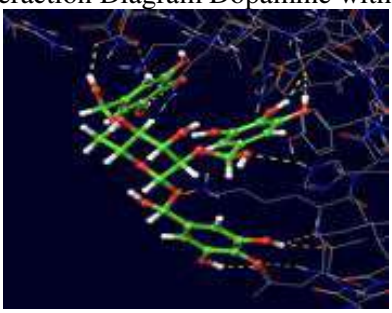

Fig 7. Electrostatic potential Map on ligand and protein at the binding site

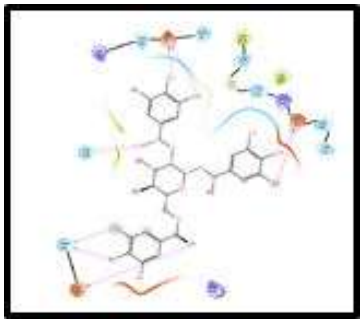

Fig 8. 2D Interaction Diagram of Dopamine with protein 3g7b

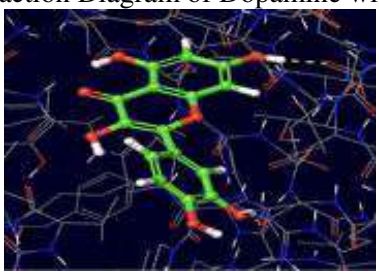

Fig 9. Electrostatic potential Map on ligand and protein at the binding site

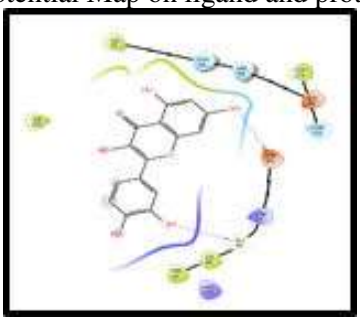

Fig 10. 2D Interaction Diagram of Dopamine with protein 3g7b

DISCUSSION:

Biofilms, which are complex communities of microorganisms encased in a matrix of extracellular polymeric substances (EPS), pose significant challenges in various fields, including medicine and industry. In the context of healthcare, biofilms formed by pathogenic bacteria like Staphylococcus mutants are implicated in a range of chronic and recurrent infections, such as those associated with medical implants and catheters(10).Their ability to resist conventional antibiotics makes biofilm-related infections difficult to treat.Actinomycetes are a group of bacteria known for their prolific production of secondary metabolites with antimicrobial properties. Over the years, these microorganisms have been a valuable source of antibiotics and bioactive compounds.This study's focus on actinomycetes as potential biofilm disruptors is well-grounded in their history of yielding compounds with antibacterial activity.(11)(12)

The study's primary objective was to evaluate the ability of actinomycetes extracts to inhibit biofilm formation by Staphylococcus mutants(13). Biofilm formation is a complex process involving attachment, growth, and maturation phases. The researchers observed that the actinomycetes extracts interfered with multiple stages of biofilm development(14). This is a promising finding, as disrupting the early stages of biofilm formation is crucial for preventing biofilm-related infections.the study explored the growth inhibitory activity of these extracts on Staphylococcus mutants. Bacterial growth is a fundamental aspect of biofilm formation, and inhibiting bacterial proliferation can indirectly hinder biofilm development. The results indicated that the actinomycetes extracts

exerted growth-inhibitory effects on Staphylococcus mutants, further supporting their potential as antibacterial agents.(15)

FUTURE SCOPE:

Understanding the precise mechanisms through which these extracts disrupt biofilm formation and bacterial growth is crucial. Additionally, exploring the safety and efficacy of these extracts in more complex biological systems, including animal models, is a logical next step. Such research could pave the way for the development of novel antimicrobial therapies and strategies to combat biofilm-related problems in diverse settings.

CONCLUSION:

In a single paragraph, the study on biofilm inhibition and the growth inhibitory activity of actinomycetes extracts against Staphylococcus mutants yields promising insights into potential strategies for combating biofilm-related infections and addressing biofilm-associated issues. The research underscores the multifaceted impact of actinomycetes extracts, which interfere with various stages of biofilm formation and exhibit inhibitory effects on the growth of Staphylococcus mutans. This discovery suggests the potential application of these extracts in the development of novel antimicrobial therapies, particularly in the challenging realm of healthcare, where biofilm-related infections pose significant hurdles. However, further investigations are imperative to unravel the precise mechanisms underlying these effects and to evaluate the safety and efficacy of actinomycetes extracts in more complex biological contexts. These endeavors hold promise for advancing our arsenal against chronic infections and biofilm-related challenges across diverse domains.

AUTHOR CONTRIBUTION:

Mr. A.S.Arjun: Literature search, data collection, manuscript writing.

Mrs.S.Sangeetha : Study design, data verification, manuscript correcting.

Dr. K.Meenakshisundaram : Research expert

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CONFLICT OF INTEREST:

None to declare.

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