ISSN: 2633-4828 Vol. 5 No.4, December, 2023

International Journal of Applied Engineering & Technology

ANTIMICROBIAL ACTIVITY OF ACTINOMYCETES EXTRACT AGAINST STAPHYLOCOCCUS AUREUS AND ITS SAFETY EVALUATION ON VERO CELLS

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ABSTRACT

Staphylococcus aureus causes human infections like skin issues and pneumonia. Actinomycetes are valuable for antibiotic discovery due to their antimicrobial activity. They produce compounds hindering bacteria, fungi, and other pathogens, with potential for new antimicrobials (1). Research employs biofilm assessment, crystal violet staining, docking studies, and agar well plate. Objective: Investigate actinomycetes extract's antimicrobial effect on Staphylococcus aureus, also testing safety on Vero cells, a mammalian line for cytotoxicity.

INTRODUCTION:

It has long been known that S. aureus is one of the most significant bacteria that endanger human health. It is the main contributor to skin and soft tissue infections like cellulitis, furuncles, and abscesses (boils). S. aureus is linked to a number of infectious oral pathologies like parotitis, angular cheilitis, mucositis, periodontitis, and infections linked to dental implants. Additionally, 0.7 to 15 percent of dental abscesses contain aureus bacteria (2). A pocket of pus that forms around a tooth as a result of a bacterial infection is known as a dental abscess. Pain, redness, and swelling around the affected tooth are possible symptoms.

Chronic diseases are primarily brought on by microbial biofilms that form on biomaterials. Gram-positive bacteria Staphylococcus aureus and Streptococcus mutans are among them and are significant pathogens that cause infections linked to dental caries (tooth decay) and other medical implants (3).

One of the significant resistant microorganisms that is frequently isolated from repeated root canal procedures is S. aureus. When the root canal is left open during root canal therapy, it significantly contributes to the etiology of primary endodontic infections and persistent infections during break sessions (4).

The antimicrobial activity of actinomycetes extracts is a subject of intense research due to their potential in combating various pathogens. Actinomycetes, a group of filamentous bacteria, are known to produce a diverse array of secondary metabolites with antimicrobial properties. These bioactive compounds exhibit the ability to inhibit the growth of bacteria, fungi, and other microorganisms.

Researchers extract these bioactive compounds from actinomycetes and subject them to testing against different target pathogens, including bacteria like Staphylococcus aureus. The effectiveness of the extract is often evaluated through techniques such as agar well plate assays, where the extract is placed in wells on an agar plate containing the target bacteria. Inhibition zones, where bacterial growth is inhibited, provide a measure of the extract's antimicrobial potency https://www.google.com/url?q=https://www.jocmr.com/uploads/paper/15ee9803e9e6d3642138c98fe986ebbb.pdf&sa=D&source=editors&ust=1705478694461483&usg=AOvVaw19dCNMKvGfIU5XUqc8wJkK

The antimicrobial activity of actinomycetes extracts is significant not only in terms of inhibiting pathogenic growth but also in the context of discovering new antibiotics. With the rise of antibiotic-resistant bacteria, actinomycetes-derived compounds offer a promising avenue for developing novel antimicrobial agents that can address the challenges posed by drug-resistant infections. As researchers continue to study and harness the antimicrobial potential of actinomycetes extracts, they contribute to the ongoing quest for effective solutions to combat infectious diseases.

MATERIALS AND METHODOLOGIES:

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 only.

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: MATERIALS & METHODS:

The structure of penta hydroxynaphthalene, kaempferol, and gallic acid 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: 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.

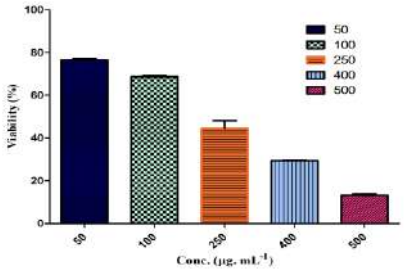
G Score = a*vdW + b*Coul + Lipo+ Hbond +Metal + BuryP + RotB + Site (1) where Van der Waals energy is represented by vdW, with coefficients a=0.065 and b=0.130. Coul is the symbol for Coulomb energy. Lipo is a symbol of lipophilic interaction.

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 via sterile syringe into 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.

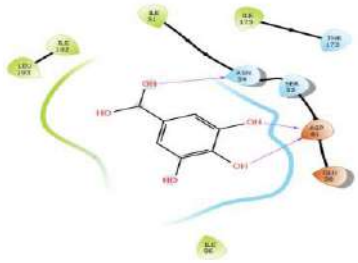
Isolation of Actinomycetes from Beach Soil

Growth inhibition of actinomycetes against staphylococcus aureus

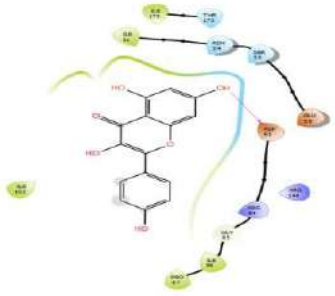
RESULTS:



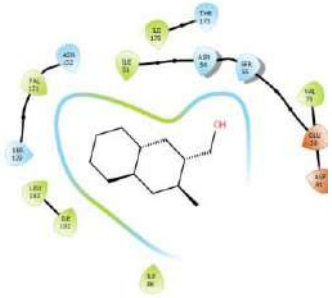
Toxicity of actinomycetes on vero cells



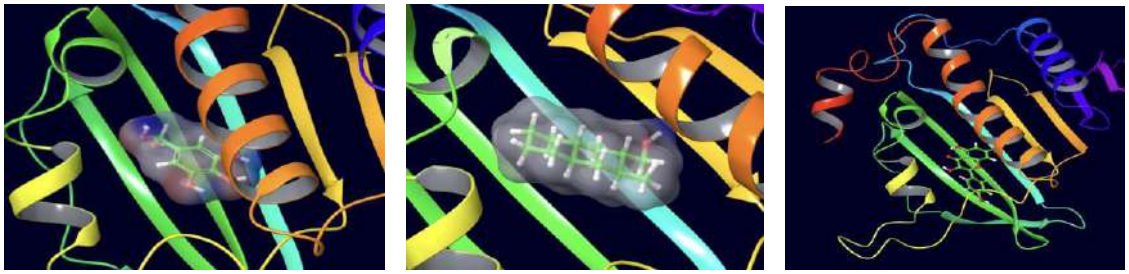
2D interaction of gallic acid with protein 3g7b



2D interaction of kaempferol with protein 3g7b



2D interaction of penta hydroxynaphthalene with protein 3g7b



Electrostatic potential map on ligand and protein at the binding site

DISCUSSION:

The research findings presented herein underscore the remarkable potential of the Actinomycetes extract as an effective antimicrobial agent against Staphylococcus aureus infections. The results reveal a substantial level of antimicrobial activity exhibited by the extract, which highlights its promise for therapeutic interventions against this notorious pathogen.

The observed significant antimicrobial activity against Staphylococcus aureus provides a compelling basis for further investigating the extract's mode of action and its potential mechanisms of inhibiting bacterial growth. The ability of the extract to hinder the proliferation of a well-established human pathogen like Staphylococcus aureus holds substantial clinical implications. It implies that the extract's bioactive compounds might possess unique properties that could target specific bacterial pathways, making it a potential candidate for drug development against S. aureus-associated infections.

Moreover, the safety evaluation conducted on Vero cells, a standard mammalian cell line utilized in cytotoxicity studies, offers a critical perspective on the extract's potential clinical viability. The non-toxic nature of the Actinomycetes extract on these cells is an encouraging finding. This suggests that the extract's antimicrobial properties are selective against bacterial pathogens without exerting harmful effects on mammalian cells. Such selectivity is a crucial factor in designing effective and safe antimicrobial agents for clinical use.

The collective implications of these findings are noteworthy. The Actinomycetes extract demonstrates a dual advantage: potent antimicrobial activity against Staphylococcus aureus, coupled with a lack of cytotoxicity on Vero cells. This combination positions the extract as a promising candidate for further exploration in pre-clinical and clinical studies. Future research avenues could delve into optimizing the extraction process to isolate and identify the specific bioactive compounds responsible for the observed antimicrobial effects. Understanding the extract's mechanism of action could aid in designing targeted interventions against S. aureus infections.

In conclusion, this study's outcomes offer an exciting glimpse into the potential of the Actinomycetes extract as a novel antimicrobial agent against Staphylococcus aureus infections. The extract's potent antimicrobial activity and its non-toxic profile on mammalian cells underscore its significance in the ongoing battle against antibiotic-resistant pathogens. By warranting further investigation, this research paves the way for potential clinical applications of the Actinomycetes extract as a valuable addition to the arsenal of antimicrobial therapies aimed at tackling Staphylococcus aureus-related health concerns.

RECOMMENDATION / SCOPE OF FUTURE RESEARCH:

Based on the findings of the study regarding the antimicrobial activity of the Actinomycetes extract against Staphylococcus aureus and its safety evaluation on Vero cells, further research could focus on isolating and identifying the specific bioactive compounds responsible for the activity. Additionally, in vivo studies could be conducted to assess the efficacy and potential therapeutic applications of the extract.

CONCLUSION:

In conclusion, the extract derived from Actinomycetes demonstrated significant antimicrobial activity against Staphylococcus aureus. Furthermore, the safety evaluation conducted on Vero cells indicated its non-toxic nature. These findings suggest the potential of Actinomycetes extract as a promising antimicrobial agent warranting further research for clinical applications against S. aureus infections.

AUTHOR CONTRIBUTIONS:

Naaziya.M: Literature search, data collection, manuscript writing.

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

Dr.Meenakshi Sundaram : Research expert

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

CONFLICT OF INTEREST:
None to declare.

REFERENCES:
1. Website [Internet]. Available from: https://www.scopus.com/record/display.uri?eid=2-s2.0-85092912484&origin=inward&txGid=fa724947bec5f9aca0838c5790627202
2. Girija SA, Jayaseelan VP, Arumugam P. Prevalence of VIM- and GIM-producing Acinetobacter baumannii from patients with severe urinary tract infection. Acta Microbiol Immunol Hung. 2018 Dec 1;65(4):539–50.
3. Kamath AK, Nasim I, Muralidharan NP, Kothuri RN. Anti-microbial efficacy of leaf extract against common oral micro-biomes: A comparative study of two different antibiotic sensitivity tests. J Oral Maxillofac Pathol. 2022 Oct 17;26(3):330–4.
4. Rifaath M, Santhakumar P, Selvaraj J. Effect of on beta catenin and Wnt mRNA expression in human colon cancer (HT-29) cells in vitro. Bioinformation. 2022 Mar 31;18(3):289–92.