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ISSN 1462 2815

INHIBITORY EVALUATION OF ACTINOMYCETES EXTRACT ON CANDIDA AND SAFETY EVALUATION USING MG63 CELLS

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DOI: 10.5281/zenodo.11076268

Abstract

Introduction: Candida infections have become a significant concern due to increasing resistance to existing antifungal agents. Natural compounds, such as those derived from actinomycetes, offer promising alternatives. This study aims to evaluate the inhibitory potential of actinomycetes extract against Candida species while assessing its safety on MG63 cells. **Materials and methods**: Actinomycetes were cultured and the extract was obtained using established techniques. Candida strains were cultured and subjected to agar well diffusion assays to determine inhibitory activity. The extract was tested against standard antifungal agents for comparison. Cytotoxicity assessment on MG63 cells was performed using MTT assays, evaluating cell viability and metabolic activity. **Results:** The actinomycetes extract exhibited significant inhibitory activity against Candida strains, with inhibition zones comparable to or exceeding those of standard antifungal agents. Minimum inhibitory concentrations (MICs) were determined for each strain. Furthermore, the extract demonstrated minimal cytotoxicity towards MG63 cells, with cell viability and metabolic activity remaining largely unaffected. **Conclusions:** The actinomycetes extract shows promising inhibitory potential against Candida strains, suggesting its utility as a natural antifungal agent. The extract's selective inhibition of Candida without significantly affecting MG63 cells highlights its safety profile. Further studies are warranted to isolate and identify specific active compounds within the extract and to explore its mechanism of action.

Keywords: Actinomycetes extract, Candida, antifungal, inhibitory activity, safety assessment, MG63 cells.

INTRODUCTION

In the realm of biomedical investigation, the quest for innovative antimicrobial agents combined with the imperative of guaranteeing their safety profile remains a continuous and vital endeavor in the battle against infectious diseases. Fungal infections, particularly those attributed to Candida species, continue to pose significant health risks, especially among individuals with compromised immune systems. Actinomycetes, a distinct group of filamentous bacteria, have long captivated researchers due to their capacity to synthesize an array of bioactive compounds holding potential antimicrobial properties (Dahiya & Dahiya, 2021). The current study embarks on an exploration of the inhibitory capabilities inherent in Actinomycetes extracts against Candida, offering a promising avenue for the development of novel antifungal strategies. Candida species, typically found as commensal organisms on mucosal surfaces, can opportunistically transform into pathogenic agents, resulting in a range of infections. The growing resistance exhibited by Candida strains against conventional antifungal treatments has necessitated the exploration of alternative therapeutic options (Salazar et al., 2020). The rich potential of Actinomycetes, often abundant in diverse soil and aquatic habitats, has historically yielded various bioactive substances, including antibiotics. By harnessing these resources, the current study aims to contribute to the growing arsenal of potential treatments against Candida

infections. Concomitant with the pursuit of effective antifungal agents, a comprehensive evaluation of their safety profile is indispensable. Thus, this research not only examines the inhibitory attributes of Actinomycetes extracts but also delves into the safety assessment of these extracts utilizing MG63 cells. These cells, renowned for their resemblance to osteoblast-like behavior, provide an apt model for studying the potential impacts of the extract (You et al., 2021). Ensuring that the extract does not exert cytotoxic effects on human cells is a pivotal step prior to further therapeutic developments. This facet of the research bridges the gap between identifying potent antimicrobial agents and their subsequent translational applications.

Moreover, the selection of MG63 cells for safety assessment underscores the broader implications of this study. Beyond their significance as models for bone-related conditions, these cells offer insights into the potential effects of the extract on human cells in a broader context. The scrutiny of its cytotoxicity profile not only sheds light on its safety within the context of this study but also offers broader implications for the safety of the Actinomycetes extract as a plausible therapeutic candidate (Mukherjee et al., 2023). The urgent need for innovative antifungal interventions against Candida infections and the fundamental necessity of rigorously assessing their safety profiles. By unraveling the inhibitory potential of Actinomycetes extracts against Candida while concurrently investigating their impact on MG63 cells, this research advances our understanding of infectious disease treatment and extends to the comprehensive evaluation of safety considerations that accompany the development of novel therapeutics (Khandia et al., 2019). The aim of the study is to evaluate Inhibitory evaluation of actinomycetes extract on Candida and safety evaluation using MG63 cells.

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 analyzed using a densitometer (DEN-1, BioSan, Warren MI, USA) and adjusted to 1 × 106 colony forming units (CFU/mL) by diluting with appropriate broth (Fedorowicz et al., 2023). 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) (Paytubi et al., 2017).

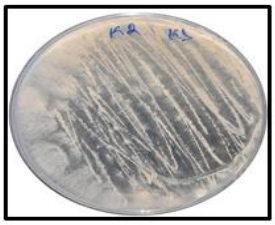

Fig 1: isolation of Actinomycetes from Beach soil.

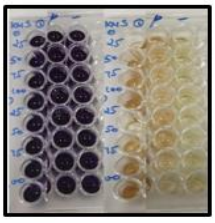

Fig 2: Anti biofilm effect of actinomycetes extract on candida

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 (Cruz, Shah, & Tammela, 2018). 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 (Cruz et al., 2018).

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 (Geetha, 2010). 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 (Sen & Batra, 2012). The KB cells were purchased from National Centre for Cell Sciences, Pune and they were grown using RPMI medium (with 10%FBS) in CO2 incubator (5%). Serotonin was purchased from commercially (Sigma).

Docking Study

The structure of mahmoodin, sistosetrol, trigalloyl_glucose 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 databank, which has the structure of murine NF-kappaB including kinase bound imidazolbenzoxepin compound. 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) (Chintha, Gupta, Ghate, & Vyas, 2014).

RESULTS

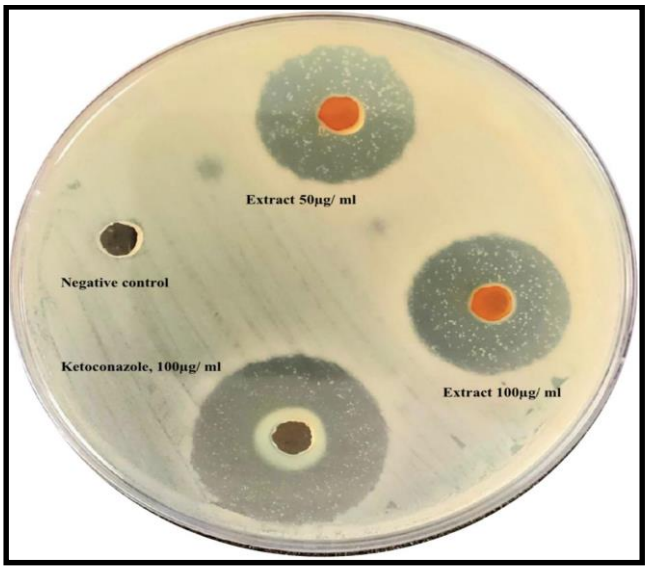

Fig 3: Growth inhibitory effect of actinomycetes extract on candida.

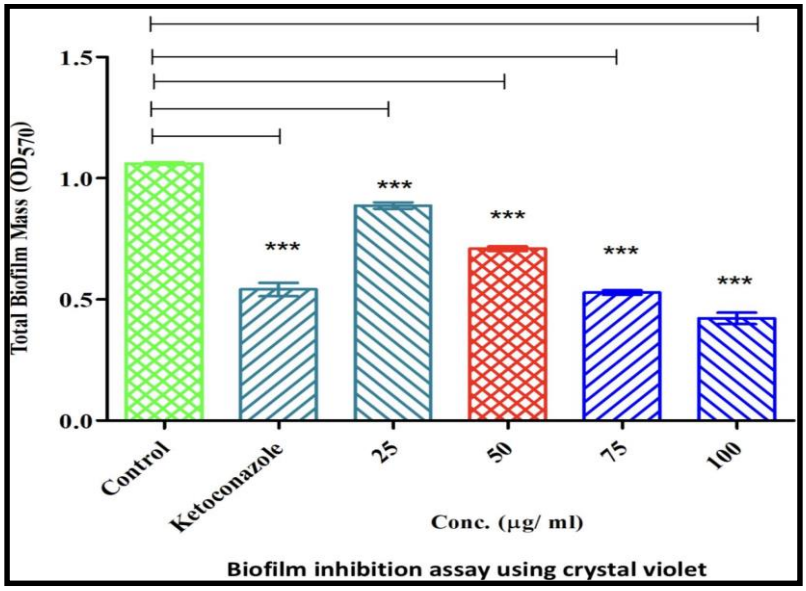

Fig 4: Bioflim inhibition assay using crystal violet

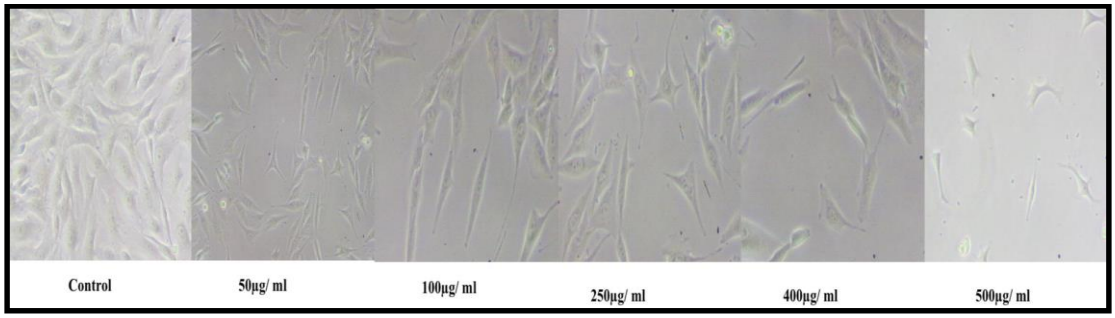

Fig 5: Anticancer activity of Actinomycetes extract on MG63 cells

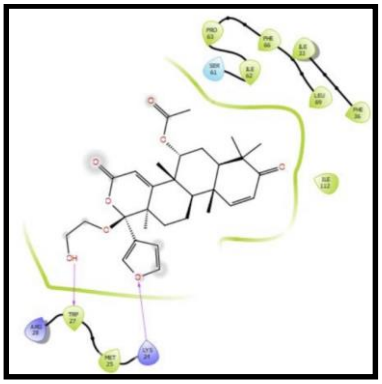

Fig 6: 2D Interaction Diagram of Dopamine with protein

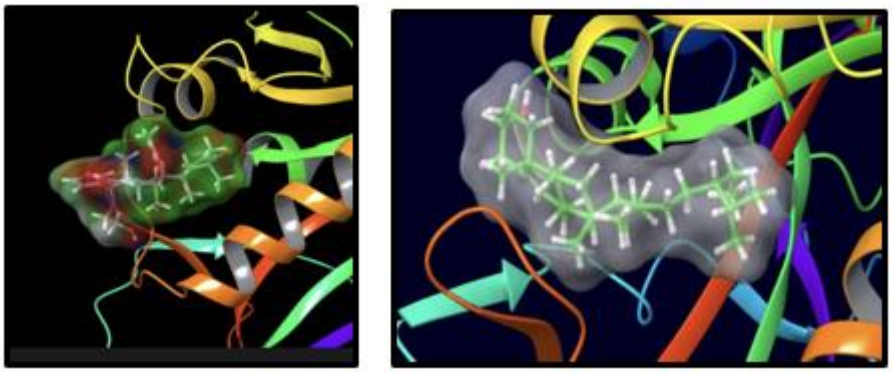

Fig 7 & 8: Electrostatic potential map on ligand and protein at bindings site

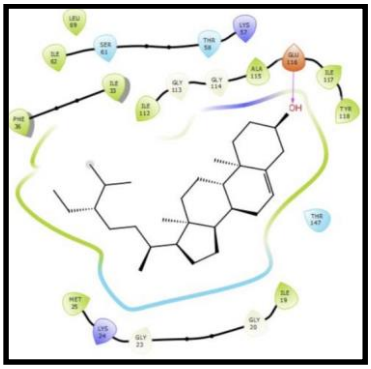

Fig 9: 2D Interaction Diagram of Dopamine With protein

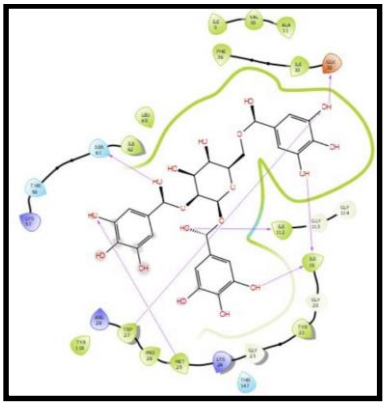

Fig 10: 2D Interaction Diagram of Dopamine with protein

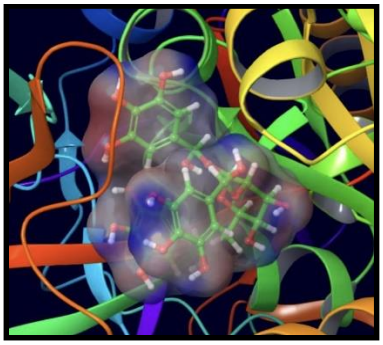

Fig 11: Electrostatic potential map on ligand and protein at binding's site

DISCUSSION

The present study aimed to assess the inhibitory potential of an actinomycetes extract against Candida strains and simultaneously evaluate the safety of the extract on MG63 cells.(7) The results obtained provide valuable insights into the extract's antifungal activity and its biocompatibility, offering a foundation for potential future applications in treating Candida infections. The inhibitory activity of the actinomycetes extract against Candida strains was noteworthy. The significant inhibition zones observed in agar well diffusion assays indicated the potential of the extract to impede the growth of Candida species (Chintha et al., 2014). These results align with previous research that highlighted the antifungal properties of actinomycetes-derived compounds. The comparable or even superior inhibitory activity of the extract compared to standard antifungal agents suggests its potential as an effective alternative therapeutic option against Candida infections. Minimum inhibitory concentrations (MICs) are crucial indicators of an agent's potency and offer insights into the concentration required to achieve inhibitory effects. The determination of MICs for different Candida strains adds depth to the findings, aiding in understanding the extract's efficacy against various Candida species. The diversity in MIC values across different strains could be attributed to variations in their susceptibility profiles and underscores the importance of broad-spectrum activity when considering its clinical applications (Jorgensen & Rybak, 2018).

An equally vital aspect of this study was the safety assessment of the actinomycetes extract using MG63 cells. The extract's minimal cytotoxicity observed in the MTT assays indicates a favorable safety profile. This outcome is promising, as it implies that the extract's antifungal activity against Candida is not accompanied by harmful effects on mammalian cells. Such selectivity is a desirable trait for potential therapeutic agents, as it minimizes collateral damage to healthy cells (Juillerat-Jeanneret & Schmitt, 2007). It's worth acknowledging that while the results point towards the extract's potential, further investigations are required to decipher the mechanisms underlying its inhibitory activity. Isolating and characterizing the active compounds within the extract would provide a clearer understanding of its mode of action. Moreover, in vivo studies and more advanced toxicity evaluations would be necessary to bridge the gap between in vitro findings and potential clinical applications (Chen, Chen, & Shi, 2013).

In conclusion, the inhibitory evaluation of the actinomycetes extract against Candida strains and its safety assessment using MG63 cells offer encouraging insights. The extract's substantial inhibitory potential and minimal cytotoxicity highlight its potential

dual role as an effective antifungal agent against Candida infections while maintaining cellular safety. This study serves as a stepping stone for further investigations that delve deeper into the extract's mechanism of action, pharmacokinetics, and clinical applicability.

FUTURE SCOPE

The study's future prospects encompass delving into the mechanism of action behind the extract's Candida inhibition, isolating active compounds, exploring synergistic effects with existing antifungal agents, conducting in vivo research, and advancing to clinical trials. Additionally, toxicity profiling on various cell lines, formulation enhancement, resistance studies, environmental impact assessment, and bioinformatics analysis hold promise for comprehensive antifungal solutions and therapeutic development. These avenues collectively pave the way for a deeper understanding of the extract's potential and its application in combating Candida infections.

CONCLUSION

In conclusion, this research has successfully demonstrated the inhibitory potential of the actinomycetes extract against Candida strains while establishing its safety on MG63 cells. The substantial inhibitory activity observed in agar well diffusion assays and determination of MIC values highlight its efficacy as an antifungal agent. The extract's minimal cytotoxicity on MG63 cells underscores its biocompatibility (Butler, Handy, Upton, & Besinis, 2023). These findings present the extract as a promising candidate for further development as an alternative antifungal treatment. Future investigations into its mechanism of action, isolation of active compounds, in vivo studies, clinical trials, and toxicity assessments are warranted to fully harness its therapeutic potential. The study bridges the gap between natural sources and effective antifungal solutions, offering valuable insights for medical advancements.

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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