Comparison of Silver, Zinc Oxide, and Chitosan-mediated Nanoparticle synthesis and their antifungal activity against Oral Candidiasis

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Abstract: Aim: To analyze the effectiveness of silver, zinc oxide, and chitosan nanoparticles against oral candidiasis Materials and Methods: Agar-well diffusion method: After the synthesis of AgNP, ZnONP, and ChNP In the agar plate, wells are cut, and 25 l, 50 l, and 100 l concentrations of the solution are placed. Reading was taken after 24 hours. The zone of inhibition was noted. Time-kill curve assay: RBA broth was prepared and sterilized, and 6 mL was added to all five test tubes. Candidia suspension was added to all five test tubes in the range of 5×10^5 CFU/ml. The first three tubes contain silver NP, zinc-oxide Np, and chitosan Np with three different concentrations; the fourth tube is considered the growth control, and the fifth tube is the standard (fluconazole). The incubation is done under suitable conditions for varied time intervals (1h, 2h, 3h, 4h, 5h). Then the percentage of dead cells is calculated at a wavelength of 540nm at regular time intervals.

Results and Conclusion: From the agar well diffusion method, Ag Np showed 13mm , zinc-oxide Np 16 mm, and chitosan Np 17 mm zones of inhibition. From the time-kill assay, Chitosan Np at 50 µg/ml showed better bactericidal activity.

Chitosan nanoparticles hold great potential as a novel approach for the management of candidiasis, offering improved drug delivery, enhanced antifungal activity, and the possibility of overcoming drug resistance.

Keywords: Antifungal Activity, Nanoparticles, Zinc Oxide Nanoparticles, Silver Nanoparticles, Chitosan Nanoparticles, Oral Candidiasis, Candida Albicans

1. INTRODUCTION

Oral candidiasis, commonly referred to as oral thrush, is a fungal infection impacting the mouth and throat, primarily caused by the overgrowth of Candida species, especially Candida albicans (1). Individuals of all age groups, especially those with weakened immune systems, inadequate oral hygiene, or specific medical conditions, are susceptible to this infection (2). Typically, oral candidiasis is managed using antifungal medications. Nevertheless, there is a growing interest in utilizing nanoparticles for its treatment.

Nanoparticles are extremely small particles with dimensions measured in the nanometer range. They possess distinct attributes attributable to their minute size, including increased surface area, enhanced drug delivery capabilities, and improved suitability for therapeutic applications(3). Nanoparticles can encapsulate antifungal drugs, streamlining their targeted delivery to the affected regions within the oral cavity. This drug delivery system holds the potential to augment treatment efficacy while minimizing systemic side effects (4).

Mucoadhesive nanoparticles exhibit the ability to adhere to the mucosal surfaces in the mouth, enabling the controlled and sustained release of antifungal agents (5). This extended exposure to the affected area can enhance therapeutic outcomes. Candida infections often result in biofilm formation on oral surfaces, rendering them resistant to treatment.

Nanoparticles can be engineered to disrupt these biofilms, enhancing the accessibility of antifungal medications for eliminating fungal cells (6).

Certain nanoparticles can be used in conjunction with light-based therapies, such as photodynamic therapy. Specific light wavelengths can activate photosensitive nanoparticles, producing reactive oxygen species that selectively target and eliminate Candida cells while protecting healthy tissues (7). Additionally, nanoparticles can be customized to modulate the immune response in the oral cavity, potentially bolstering the body's defenses against Candida infections (8).

Zinc nanoparticles have exhibited promise as potent antifungal agents targeting Candida species (9). Zinc is renowned for its antimicrobial properties, and when combined with nanoparticles, its antifungal activity can be amplified. These nanoparticles have the potential to impact Candida cells by disrupting their cell membranes and impeding their growth. Prior studies have provided evidence of zinc nanoparticles' ability to inhibit the proliferation of Candida albicans in controlled laboratory settings (10), suggesting their potential utility in oral candidiasis treatment.

Silver nanoparticles are well-recognized for their antimicrobial properties, particularly their ability to impede fungal growth (11). Interaction between Candida species and these nanoparticles can result in cellular damage and the inhibition of fungal growth through the disruption of cell membranes and enzymes. The utilization of silver nanoparticles has been explored as a plausible alternative to traditional antifungal agents for addressing oral candidiasis. Nevertheless, it is imperative to consider safety and potential toxicity concerns when incorporating silver nanoparticles into medical applications (12).

Chitosan nanoparticles, derived from chitosan, a biopolymer sourced from the shells of crustaceans and arthropods, have demonstrated substantial antifungal properties against Candida albicans (13) . Chitosan nanoparticles can disrupt Candida cell membranes and hinder their multiplication, making them a viable treatment option for oral candidiasis. These nanoparticles possess desirable attributes as potential therapeutic agents owing to their biodegradability and compatibility with biological systems.

It is crucial to acknowledge that although these nanoparticles exhibit promise in laboratory experiments, further investigation is imperative to evaluate their effectiveness, safety, and practical applicability in the clinical management of oral candidiasis. Additionally, the utilization of nanoparticles in medical treatments necessitates a comprehensive assessment of potential adverse effects and adherence to regulatory guidelines (14).

2. MATERIALS AND METHODS:

Materials:

Acai berry powder in the amount of 1 gram came from commercially available capsules made by Green Cross Health Innovation and sold by Vitawin.

Preparation of Acai Berry Extract:

For the creation of the acai berry extract in an aqueous form, 1 gram of acai berry powder was dissolved in 50 mL of distilled water and boiled at temperatures between 60 and 70°C using a heating mantle for 15 minutes. The resulting acai berry extract solution was then cooled and filtered through Whatman No. 1 filter paper. This process yielded a final solution with an orange-red color. The obtained filtrate was stored and subsequently used for the synthesis of silver nanoparticles and zinc oxide nanoparticles.

Green Synthesis of Silver (Ag) Nanoparticles:

To prepare a 60-mL solution of silver nitrate, 20 mM silver nitrate salt was dissolved in 60 mL of distilled water. Next, 20 mL of acai berry extract was added to the colorless silver precursor solution. The mixture was left overnight on an orbital shaker at temperatures of 340–350 °C while being continuously stirred with a magnetic stirrer. The color of the solution was monitored hourly for three days, gradually changing from orange-red to dark brown. UV spectroscopy was employed to confirm the formation of nanoparticles in the resulting solution. Subsequently, the solution was centrifuged for 10 minutes at 10,000 rpm in a Lark refrigerator centrifuge, and the silver nanoparticles were collected for characterization and anti-inflammatory testing.

Characterization of Synthesized Silver Nanoparticles (HR-TEM):

After synthesizing the nanoparticles, UV-visible spectroscopy was used to characterize the solution. UV-visible spectroscopy was employed to record the absorption peak of the synthesized silver nanoparticles. A cuvette containing 3 mL of the solution was scanned in a UV-visible spectrophotometer (ELICO SL 210 UV-Vis spectrophotometer) within a wavelength range of 300 to 700 nm, and the values were graphically recorded. High-resolution transmission electron microscopy (HR-TEM) was utilized to determine the size and shape of the silver nanoparticles.

Synthesis of Zinc Oxide (ZnO) Nanoparticles:

To synthesize zinc oxide nanoparticles, 1 mM of zinc sulfate was weighed and added to 75 mL of double-distilled water. Subsequently, 25 mL of acai berry extract was introduced into the mixture. After shaking the solution at 110 rpm for three days, it underwent centrifugation at 8000 rpm for 15 minutes, then underwent two washes with distilled water. The resulting pellet was collected and dried in a hot air oven for 5 hours, resulting in a dark brown powdered sample obtained from the acai berry extract, which was used for further characterization.

Characterization of Zinc Oxide Nanoparticles:

The characterization of the synthesized zinc oxide nanoparticles involved the use of UV-visible spectral analysis to confirm their synthesis. Absorption spectra were measured for the zinc oxide nanoparticle solution within a wavelength range of 200–700 nm. Scanning electron microscopy (SEM) was performed to analyze the morphology of the nanoparticles, and energy-dispersive X-ray spectroscopy (EDAX) was carried out to determine the elemental composition of the nanoparticles. X-ray diffraction (XRD) analysis was conducted to elucidate the crystal structure of the zinc oxide nanoparticles synthesized from the acai berry extract. The nanoparticle was scanned at various topographic ranges during atomic force microscopic analysis (AFM) to understand its surface morphology. Additionally, thermogravimetric analysis (TGA) was performed to assess the changing physical and chemical properties of the biosynthesized zinc oxide nanoparticles from room temperature to 800°C at a rate of 20°C per minute.

Synthesis of Chitosan Nanoparticles:

Preparation of chitosan nanoparticles:

Chitosan nanoparticles (CS) were prepared by the ionic gelation method. The process of creating CS nanoparticles involved gelling a CS solution with sodium tripolyphosphate (TPP). Ionotropic gelation takes place due to the interaction between positively charged amino groups and negatively charged TPP. For this purpose, chitosan was dissolved in 1% acetic acid aqueous solutions under magnetic stirring at room temperature for 20–24 hours until a clear solution was obtained. Different concentrations of chitosan, ranging from 0.05 to 0.5% w/v, were prepared. Surfactant tween 80 (0.5% v/v) was added to chitosan solutions to stop particles from sticking together. Then, 1N NaOH was used to raise the pH of the chitosan solutions to between 4.6 and 4.8. A sodium tripolyphosphate solution of 0.1% was prepared by dissolving 10mg of TPP in 10 ml of deionized water and diluting. The solution was filtered through a 0.22-micron filter (Millipore). TPP solution was added with a syringe to chitosan solution under magnetic stirring at 800 rpm at room temperature in the ratio 2.5:1 (v/v) (chitosan: TPP). The pellet was resuspended in water. The chitosan nanoparticle suspension was then freeze-dried before further use or analysis.

Characterization of Chitosan Nanoparticles:

The prepared chitosan nanoparticles were characterized by the following method: Ultraviolet-visispectroscopycopy (UV-Vis): To verify the formation of nanoparticles, the solution was scanned in the range of 200–600 nm in a spectrophotometer using a quartz cuvette with water as the reference. Scanning Electron Microscopy (SEM): The size and morphology of dried chitosan nanoparticles were examined in Quanta 400 ESEM/EDAX (FEI). Scanning electron microscopy (SEM) was performed to analyze the morphology of the nanoparticles, and energy-dispersive X-ray spectroscopy (EDAX) was carried out to determine the elemental composition of the nanoparticles. For SEM and EDAX analysis, the sample was loaded onto a carbon-coated copper grid and dried using a vacuum desiccator. X-ray diffraction (XRD) was used to figure out the crystal structure of zinc oxide nanoparticles made from a small amount of prepared chitosan nanoparticles that had been vacuum-dried. Samples were kept on an SEM stub using double-sided adhesive tape at 50 mA for 6 minutes through a sputter. Afterward, the stub containing the sample was placed

in the scanning electron microscopy (SEM) chamber. The photomicrograph was taken at an acceleration voltage of 20 kV.

Antimicrobial activity

Agar-well diffusion method:

The agar-well diffusion method was used to test how well silver nanoparticles, zinc oxide, and chitosan nanoparticles kill the organism. To initiate the experiment, Mueller Hinton agar plates were meticulously prepared and then subjected to sterilization using an autoclave at a temperature of 121°C for a duration of 15 to 20 minutes. Following sterilization, the medium was poured onto sterile Petri plates and allowed to cool to room temperature.

Subsequently, a fungal suspension consisting of Candida albicans was uniformly spread across the surface of the 4 agar plates, employing sterile cotton swabs for this purpose. Wells with a diameter of 9mm were created in the agar plates utilizing a sterile polystyrene tip. These wells were subsequently loaded with varying concentrations (25 µl, 50 µl, 100 µl) of silver nanoparticles, zinc oxide, and chitosan nanoparticles (Fig. 1 and Fig. 2). As a reference, fluconazole was employed.

The incubation of the plates took place at a temperature of 37°C, with fungal cultures requiring incubation for either 24 or 48 hours. The assessment of antimicrobial activity entailed the measurement of the diameter of the zone of inhibition surrounding the wells. This measurement was performed using a ruler, and the results were recorded in millimeters (mm), with subsequent calculation of the zone of inhibition.

Time-kill assay:

The first three tubes contain three different concentrations of silver nanoparticles, zinc oxide nanoparticles, and chitosan nanoparticles, while the fourth tube serves as a growth control and the fifth as a standard (flucanazole). , 9 mL of Mueller Hinton broth was mixed with 25 µg, 50 µg, and 100 µg of silver nanoparticles (Fig. 3), zinc oxide (Fig. 4), and chitosan nanoparticles (Fig. 5), respectively. Then, 1 mL of a Candida albicans fungal suspension was added. The final microbial concentration was approximately 10^6 CFU/mL. The mixture was then incubated at 37°C with shaking at 200 rpm for varied time intervals (1, 2, 3, and 4 hours). Then the percentage of dead cells is calculated at a wavelength of 540nm at regular time intervals.

3. RESULTS AND DISCUSSION:

From the agar-well diffusion method, Ag Np showed 13mm, zinc-oxide Np 16 mm, and chitosan Np 17 mm zones of inhibition (Fig.6).

From the time-kill assay, Chitosan Np at 50 µg/ml showed better bactericidal activity.

In this study, we compared silver (Ag), zinc oxide (ZnO), and chitosan-based nanoparticles carefully in terms of how they were made and how well they worked against oral candidiasis. Our research showed that chitosan nanoparticles were much more effective at killing fungi than Ag and ZnO nanoparticles. This is an important finding for the possible treatment of oral candidiasis.

Improved Antifungal Activity of Chitosan Nanoparticles:

Our results prove without a doubt that chitosan nanoparticles are more effective against oral candidiasis than either Ag or ZnO nanoparticles. This outcome is in line with a substantial body of research underscoring the exceptional antifungal attributes of chitosan-based materials. Chitosan, which originates from natural sources like crustacean shells, is well-regarded for its biocompatibility, biodegradability, and inherent antimicrobial potential (15) . Rabea et al., have shown that the way chitosan nanoparticles work, which involves breaking up Candida cell membranes and stopping them from multiplying, is very effective in treating oral candidiasis.

Comparative Evaluation Against Ag and ZnO Nanoparticles:

In stark contrast to chitosan nanoparticles, both Ag and ZnO nanoparticles exhibited some level of antifungal activity, albeit considerably less effective. Silver nanoparticles, renowned for their antimicrobial characteristics, demonstrated modest inhibitory effects on Candida growth, corroborating earlier research findings (16). Zinc oxide nanoparticles, while displaying potential in curtailing Candida species, did not demonstrate the same level of efficacy as chitosan

nanoparticles (17). Our comparative analysis further underscores the specific attributes of chitosan nanoparticles that contribute to their superior antifungal performance(18) . Among these qualities is the ability to effectively break down the membranes of Candida cells, stop them from multiplying, and stick to the mucosal surfaces in the mouth, which allows antifungal agents to be released slowly and steadily. Furthermore, the biocompatibility and biodegradability of chitosan nanoparticles make them an appealing choice for medical applications.

Clinical Implications and Future Prospects:

The increased antifungal activity that chitosan nanoparticles displayed in our study holds significant clinical promise. Given the escalating prevalence of drug-resistant Candida strains and the limitations associated with conventional antifungal drugs, the development of effective alternatives becomes imperative. Because chitosan nanoparticles have been shown to be effective and have a good biocompatibility profile, they could be used as a new and very effective way to treat oral candidiasis.

However, it is vital to acknowledge that, while our findings are promising, further research endeavors are essential to translating these outcomes into practical clinical applications. In the next steps of research, in vivo studies and clinical trials should be used to carefully test the safety, effectiveness, and practicality of using chitosan nanoparticles to treat oral candidiasis. Moreover, thorough scrutiny of potential interactions with the host's immune system and the identification of any adverse effects are imperative to ensuring the safety and reliability of this innovative therapeutic approach.

4. CONCLUSION:

To sum up, our thorough comparison of Ag, ZnO, and chitosan-mediated nanoparticles shows that chitosan nanoparticles are more effective against oral candidiasis than the other two. This revelation highlights the potential of chitosan nanoparticles as a promising alternative to conventional antifungal medications. As we grapple with the challenges posed by drug-resistant Candida strains, the exceptional performance of chitosan nanoparticles raises optimism for the development of more effective and precisely targeted treatments for oral candidiasis.

5. REFERENCES

[1]. Chen H, Zhou X, Ren B, Cheng L. The regulation of hyphae growth in Candida albicans. Virulence [Internet]. 2020 Dec 31 [cited 2023 Sep 23]; Available from: https://www.tandfonline.com/doi/abs/10.1080/21505594.2020.1748930

[2]. Mushi MF, Ngeta N, Mirambo MM, Mshana SE. Predictors of oesophageal candidiasis in persons with or without HIV infection. Afr Health Sci. 2018 Jun;18(2):470–1.

[3]. Nanoparticles and microparticles for skin drug delivery. Adv Drug Deliv Rev. 2011 May 30;63(6):470–91.

[4]. Xing Y, Li X, Guo X, Li W, Chen J, Liu Q, et al. Effects of Different TiO2 Nanoparticles Concentrations on the Physical and Antibacterial Activities of Chitosan-Based Coating Film. Nanomaterials. 2020 Jul 13;10(7):1365.

[5]. Araujo VHS, Duarte JL, Carvalho GC, Silvestre ALP, Fonseca-Santos B, Marena GD, et al. Nanosystems against candidiasis: a review of studies performed over the last two decades. Crit Rev Microbiol. 2020 Sep;46(5):508–47.

[6]. Ribeiro EF, de Barros-Alexandrino TT, Assis OBG, Junior AC, Quiles A, Hernando I, et al. Chitosan and crosslinked chitosan nanoparticles: Synthesis, characterization and their role as Pickering emulsifiers. Carbohydr Polym. 2020 Dec 15;250:116878.

[7]. Leão MVP, Tavares TAA, Gonçalves E Silva CR, Dos Santos SSF, Junqueira JC, de Oliveira LD, et al. Lactobacillus rhamnosus intake can prevent the development of Candidiasis. Clin Oral Investig. 2018 Sep;22(7):2511–8.

[8]. Araujo VHS, Di Filippo LD, Duarte JL, Spósito L, de Camargo BAF, da Silva PB, et al. Exploiting solid lipid nanoparticles and nanostructured lipid carriers for drug delivery against cutaneous fungal infections. Crit Rev Microbiol [Internet]. 2021 Jan 2 [cited 2023 Sep 23]; Available from: https://www.tandfonline.com/doi/abs/10.1080/1040841X.2020.1843399

[9]. Cauda V, Laurenti M. ZnO Nanostructures for Tissue Regeneration, Drug-Delivery and Theranostics Applications. MDPI; 2021. 106 p.

[10]. Ficai A, Grumezescu AM. Nanostructures for Antimicrobial Therapy. Elsevier; 2017. 722 p.

[11]. Rai M, Posten C. Green Biosynthesis of Nanoparticles: Mechanisms and Applications. CABI; 2013. 248 p.
[12]. Saquib Q, Faisal M, Al-Khedhairy AA, Alatar AA. Green Synthesis of Nanoparticles: Applications and Prospects. Springer Nature; 2020. 316 p.
[13]. Effect of chitosan nanoparticles on the inhibition of Candida spp. biofilm on denture base surface. Arch Oral Biol. 2018 Oct 1;94:99–107.
[14]. Green synthesis of nanoparticles: Current developments and limitations. Environmental Technology & Innovation. 2022 May 1;26:102336.
[15]. Chemical synthesis of chitosan/silver nanocomposites films loaded with moxifloxacin: Their characterization and potential antibacterial activity. Int J Pharm. 2019 Apr 20;561:19–34.
[16]. Pal S, Tak YK, Song JM. Does the Antibacterial Activity of Silver Nanoparticles Depend on the Shape of the Nanoparticle? A Study of the Gram-Negative Bacterium Escherichia coli. Appl Environ Microbiol [Internet]. 2007 Mar 15 [cited 2023 Sep 24]; Available from: https://journals.asm.org/doi/10.1128/AEM.02218-06
[17]. Better safe than sorry: Understanding the toxicological properties of inorganic nanoparticles manufactured for biomedical applications. Adv Drug Deliv Rev. 2010 Mar 8;62(3):362–74.
[18]. Ing LY, Zin NM, Sarwar A, Katas H. Antifungal activity of chitosan nanoparticles and correlation with their physical properties. Int J Biomater. 2012 Jul 8;2012:632698.

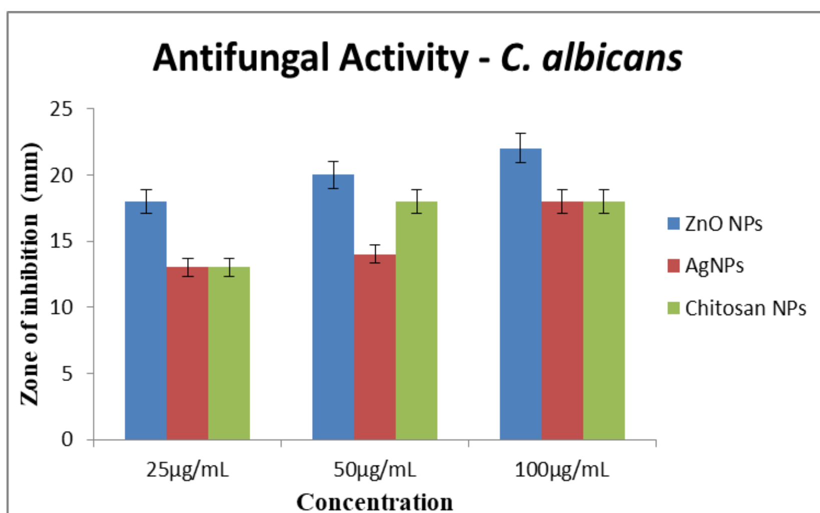


Fig. 1

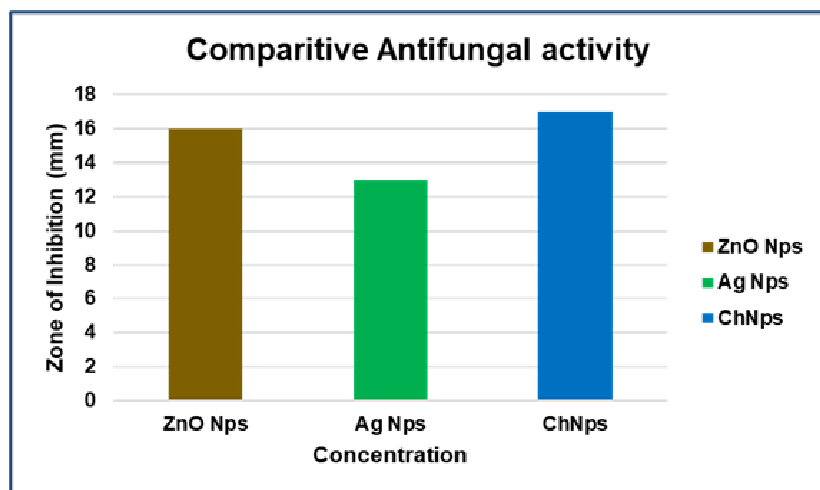


Fig. 2

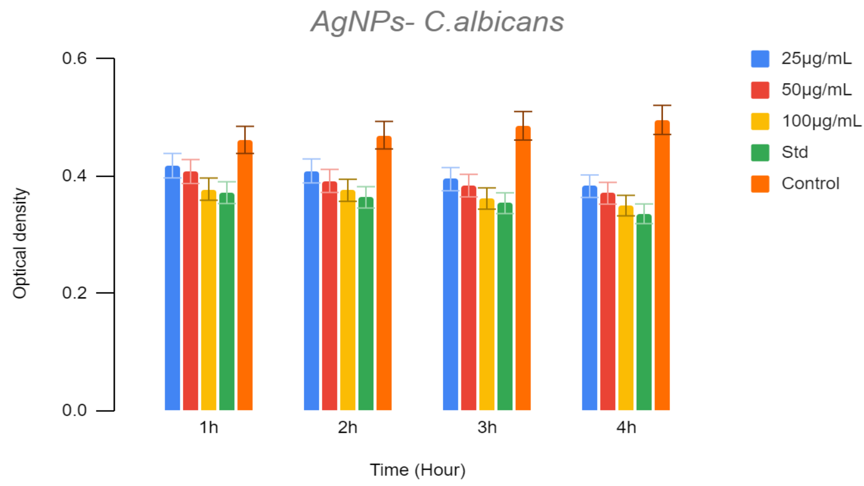
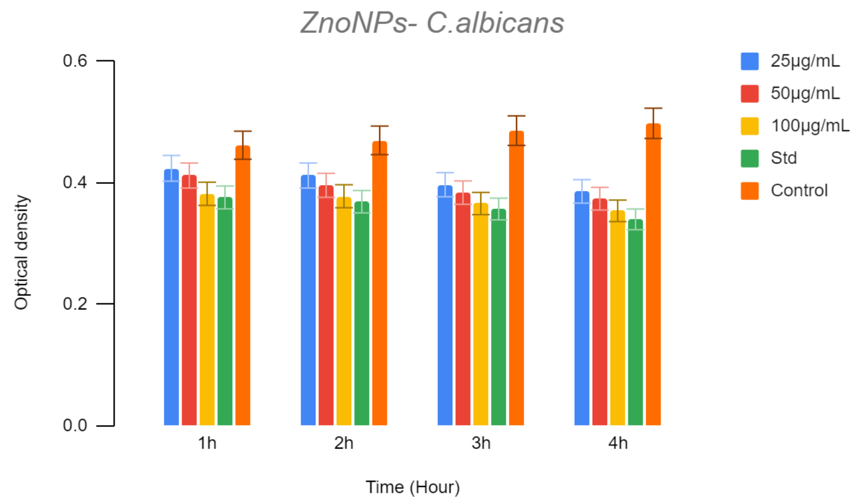


Fig. 3



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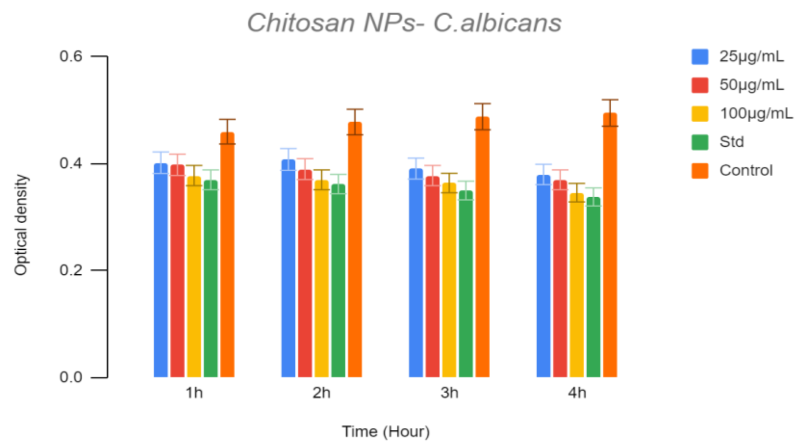

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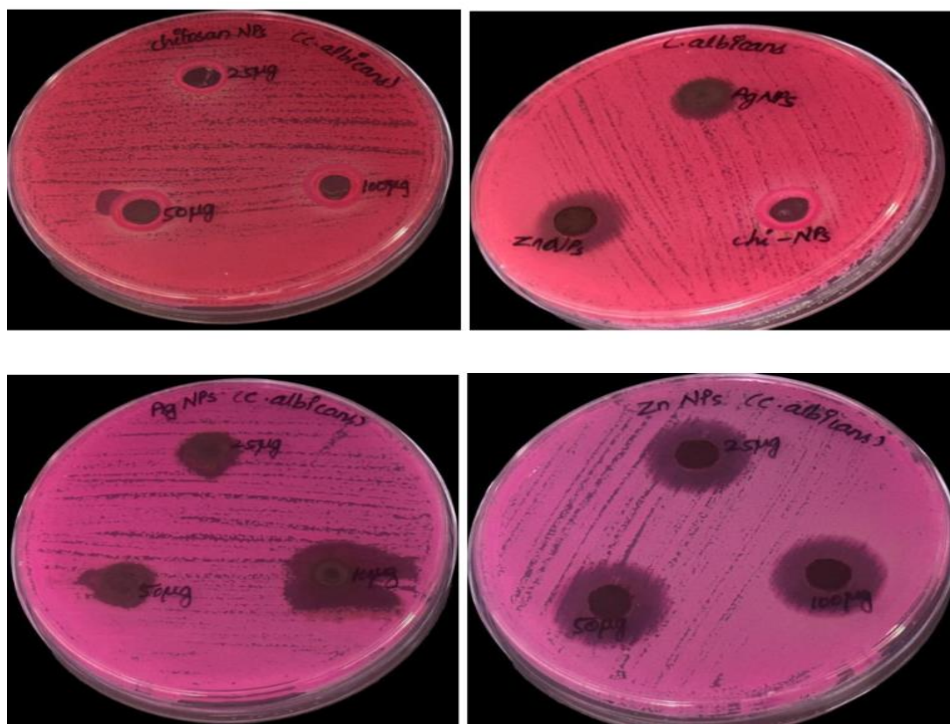

Agar well diffusion method (Fig.6)

DOI: https://doi.org/10.15379/ijmst.v10i4.2411

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