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Cytotoxicity, Anti-microbial, and Antifungal Activity of Zinc oxide Nanoparticles Synthesized Using Guilandinabonduc Herbal Formulation

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The recent highlights of nanoparticles (NPs) are playing a wide range of biomedical applications because of their low toxicity due to their unique physical and biological futures. This study aimed to synthesize and characterize zinc oxide nanoparticles (ZnONPs) using Guilandinabonduc plant preparation. It is efficient and helps in drug delivery and biomedical applications such as antimicrobial activity and to evaluate their cytotoxic effect.G. bonduc (GB) seed extract was utilized in the green synthesis of GB-ZnO NPs, and the agar well diffusion method was employed to evaluate the NPs' antibacterial activity, while the brine shrimp mortality test method was used to evaluate their cytotoxic impact. The Staphylococcusaureus zone of inhibition, as measured by the antibacterial activity, was 17.5 mm when compared to the standard (Antibiotic). The cytotoxic impact shows that there are around 90% of living nauplii at the lowest concentration of 5 µl and 70% of live nauplii at the maximum concentration of 80 µl, demonstrating the low toxicity of the synthesizedZnONPs.The investigations effectively synthesized and analyzedZnONPs from G.bonduc plant preparations. Our findings underscore the necessity of greater discreet usage of nanoparticles in biomedical research. More research is required to investigate the possible applications and ensure their secure and productive implementation.

Keywords: Antibacterial activity, Antifungal activity,Brine shrimp lethality assay,green synthesis, Zinc oxide.

1. Introduction

Nanomaterials are produced all over the world due to their various applications, which include medicament, medications, wound healing, catalytic, magnetic, optical, and electricity abilities. (Gunalan et.al.,2012) They have a lower surface-to-volume magnitude connection than their associated bulk materials, which increases once their measurements are remittent (Ohno et.al.,2010). Place them at the border among tiny molecules and bulk substances. The development of nanoparticles inside the commodities, medical, food, space, chemical, and associated degreed beauty products sectors has created the need for an environmentally friendly and property-producing technique (Mansoori et.al.,2005, Rao MD et.al.,2016). Metallic Examples of oxides and dioxides include philosopher's wool, Ag, Au, as well as titania, which are getting plenty of interest due to their many possibilities and usages (Dobrucka et al.,2016).

Nanoparticles are synthesized using a variety of chemical and physical methods. Until now, optical device ablating, microwave radiation, and vapour accumulation have been recorded (Sathyanarayana et.al.,2018). These procedures all contain toxic substances that will disturb the diversity of the environment, as well as forces that cause bulk fragments to condense, disperse, or split into nanoparticles (Dhandapani et.al.,2014). A growing benefit associated with technology is that the creation of nanomaterials employs natural processes, aided by bound technological advances devices, which provides a safe, economical, and ecologically sound analysis method. It has come into contact with vegetation, fungal organisms, yeast, algal growth, microbes, and human beings. The proteins and other metabolic products can convert elements into metal particles (Krupa et.al.,2016). Biological sciences rather than physical or chemical procedures are better at producing nanoparticles (Kharissova et.al.,2013). Total untrained chemistry has produced a variety of metal substance nanoparticles, including TiO_2, CuO, and ZnO. ZnO, an associate degree transistor device, has gained popularity among them due to its ease of production, low cost, and security of synthesis. The creation of nanoparticles that target plants holds great promise across all biological systems. Plant-based nanoparticle production is simple, ecologically sound, and offers therapeutic advantages. Metal particles can be reduced by terpenoids, polyphenols, sugars, alkaloids, synthetic resin acids, and molecules of protein (Parveen et.al.,2016).

Nanotechnology is manufactured utilizing diverse plant ingredients, including fruits as well as leaves of various species, such as aloe, hibiscus, garlic, onion plant, herbaceous plants, Moringaoleifera, and tea has been unquestionable in numerous investigations devoted to the untrained creation of ZnO nanoparticles using extracts from plants (Iravani et.al.,2015). Nanotechnology is tiny particles with improved beneficial reactivity, thermal insulation, and chemical stability, resulting in an enormous surface-per-unit volume ratio (Thema et.al.,2015).Nanoparticles are among the most effective applicants for attacking microbes due to their unique belongings of having a higher surface area than volume ratio (Matinise et.al.,2017).Green nanotechnology is a resulting growing and ecologically sound substitute to chemical-based nanotechnology (Stan et.al.,2015). Green technologies serve a significant part in the synthesis of nanoparticles (Vijayakumar et.al.,2016). The methods of physics and chemistry used to create nanoparticles have been substituted with eco-friendly green synthesis based on plants, herbal extracts, and microbes (Sujatha et.al.,2021). Biological zinc oxide nanoparticles demonstrated high decomposition efficacy (Rajeshkumar et.al.,2018). In addition to being non-toxic, zinc oxide, a component of semiconductors, has good photocatalytic properties and high reactivity (Santhoshkumar et.al.,2017),(Dobrucka et.al.,2016) Zinc oxide nanoparticles are known as low inorganic nanoparticles, with their utilization in infections of the urinary tract (Thi et.al.,2020)(Thema et.al.,2015).Zinc oxide nanoparticles lead investigations due to their broad range of potential uses and attributes. (Davar et.al.,2015).

Green synthesized zinc oxide nanoparticles demonstrated strong antimicrobial and antifungal properties. (Ghosh et.al.,2021). The chemical production of nanoparticles requires a high vacuum and utilizes high energy, whereas biosynthesis is a promising and effective method based on plant extracts (Ramesh et.al.,2015). Given the growing demand for sustainable nanoparticles, green synthesis is the best method for synthesizing metal nanoparticles (Ayyadurai et.al.,2022).Green and eco-friendly products have gained popularity due to their use of plant or fruit-based extracts and avoidance of hazardous substances (Kumar et.al.,2024). Nanoparticles have potential uses in image processing, diagnosis, medication delivery systems, and other medical fields, including biocompatibility, accessibility, strength, and immunity cytotoxic effect of Guilandinabonduc-mediated ZnO NPs on the brine shrimp larvae. Higher concentrations of ZnO NPs resulted in increased mortality rates, indicating the low toxic nature of the nanoparticles (Miri et.al.,2019). Zinc oxide nanoparticles are utilized in protection and cosmetics because they have in effect UV-A and UV-B consumption attributes.As a kind of semiconductor using a wide band gap, zinc oxide nanoparticles exhibit essential spectral characteristics beyond their original capacity G. bonduc is considered a primary candidate for its role in polycystic ovarian syndrome and additionally drug discovery.

Using herbal formulation extracts made from GuilandinaBonduc seed extract, ZnONPs are synthesized and characterized in the current work utilizing the first in vitro investigations. Analysis techniques such as UV-visible spectra analysis will be used to characterize the synthesized nanoparticles. The cytotoxic effects of these nanoparticles will be evaluated using the brine shrimp lethality test technique, and the paper outlines the initial in vitro experiments of assessing the antibacterial. Important new information on the potential of medicinal plants for the synthesis of ecologically safe nanoparticles and their wide range of applications in many disciplines is provided by this study.

2. Materials and Methods

2.1 Preparation of Guilandinabonduc and herbal formulation:

1g Guilandinabonduc seed powder has been weighed and 100 mL of water is prepared from using distilled water. The aqueous composition was heated on a mantle at 60-70°C for about fifteen minutes. The boiled solution was filtered using muslin cloth and used for the nanoparticle synthesis.

2.2 Green Synthesis of Zinc Oxide Nanoparticles (ZnoNPs):

By dissolving 1 mM of zinc nitrate (0.594 gm) in 50 mL of distilled water. The solution was combined with 50 mL of processed herbal formulation. An orbital shaker was used to rotate the reaction mixture for 24-48 hours at 110 rpm. The nanoparticle synthesis was monitored regularly using a UV-visible double-beam spectrophotometer. Following that, 14 milliliters of solution were placed in six tubes, and the nanoparticle solution was centrifuged at 8,000 rpm for 10 minutes. The centrifugation step is used for separating the ZnONPs pellet from the supernatant. The pellet was collected and stored in a sealed Eppendorf tube, while the supernatant was eliminated.

2.3 Antimicrobial Activity

G.bonduc-mediated ZnO nanoparticles have huge antimicrobial effects on E. faecalis, S. aureus, and Pseudomonas aeruginosa. For this activity, Mueller Hinton Agar was used to identify the zone of inhibitory activity. Muller Hinton Agar was prepared and sterilized at 121 degrees Celsius for fifteen pounds. Before swabbing the test microorganisms, the apertures were made with a clean 9 mm polyethylene tip. These nanoparticles were added at varying quantities, and the plates were incubated for 24 hours at 37°C. Following the incubating period, the region of the restriction was identified. The inhibited bacterial zone was assessed encircling all the wells was measured and documented in millimetres and the zone of inhibition was represented as Figure 2.

2.4 MIC assay of Guilandinabonduc mediated ZnONPs against C. albicans.

In the current investigation, ZnONPs are synthesized utilizing the Guilandinabonducextract procedure, which is a natural and environmentally benign technique, thataddresses the rising need for ecologically friendly methods. ZnONPsantifungal properties in opposition to Candida albicans were studied. The existence of inhibitory zones in the culture medium diffusing disc assay as well as the MIC values, indicate that the ZnO NPs have potent antifungal abilities. These findings indicate that Guilandinabonduc-mediate ZnONPs have a huge impact to treat a wide variety of fungal infections, such as those caused by clinically important species such as Candida albicansand Aspergillusniger. The agar well diffusion assay uses C. albicans as a test for the infectious agent. The solution is prepared using rose bengal agar.The sterile medium was swabbed with test organisms, and then nanoparticles in different concentrations were introduced to each well. The plates were maintained at 28°C for 48 to 72 hours. Afterward the incubation period, the zone of limitations was identified as shown in Figure 3.

2.5 Cytotoxic effect -Brine Shrimp Lethality Assay:

200 millilitres of filtered water were used to dissolve 2 grammes of iodine-free salt. 10–12 mL of saline water were added to each of the six well ELISA plates. Ten nauplii (20µL, 40µL, 60µL, 80µL, and 100µL) were added to each well. After that, these nanoparticles are mixed in the proper proportions. As shown in Figure 4, the plates were first cultured for twenty-four hours. The ELISA plates were checked for signs of live nauplii after a 24-hour period.

3. Results

3.1 Antibacterial activity of Guilandinabonduc - mediated zinc oxide nanoparticles:

Figure 1 illustrates the antibacterial properties of Guilandinabonduc-mediated ZnO nanoparticles at both high (100µg/mL) and low (25µg/mL) concentrations on E. faecalis, S. aureus, S. mutans, and pseudomonas. The antibacterial activity of the nanoparticles against E. faecalis, S. mutans, S. aureus, and Pseudomonas was demonstrated on Agar plates A, B, and C, respectively. Around the NPs, a zone of inhibition is seen. The chemically produced ZnONPs were found to have a minimum inhibition zone of 7.5 mm and a maximum inhibition zone of 17 mm against strains of S. aureus bacteria. 5 mm of inhibition was also seen against these strains. (D) Zinc oxide nanoparticle inhibitory region's anti-fungal capabilities against Candida albicans on rose bengal agar with positive control

Figure 1: The antibacterial activity was measured by the agar well diffusion method with GB-ZnONPs and the zone of inhibition was measured, the zone of inhibition since the GB-ZnONPs were taken in different concentrations (25, 50, 100µg/mL) against E. faecalis, S. aureus, S. mutant, and Pseudomonas

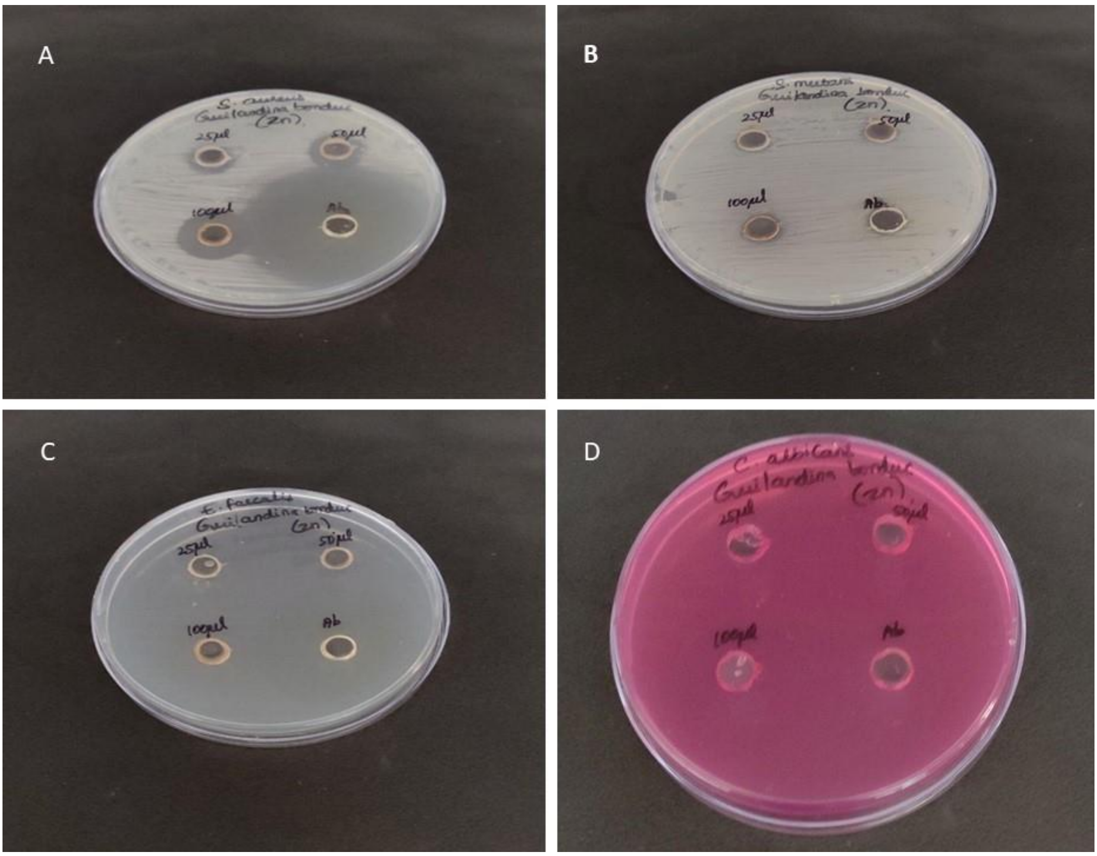


Figure 2: The graphical representation of Antibacterial Activity of G.bonduc mediated Zinc oxide Nanoparticle zone of inhibition

The antimicrobial activity depicts the highest zone of inhibition at 100µL concentration (µL-microlitre; mm-millimetre; Ab-Antibiotic) E. faecalis, S. aureus, S. mutans, pseudomonasaeruginosa.

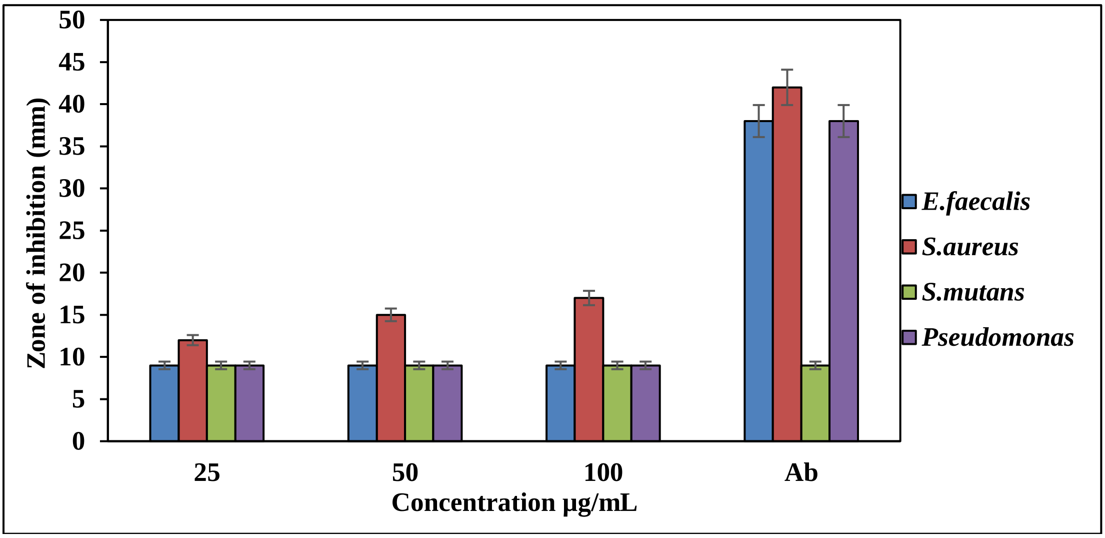


Figure 3: Graph showing antifungal activity of MIC assay of G. bonduc mediated ZnONPs positive control. The agar well diffusion assay uses C. albicans as a test for the infectious agent. The solution is prepared using rose Bengal agar.

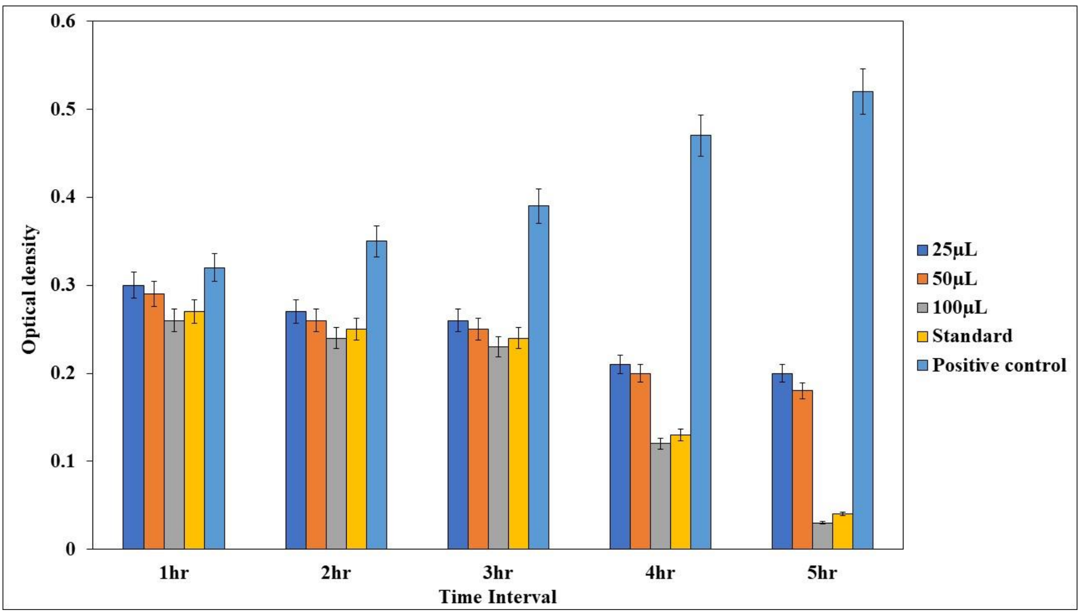
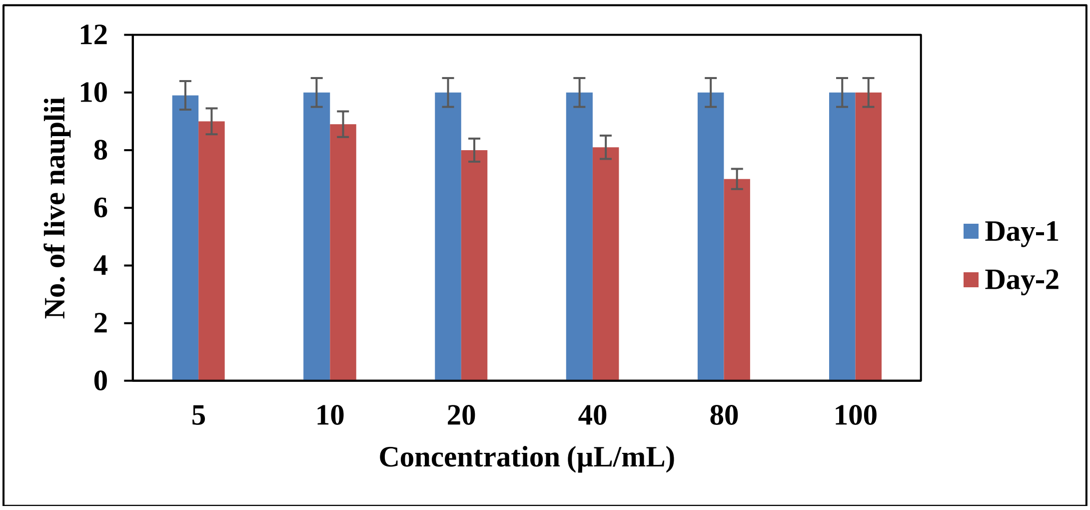


Figure 4: The graph showing cytotoxicity of G. bonduc-mediated ZnO nanoparticles



4. Discussion

ZnONPs were synthesized in the current work, and then their antibacterial, structural, and morphological characteristics were assessed at two distinct doses. Additionally, it was shown that these NPs' inhibitory effects were detected when the zone of inhibition was evaluated at different doses against S. aureus, S. mutans, and Pseudomonas aeruginosa. For many years, the tropical herb Guilandinabonduc has been utilized in traditional medicine. Because of its cytotoxic impact, Guilandinabonduccan destroy or harm cells.

The sol-gel approach was effectively used in the previous work to synthesize ZnO-SnO2 NPs. It was successfully determined that NPs were present. Against the examined pathogens, the produced NPs demonstrated a strong antibacterial activity. et al., 2019 [Miri]. This research looked at the rapid, inexpensive, and simple synthesis of ZnONPs using P. farcta extract. The synthesized nanoparticles have a hexagonal shape and a sheet form, and their size ranges from 40 to 80 nm, according to the results. When applied to Candida albicans, the synthesizedZnO-NPs demonstrated a respectable antifungal activity. According to Rifaath et al. (2023), the cytotoxicity of synthesizedZnO-NPs is dependent on their concentration, as seen by the WST-1 findings. The IC50 of MCF7 cells is 90 µg/ml.

In these studies, chemically synthesized ZnONPs expulsions at various concentrations (2, 4, 6 mM, and 4, 8, and 12 mM) towards different bacterial and fungal pathogens have been evaluated using the well and the diffusion on disc agar methods. The presence of a barrier to growth confirms the theory that ZnO nanoparticles kill infectious agents by disrupting the membrane and rapidly producing surface oxygen species. It is interesting to consider that the dimension of the area of inhibition differed depending on the pathogen type, analysis method, and ZnO nanoparticle levels.

The anti-fungal activity of ZnO NPs against Candida albicans was investigated. The presence of zones of inhibition in the agar disc diffusion assay, as well as the MIC values, indicate that the ZnO NPs have potent antifungal abilities. These findings suggest that Guilandinabonduc-mediated ZnO NPs can treat a wide range of fungal infections.

To evaluate the figure showing the cytotoxic potential of synthesized ZnONPs, a brine shrimp lethality test was conducted. The nanoparticles gave maximum inhibition of 90% which was in agreement with the reports as smaller nanoparticles induce a higher level of cytotoxicity clinicallythese investigations were carried out in vitro, indicating that they were done on cells in a lab rather than on living organisms. Therefore, more study is needed to determine the possibility of cytotoxic consequences of Guilandinabonduc in humans.

5. Conclusion

Therefore, this green production of zinc oxide nanoparticles utilizing Guilandinabonduc has the potential to be commercialized for S.aureus illnesses and infections. The results of this research emphasize the efficacy of Guilandinabonduc-mediated ZnO NPs as fungicides.Green synthesis methods have similar or higher efficacy than conventional drugs, making them intriguing possibilities for further research. The synthesized ZnO NPs were dependent upon the concentration of cytotoxic effects on brine shrimp larvae, with greater levels leading to greater mortality rates. These findings highlight the toxicity of Guilandinabonduc-mediated ZnO NPs and support the possibility of toxicity in other systems of life. The brine shrimp lethality assay proved a useful instrument for unreliable cytotoxicity screening of nanoparticles, offering a simple and cost-effective method. However, more research is required to elucidate the fundamental processes causing the reported cytotoxic effects. In addition, the biodegradability and safety of Guilandinabonduc-mediated ZnO NPs must be evaluated in more complex biological systems, such as cell lines and model organisms.

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