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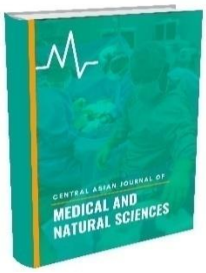

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Biogenic Zirconium Nanoparticles Synthesized from Silybum marianum: A Potent Antibacterial Agent Against Gingivitis bacteria

1. **Ranya Mohammed Hardan**
2. **Ashwaq Talib Hameed***
3. **Wafaa Tali Radef**

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[1,2,3] Department of Biology, College of Education for women, University Of Anbar, Anbar, Iraq

*Corresponding author Email: ashwaq.talib@uoanbar.edu.iq

Abstract: The present paper incorporates the preparation of Zirconium Dioxide Nanoparticles (ZrO_2NPs) through the green synthesis using extracts from the leaves of the wild Silybium marianum plant growing in Anbar regions. The plant has special compounds that can help reduce and stabilize nanoparticles. This can also make the nanoparticles less harmful. So, the nanoparticles have been characterized utilizing: Atomic Force Microscopy (AFM), Fourier Transform Infrared Spectroscopy (FT-IR), Ultraviolet-Visible Spectroscopy, Scanning Electron Microscopy (SEM) in conjunction with Energy Dispersive Spectroscopy (EDS) analysis as well as X-Ray Diffraction (XRD). According to the analysis conducted with the X-Ray Diffraction SEM, it was observed that the nano-zirconium particles possessed a round shape and were estimated to be around 55 in dimension.91 nanometers. The ZrO_2NPs were used as antibacterial agents against bacteria that cause gum inflammation. In order to determine the levels of nano-zirconium, different quantities were tested at concentrations of 200, 250, and 300 µg/ml. The concentration of 250 µg/ml showed the highest diameter of inhibition against the behavior of the isolated bacteria P.mirabilis, S.mitis. Conversely, the lowest inhibitory diameter was at the concentration of 200 µg/ml. The study demonstrated the ability of ZrO_2NPs in inhibiting the activity of bacteria causing gum inflammation.

Key words: Zirconium nanoparticles، Silybum marianum extract، Antibacterial activity، Nanoparticle synthesis, Characterization techniques.

Introduction

Nanoparticles are nanoscale particles with dimensions typically ranging from (1-100) nanometers. In this size range, small particles called nanoparticles have different characteristics compared to atoms, molecules, and larger materials. These differences occur in how they look, how they react with other substances, and how they affect living things (Taghiyari *et al*., 2017). Tiny particles called nanoparticles can be made from many different materials. Some examples of these materials are biological particles, carbon, organic substances, polymers, non-oxidized ceramics, silicates and metal oxides. Metallic nanoparticles, due to their exceptional physical and chemical properties, have found effective applications in various fields, including healthcare, biology, and cellular transport (Gnanasangeetha and Suresh, 2020).

Several methods exist for nanoparticle production, but many of them involve high costs, high energy consumption, and the presence of stability and reduction factors that may result in toxicity in the final nanoparticle solution. Common methods include chemical and physical approaches. Therefore, bio-based sources such as bacteria, fungi, yeasts, algae, and plants have proven to be effective in promoting the transformation of materials into smaller particles through a biogenic synthesis process. This process imparts unique optical, chemical, and environmentally friendly properties to the resulting nanoparticles (Senthil Kumar and Siva Kumar, 2014), zirconium is a type of metal that belongs to the titanium family. It is classified as a transition metal and has an atomic number of 40. It can capture neutrons and is often used as a metallic layer on fuel rods in nuclear reactors (Kazado *et al*., 2021). Zirconium dioxide, also known as zirconia, is a very stable compound made from zirconium through different ways (Hassan and Jalil, 2022). ZrO2 can have different crystal structures like monoclinic, tetragonal, and cubic, depending on how it is made (Zhang et al., 2018). Due to its extremely low toxicity and wide bandgap energy, ZrO_2 has demonstrated significant applications in the development of high-temperature-resistant ceramic alloys (Chen *et al*., 2021؛Awad and Hameed,2021). Biological synthesis, particularly using plants, has emerged as a green approach for the nano-scale synthesis of ZrO_2. This method provides an efficient, controllable, and environmentally friendly approach, utilizing low-costs and locally available biological sources like plants, fungi, algae, and bacteria (Shafey, 2020). iological synthesis means getting biomolecules from natural sources. These biomolecules are important because they can both reduce and stabilize things very well. This causes the creation of really good amounts of ZrO_2 particles that are very small (Bandeira et al., 2020). Additionally, these natural structures can be used instead of harmful and costly chemicals or energy-intensive tools (Agrawal *et al*., 2017؛Hameed *et al*.,2021), the biogenic synthesis of ZrO2 nanoparticles aligns well with the principles of sustainable and green chemistry by reducing potential risks associated with chemical and physical methods, minimizing the generation of hazardous intermediate substances, and avoiding secondary pollution (Jadoun *et al*., 2021؛Dawood *et al*., 2022). Making ZrO2 nanoparticles using plants is easier and cheaper than using other green substances like bacteria, fungi, and algae. Plants are widely available and easy to find in many places. Using plants as biological templates has many advantages (Saravanan *et al*., 2021).

Materials and Methods

Plant Collection:

leaves of the *S. marianum* plant were collected during their growth period from various areas in the city of Ramadi, Anbar Province, between December and the end of February 2023. Standard collection practices were followed, considering the plant's saturation with sunlight during the collection period (Khan *et al*., 2018). Plant parts, specifically leaves, were selected without tears or diseases. The collected plant material was washed with tap water to remove mud and impurities, and left to air-dry completely at room temperature in a dry and well-ventilated environment. After drying, the plant material was finely ground using an electric grinder, as depicted in Figure 1. The ground material was stored in an incubator at room temperature (25°C), away from moisture and light, until further use. The plant was classified in the botanical herbarium of the College of Education for women - University of Anbar by Dr. Ashwaq T. Hameed based on its morphological characteristics, following the Iraqi Plant Encyclopedia.

This detailed methodology ensures the proper collection and preparation of the *S. marianum* plant for subsequent use in the synthesis of nanoparticles. The adherence to standard collection practices and the involvement of an expert for plant classification enhance the reliability and accuracy of the study

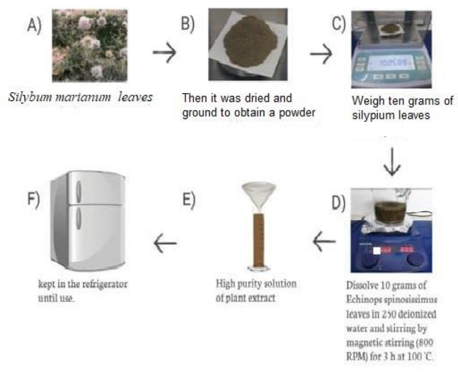


Figure 1: Schematic diagram of illustrating the steps in the Preparation of Plant for Extraction

Sample Collection - Bacterial Swabs:

One hundred swabs were collected from the gums of patients of various ages at the Specialized Health Center for Dentistry in Ramadi\ Iraq . The samples were obtained using sterile cotton swabs containing a transport medium, swabbing from the affected gum area to ensure the vitality of the isolates. Subsequently, the samples were transported to the laboratory of the College of Education for women within a period not exceeding two hours for culturing, purification, and diagnosis.

Preparation of Zirconium Nanoparticles (ZrO_2 NPs:

Zirconium nanoparticles were prepared by dissolving 2.5 grams of zirconium nitrate ($ZrO_2(NO_3)_3.8H_2O$) in 100 ml of ethanol. The mixture was stirred using a magnetic stirrer for 30 minutes at 100°C. Then, 100 ml of the plant extract from *S. marianum* was added to the nitrate solution with continuous stirring for 60 minutes at 100°C. After some time, a yellow precipitate formed, which was washed several times with distilled water and ethanol before being separated using a centrifuge and dried in an oven to complete the calcination process. The powder was treated for two hours at a temperature of 500°C, causing the color of the powder to change to white (Kokila *et al*., 2015

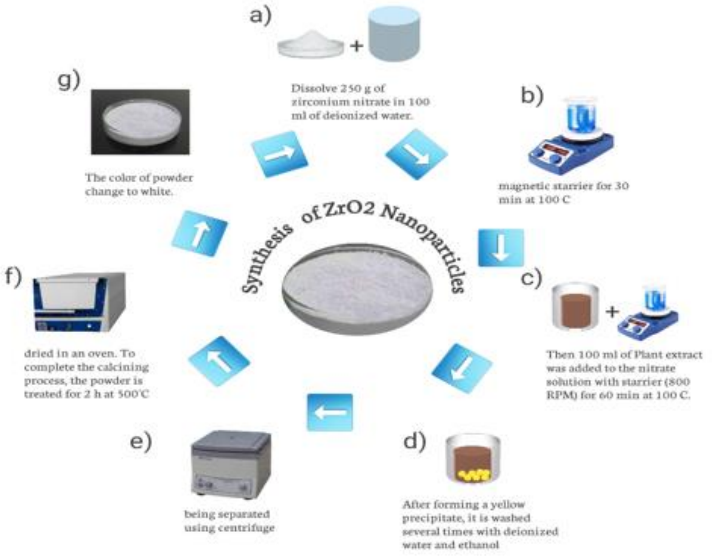


Figure 2: Schematic diagram illustrating the steps in the preparation of ZrO2 nanoparticles.

Characterization of Nanoparticles:

UV-Visible Spectroscopy Analysis: UV-Visible spectra of ZrO_2 nanoparticles (NP_S) were periodically recorded using a Shimadzu UV-1800 spectrophotometer, in Japan. The absorption spectra for all tested samples were obtained in the range of 190 to 1100 nanometers. Measurements were taken 24 hours after preparation using distilled water for instrument calibration (Ganguly *et al*., 2013). Fourier-Transform Infrared Spectroscopy (FTIR) Analysis: FTIR measurements were performed to identify functional groups present in plant extract particles and their potential involvement in nanoparticle synthesis. The test samples were independently dried, mixed with potassium bromide, and pelletized for FTIR spectroscopy using a TENSOR27, Bruker Optik GmbH. Spectra were recorded in the range of 400 to 4000 cm^{-1} . X-Ray Diffraction (XRD) Analysis: The crystalline structure was determined using XRD-6000 (Shimadzu, Japan) to study the structural properties of the nanoparticles. The device was equipped with a cu(ka) radiation source with a wavelength (λ) of 1.5405Å, a voltage of 60 kV, and a current of 80 mA. The scanning speed was set at 5 degrees per minute in the θ2 degree range from 20° to 80°. Measurements were performed on dried samples using a paper filter. Dynamic Light Scattering (DLS) Analysis: DLS (Brookhaven NanoBrook 90 Plus, USA) was employed to determine the hydrodynamic diameter of nanoparticles in a water medium.

Results and Discussion

Preparation of Zirconium Nanoparticles by *S, marianum* Plant Extract:

In this study, alcoholic extracts of Silypium marianum plant leaves were used to prepare zirconium nanoparticles to verify their antibacterial effectiveness. Plants contain various compounds and small molecules in different parts, such as alkaloids, flavonoids, tannins, amino acids, and phenols, which may act as reducing and stabilizing factors for nanoparticle formation. The color of the mixture turned dark brown, indicating the potential formation of nanoparticles. This color change may be due to the appearance of the Surface Plasmon Resonance (LSPR) band, a characteristic feature of metallic nanoparticles like copper, silver, gold, zirconium nitrates, and zinc. The final yield of zirconium nanoparticles after drying was 85 milligrams (Silva *et al.*, 2019; Naya *et al.*, 2023)

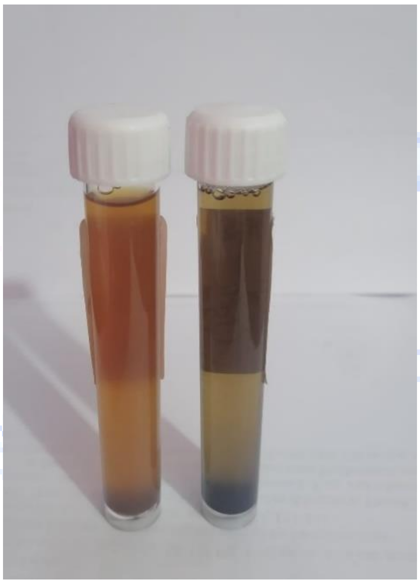


Figure 3: Right: Methanolic Solution of *S. marianum* extract, Left: Zirconium Nanoparticles.

The absorption results for zirconium nanoparticles formed at room temperature using the *S. marianum* plant extract showed a gradual decrease in the absorption spectrum, accompanied by a shift in the wavelength to 332 nanometers and 377 nanometers. The presence of active components in the plant extract for the formation of zirconium nanoparticles led to an increase in absorbance intensity (Figure 4). It is also observed that the Plasmon peak at 377 nanometers shifted slowly towards the lower wavelength. This indicates the size and shape of the formed nanoparticles (Kumaresan et al., 2018).

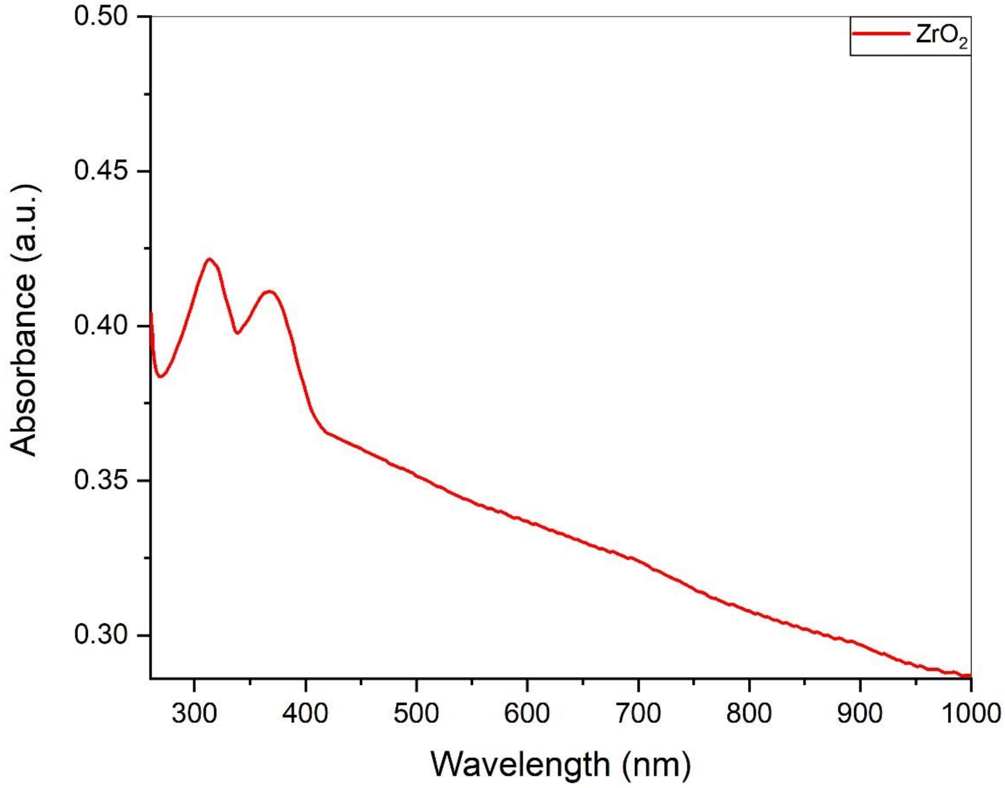


Figure 4: UV-Visible Spectra of Zirconium Nanoparticles and Methanolic Plant Extract

The UV-Visible spectra illustrate the characteristic absorbance peaks of zirconium nanoparticles, demonstrating the successful synthesis using the Silypium marianum plant extract. The shift in peak position and intensity provides valuable information about the size and morphology of the formed nanoparticle.

The XRD analysis results for zirconium nanoparticles prepared from the Silypium marianum plant extract revealed diffraction peaks at 27.6 degrees, 32.6 degrees, 35.3 degrees, 47.8 degrees, and 57.4 degrees. These peaks are indicative of the cubic phase of zirconium nanoparticles (Selvam *et al*., 2023), the XRD pattern confirms the crystalline nature of the synthesized ZrO2 nanoparticles. The distinct diffraction peaks correspond to specific crystallographic planes, indicating the crystal structure of the nanoparticles. Using the Debye-Scherrer formula, the calculated particle diameter was found to be 15.073 nanometers (Vorokh *et al*., 2018). This suggests that the nanoparticles exhibit a well-defined crystalline structure in the cubic phase. The XRD analysis provides valuable insights into the crystallographic properties and phase composition of the synthesized ZrO2 nanoparticles.

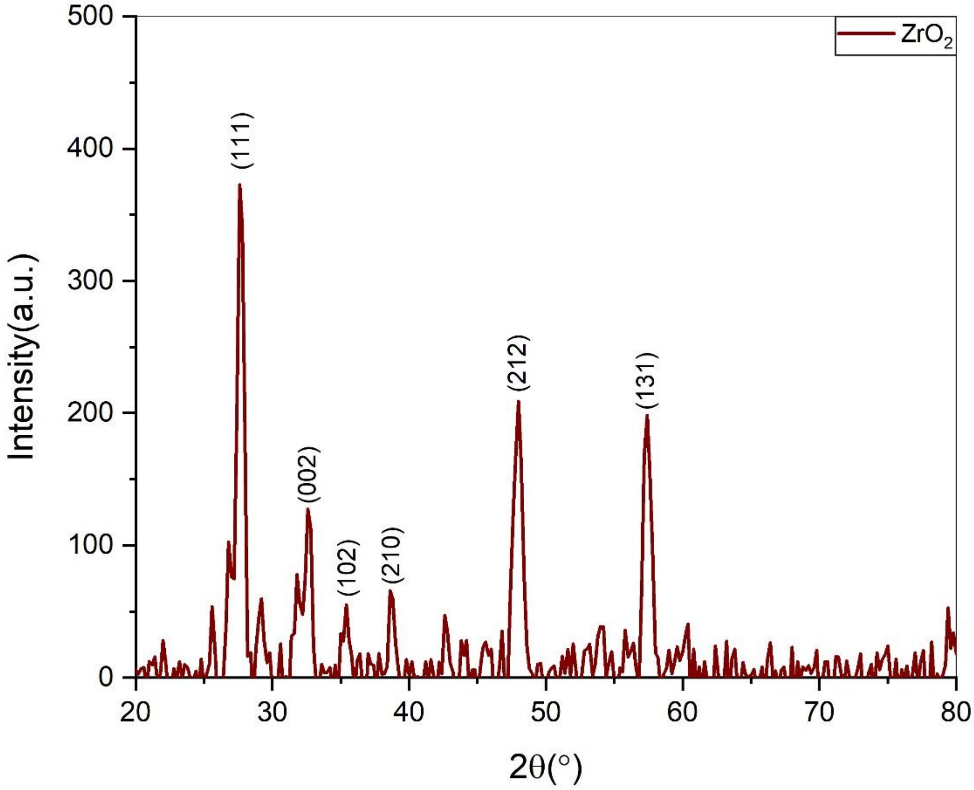


Figure 5: X-Ray Diffraction (XRD) Analysis of Zirconium Nanoparticles

The composition of the zirconium nanoparticles was analyzed using Energy Dispersive X-ray Spectroscopy (EDS). The elemental composition of the sample showed a percentage of zirconium (20.3%), oxygen (40.0%), sodium (23.8%), carbon (12.4%), chlorine (0.8%), potassium (2.2%), and silicon (0.6%) %) (Chowdhury *et al.*, 2023).

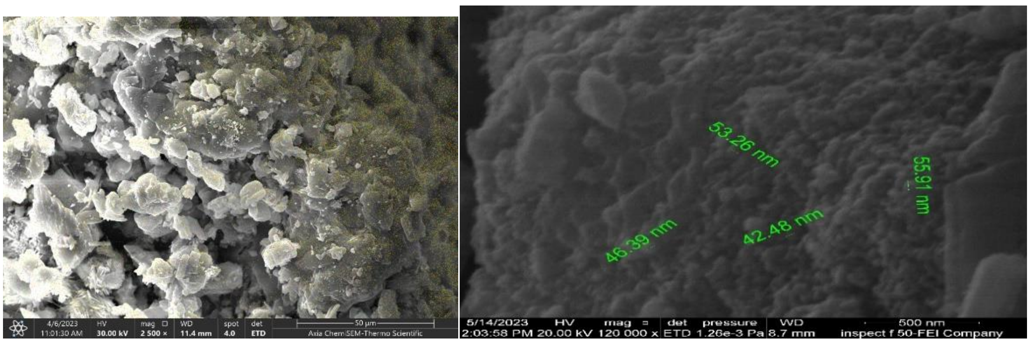


Figure 6: The appearance of zircon nanoparticles under a scanning electron microscope. 200,000X magnification power

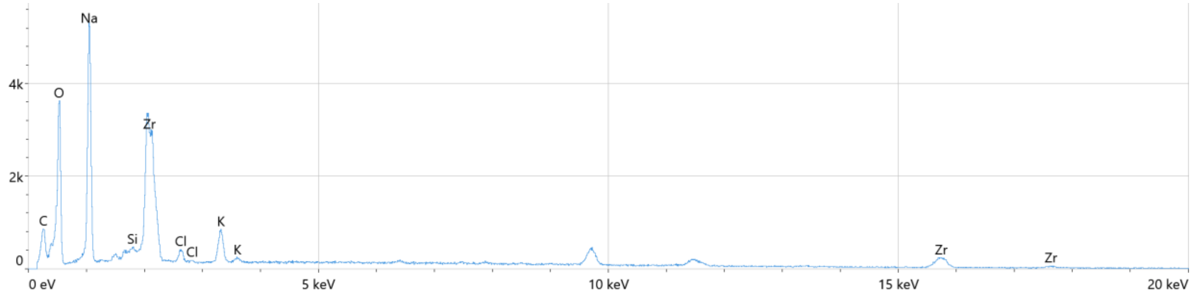
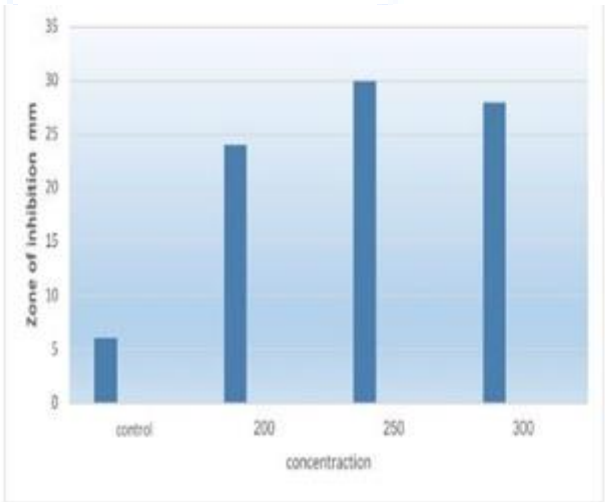


Figure 7: Elemental analysis of the zircon nanoparticle model using an EDS device

Antibacterial activity

In Figure 8, the antibacterial activity of biologically synthesized zirconium nanoparticles from *S. marianum* is demonstrated at six different concentrations (9.37, 18.75, 37.5, 200, 250, 300 µg/ml). The study reveals the inhibitory effectiveness of zirconium nanoparticles against antibiotic-resistant bacterial strains (*P. mirabilis, S. mitis*) using the agar well diffusion method. The results indicate that zirconium nanoparticles exhibit significant inhibitory effects on bacteria, as evidenced by the measured inhibition zones around the wells on Mueller-Hinton agar medium. The highest inhibition zone diameter was observed at a concentration of 250 µg/ml against *S. mitis*, with a diameter of 30 mm. Similarly, the highest inhibition zone diameter against P. mirabilis was 27mm at a concentration of 250 µg/ml ,These findings align with previous research by Khan et al. (2020)

The antibacterial efficacy of zirconium nanoparticles against microbes involves a variety of minerals, including nano-sized particles of zirconium, gold, silver, zinc, titanium, and copper. These nanoparticles interfere with the microbial synthesis of proteins and the production of DNA and RNA, in addition to disrupting the functions of the bacterial cell membrane (Sanchez *et al*., 2020**).**



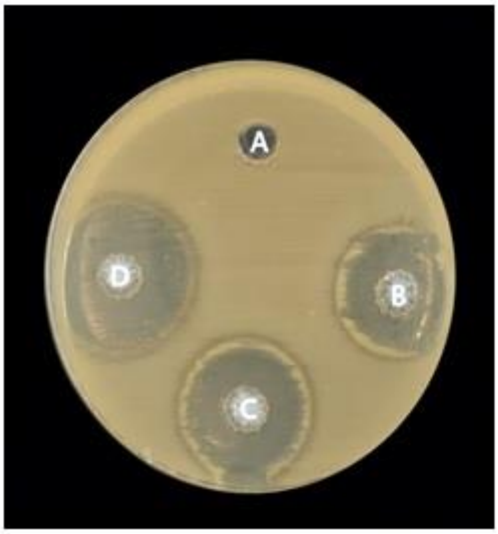
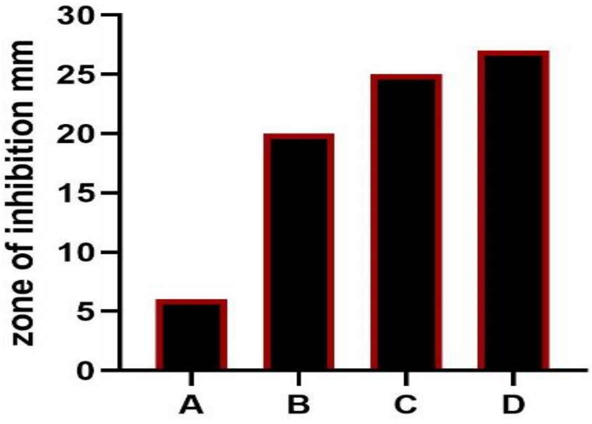


Fig8.: Antibacterial activity of (ZrO_2) against *S. Mitis.* A, control . B, 200 µg/ml. C, 250 µg/ml. D, 300 µg/ml.



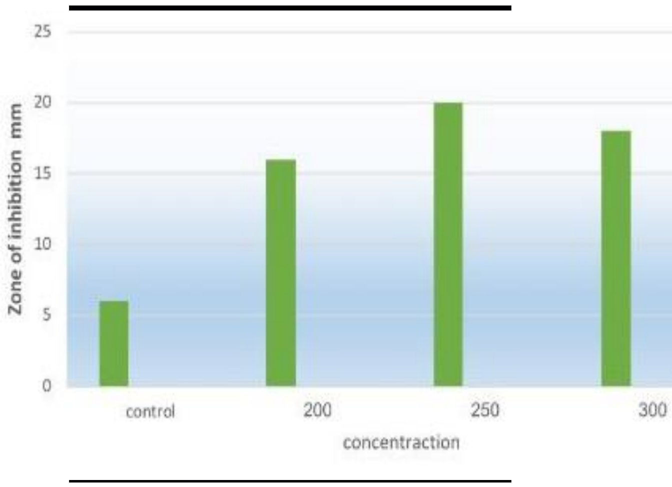


Fig 9.: Antibacterial activity of (ZrO_2) against *P. mirabilis.* A, control . B, 200 µg/ml. C, 250 µg/ml. D, 300 µg/ml.

Conclusion

Biologically synthesized zirconium nanoparticles from *S. marianum* demonstrated potent antibacterial activity against antibiotic-resistant strains *(P. mirabilis, S. mitis, P. aeruginosa*). The nanoparticles exhibit concentration-dependent inhibitory effects, with the highest efficacy observed at 250 µg/ml. The study suggests the potential of these nanoparticles in combating bacterial infections, attributing their effectiveness to interference with protein synthesis, DNA, RNA production, and disruption of bacterial cell membranes. These findings underscore the promising application of S. *marianum*-derived zirconium nanoparticles in developing novel antibacterial agents.

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