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Effect of Biosynthesized ZnS Nanoparticles against Multi-Drug Resistance *Staphylococcus aureus* Isolated from Infected Wound

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Abstract: this paper describes the biosynthesis, characterization, evaluation and antibacterial activity of zinc sulfide nanoparticles (ZnS NPs) made using a low-cost and straightforward method. With the help of a brand-new, non-toxic, environmentally acceptable biological substance called pomegranate peel extract, bio-inspired ZnS NPs were created. By using a UV-Vis spectrophotometer and Atomic Force Microscopy (AFM), the produced nanoparticles' structural, morphological, and optical characteristics have been determined. The UV-Vis spectra of the produced ZnS NPs had a peak at 270 nm that served as an identifying feature. AFM was used to examine the sample's surface morphology. The findings demonstrated the ZnS NPs' antibacterial efficacy against Gram-positive bacteria (*Staphylococcus aureus*) isolated from wounds and resistant to antibiotics.

Keywords: nanoparticles, antibacterial, wound swab, AFM

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Introduction

Antibiotics are typically used to treat infections brought on by bacterial pathogens (1, 2). Antibiotics are compounds that are antibacterial and halt the growth of bacterial infections by a number of methods, such as inhibition of enzyme activity and disruption of DNA, RNA, and protein production (3). The bacterial cell membrane structure is finally disrupted by these mechanisms, which leads to cell death (2, 3). Recent studies have revealed that bacterial infections are becoming less susceptible to a variety of antibiotics (4, 5, 6), which reduces the effectiveness of these medications. Certain bacterial strains can develop antimicrobial resistance, which is the opposite of how antibiotics function. Since they have unique physical and chemical characteristics compared to bulk materials, nanomaterials are widely used in medicine. Numerous nanomaterials are created in order to find their uses in a wide range of commercial products, including electronics, drug delivery, cosmetics, protective gear, and sporting

goods. (7,8) ZnS is frequently employed in biological domains because it is nontoxic, biosafe, and biocompatible (9-10). Due to its deeper conduction and valence bands than other semiconductors, the first and most extensively investigated semiconductor material, ZnS, has become known as a possible antibacterial material. ZnS nanostructures have received a lot of attention lately because they can be used for biological tagging and detection. ZnS nanomaterials have been made using a variety of methods, including the precipitation method (11), the hydrothermal method (12), and the thermal evaporation method (13). Due to the difficulties of scaling up the synthesis process, the separation and purification of nanoparticles, the high energy consumption, and the hazardous by-products, these methods and routes have several drawbacks.(23) Researchers working on materials still face difficulties in creating environmentally friendly processes for synthesizing zinc sulfide nanoparticles (ZnSNPs).Therefore, the aim of this study is to synthesize ZnS NPs by a green approach based on eco-friendly biological material , abundantly available and as a waste; pomegranate peel extract and nanoparticles have been characterized utilizing UV-vis and Atomic Force Microscopy (AFM) and investigate its antibacterial activity against multiple antibiotic resistance *S.aureus* .

Materials and methods

Isolation and identification of clinical isolate

78 samples were randomly isolated from male and female patients with infected wounds at Medical City Hospital in Baghdad between December 2021 and March 2022. Each patient's age (1-83 years), sex, and other vital information were recorded. The samples were cultured for 24 hours at 37°C on blood and MacConkey agar. The developing colonies were then raised on a certain medium. Following differentiation using morphological and biochemical tests like the oxidase test, catalase test, and IMViC, the identification method described by (14) is used.

Antibiotic susceptibility testing by disk diffusion method

For tests of antimicrobial susceptibility, standardize the inoculum density using the McFarland standard No. 0.5 solution. This test was carried out with the disk diffusion method on Muller-Hinton agar in accordance with the standards of the Clinical and Laboratory Standards Institute (CLSI) (15). In order to compare the suspension to the McFarland standard solution, it was streaked with 0.1 on a Mueller Hinton agar plate and then infected with sterile swabs. It was then kept at room temperature for five minutes after that. Five antimicrobial discs were placed on each plate using sterile forceps. The plate was then kept at 37°C for a further 18 to 24 hours. The findings Results were noted, and they were compared to the basic standards for the CLSI document (15).

Preparation of ZnS nanoparticles by using the Pomegranate peel extract

Both zinc acetate dihydrate (99%, Zn (CH3COO)2. 2H2O) and sodium sulfide nonahydrate (99%, Na2S. 9H2O) were bought from Thomas Baker. The pomegranate peel washed and allowed to dry for two weeks in the shade then weighing 50g of peel and blended with blender and soaked in 500ml of double-

distilled water for 72 hours. The mixture was then centrifuged and filtered to remove biomaterials. At room temperature, 100 ml of double-distilled water was used to dissolve 2.4g of sodium sulfide nanohydrate. Then, for 10 minutes at 45 °C, 10 ml of pomegranate peel extract was applied dropwise over a magnetic stirrer. The extract spontaneously changed the color of the mixture to yellow as soon as it came into contact with sulfide ions. After that, the pomegranate-sulfide mixture was gradually infused with a 10% zinc acetate solution. As a result, uniform yellow-white suspended particles developed, indicating the formation of monodispersed zinc sulfide nanoparticles. After that, they were washed three times with deionized water to remove any biological materials (18).

Characterization of synthesized ZnS nanoparticles

Using a UV-visible spectrometer (Bio Rad, USA), UV-Vis analysis was performed to determine how nanoparticles form and maintain stability. and by determined the surface topography and morphology using an atomic force microscope (SHIMADZU 8400S, Singapore) (AFM). The atomically thin nanoparticle surfaces may be seen in two or three dimensions due to AFM. The shape and microstructure of the created ZnS nanoparticles were examined using field emission scanning electron microscopy (FE-SEM) analysis.

Antimicrobial activity of synthesized ZnS nanoparticles

An agar well diffusion assay (16) was used to test the antibacterial effectiveness of the biosynthesized ZnSNPs against multidrug resistant *staphylococcus aureus* that was isolated from an infected wound. Using a sterile cotton swab, the tested bacteria were evenly swabbed onto Mueller Hinton agar plates. Next, a sterile well borer was used to make four 6 mm-diameter wells. ZnSNPs solutions diluted to four concentrations (400, 200, 100, 50, 25) µg/ml. 100 microliters were added to the appropriate well. After 24 hours of incubation at 37° C, the diameter of the inhibitory zone was measured (17).

Results and discussion

Isolation and identification

Only 4 out of the 78 wound swab samples were negative for bacterial growth. These events may be caused by a variety of variables, such as unidentified agents like viruses and anaerobic bacteria, which were not included in this experiment. Using biochemical tests, 75 isolates were found and identified. Like in Figure 1, 16 were *S. aureus*, 50 were gram-negative bacteria, and 9 were other gram-positive bacteria.

Figure -1 Distribution of bacterial isolates

Antimicrobial susceptibility test

According to the current study's findings, the majority of bacterial isolates were resistant to antibiotics (100%) Penicillin, (90%) Tetracycline, (80%) Azithromycin, (80%) Oxacillin, (30%) Ciprofloxacin, (70%) Gentamicin, (40%) Vancomycin, (30%) Norfloxacin, (30%) Rifampicilin (60%). Trimethoprim/sulfamethoxazole (SXT) as shown in Figure 2. This result agreement with (21). This is because bacteria have the capacity to produce enzymes that can break down or modify drugs, change the antibiotic's target through the expression of genes that encode an alternative antibiotic target, prevent the uptake of antibiotics, or act as efflux pumps that push antibiotics out of the body of bacteria (22).

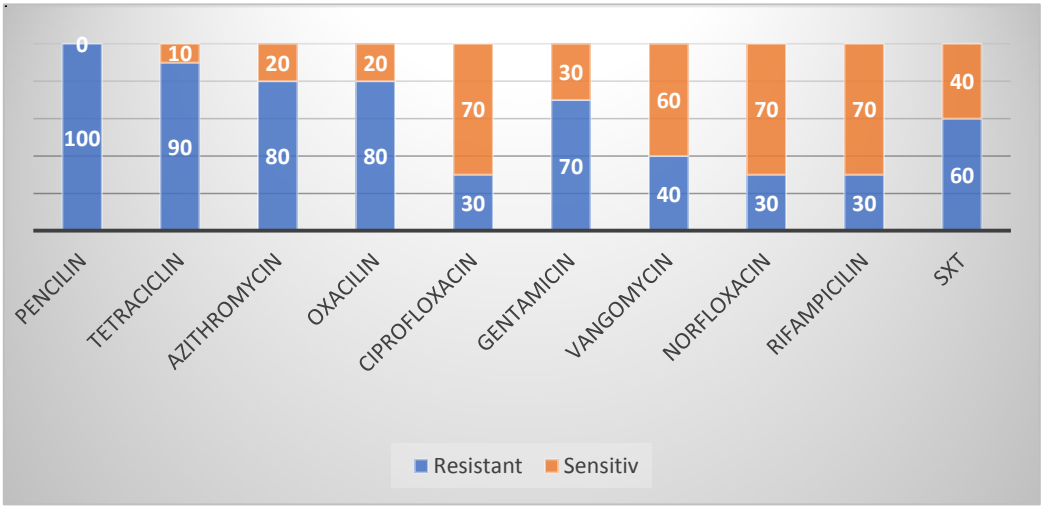

Figure -2 Antibiotics susceptibility against resistant bacteria

Synthesis and characterization of ZnS nanoparticles by pomegranate peel extract

In the current study as in figure 3, a clear change in color and the development of precipitates served as indicators of the biogenesis of ZnS nanoparticles from pomegranate peel extract. Bioretention includes converting metal ions into metal nanoparticles with the aid of phytochemicals such as polyphenols, polysaccharides, alkaloids, vitamins, and amino acids (17). Flavonoids can act as reducing agents for metal ions (18).

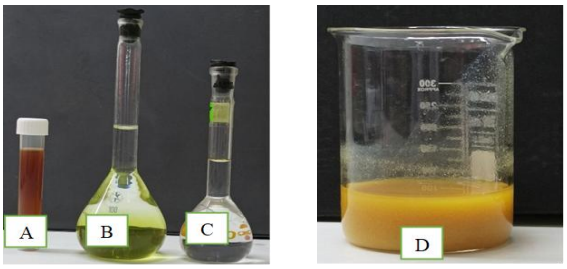

Figure -3 (A) Pomegranate peel extract, (B) Sodium sulfide (C) Zinc acetate, (D) ZnS nanoparticles

Ultraviolet (UV) spectra

The optical properties of the nanoparticles were examined using a UV-Visible spectrometer. The figure 4 displays the sample's absorbance in the nanoparticles. This result is lower by a peak at 270 nm wavelength compared to the absorption spectra result. (20).

Atomic force microscope (AFM)

An atomic force microscope (AFM) was employed to find the surface topography and morphology. The atomically thin nanoparticle surfaces may be seen in two and three dimensions due to AFM. The figure 5 shows the calculated average particle diameter at the nanoscale. The ZnS NPs generated by pomegranate peel extract were examined using AFM. The result shows that the average size of ZnS NPs was 20.10 nm (20).

Field emission scanning electron microscopy (FE-SEM)

According to the FESEM data, which are shown in Figure 6 the ZnS nanoparticles have a spherical shape, a homogenous and uniform distribution, and an average diameter under 40 nm.

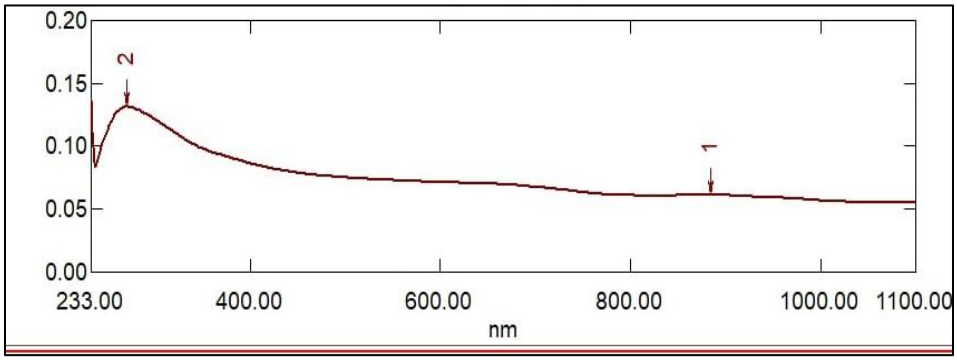

Figure -4 Absorbance peak of ZnS nanoparticles using UV-visible spectrometer

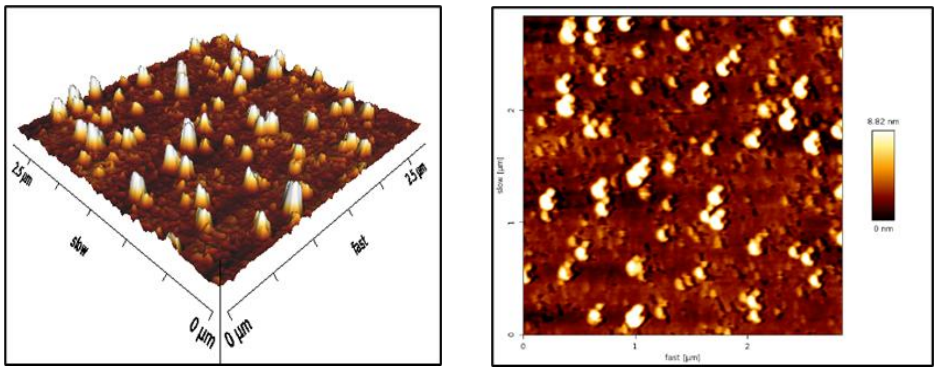

Figure -5 Atomic Force Microscopy illustrate 2D and 3D Topological of ZnS nanoparticles

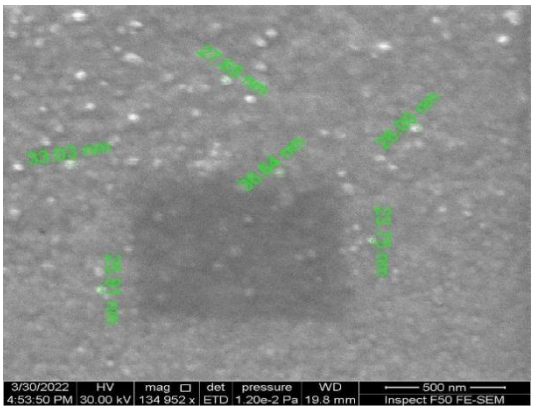

Figure -6 FE-SEM image of ZnS nanoparticles

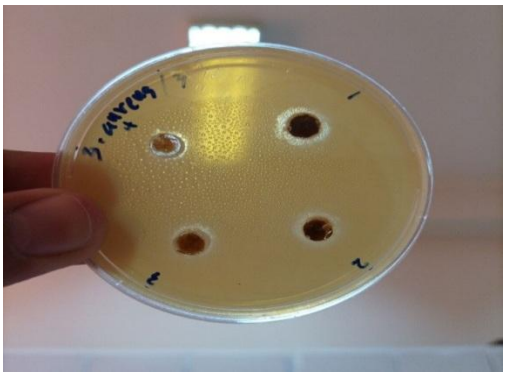

Figure -7 Plates showing the zones of inhibition upon testing biosynthesized nanoparticles against bacterial strains.

Antimicrobial activity Assay

Antimicrobial efficacy of ZnS nanoparticles

The ZnS nanoparticles' antimicrobial effectiveness was tested against the investigated pathogenic bacteria at concentrations of (400, 200, 100, 50, 25) µg/ml. as in the figure 7 and table 1 the diameter of inhibition zone increases with higher concentration of ZnSNPS. The extremely broad surface region of ZnSNPs, which allows for better contact with dangerous bacteria (25), is what causes their strong bactericidal activity. The ultra-fine size and greater surface area of ZnS nanoparticles may contribute to their impact, while their positively charged Zn+ ions attach to the negatively charged compounds present in bacterial cell walls, deactivating the cellular enzymes as a result and causing membrane permeability disruptions (15). The possibility that they might encourage the creation of reactive oxygen species is the second strategy (ROS). ROS production goes up when Zn ions, especially hydroxyl and singlet oxygen radicals, are present (24).

Table -1 Inhibition zones value (mm) produced by ZnS nanoparticles

No	ZnS NP concentration µg/ml	Diameter of inhibition zone (mm)
1	50	0
2	100	7
3	200	10
4	400	13

Conclusion

Pomegranate peel extract, an agricultural byproduct, was used to quickly and effectively manufacture ZnS nanoparticles and might be used for large-scale production. ZnS nanoparticle concentrations were tested against dangerous microorganisms. (400, 200, 100) µg\ml nanoparticles inhibited bacterial growth. The generation of ROS on the bacterial cell membrane is hypothesized to limit bacterial growth by damaging the membrane and causing protein dysfunction. Antibacterial ZnS nanoparticles are a promising alternate technique to stop drug resistance. This approach could be used in medical equipment and antimicrobial systems.

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