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Evolution the Synergistic effect of ZnS nanoparticles with antibiotic against multi-drug resistance bacteria

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Abstract

Wound swab samples that were obtained from infected wounds were used for the isolation and identification of bacterial isolates based on their appearance and biochemical properties. Staphylococcus aureus, gram-negative bacteria, and other gram-positive bacteria. Using the disk diffusion method, antibiotic susceptibility tests for S. aureus isolates were conducted against several antibiotic types. The results showed varying levels of resistance to various antibiotics; the ones with the highest level of resistance were chosen for more research. Meanwhile, zinc sulfide nanoparticles were synthesized using a quick and affordable green technique that used pomegranate peel extract. By using accepted techniques, the phytochemicals in pomegranate peel extract were tested. The outcomes confirmed that it contains flavonoids. These components served as capping, stabilizing, and reducing agents for ZnSNPs. The size and morphology of ZnS NPs were characterized by using Scanning electron microscopy (FE-SEM), UV-Vis spectrophotometer, atomic force microscopy (AFM), X-ray Diffraction analysis (XRD), and the results were 40nm, 270nm, 20nm, 90nm respectively. Evaluation of the synergistic effect of the synthesized nanoparticles revealed that the antibiotic activity. (Ciprofloxacin, azithromycin, tetracycline, gentamycin, and penicillin) enhanced in the presence of ZnS nanoparticles against pathogenic isolates chosen for the analysis.

Keywords: ZnS nanoparticles, S. aureus, antibacterial, wound swab, antibiotics

INTRODUCTION

Antibiotics are antibacterial substances that stop the development of bacterial infections by a variety of mechanisms [30]. Recent research has shown that bacterial infections are showing resistance to a range of antibiotics [18], which limits the efficacy of these drugs. The need for more effective agents and strategies to combat antimicrobial resistance has been sparked by the rising prevalence of bacterial resistance among pathogenic bacteria, which is most frequently brought on by the inappropriate or misuse of antibiotics [23]. Nanoparticles are tiny particles with a diameter of roughly 1-100 nm and a variety of uses [36]. Formulated metal nanoparticles, particularly those made from plant extracts, have recently been investigated as alternatives to conventional biocontrol agents [30,34]. Additionally, when used as therapy agents for illnesses brought on by multidrug-resistant Gram-negative and Gram-positive bacteria, these biosynthesized metal nanoparticles have shown to be extremely beneficial [15]. Plant extracts serve as reducing, stabilizing, and capping agents in the production of nanoparticles [25,26]. According to reports, the pomegranate plant contains significant amounts of organic acids, polyphenols, flavonoids, anthocyanins, alkaloids [20]. The efficacy of produced metal nanoparticles, however, is strongly correlated with their stability, size, size distribution, surface functionality, morphology, form, and the type of material employed in synthesis [24]. This is due to the fact that tiny nanoparticles can interact directly with the bacterial pathogens' cell membranes [32]. As a result, the synergistic interaction will enable the use of minimal concentrations of metal nanoparticles because they have low toxicity for humans when used with standard antibiotic dosages [22]. In order to develop new therapeutic agents, it may be beneficial to increase the synergistic action of bio-synthesized metal nanoparticles and antibiotics against multidrug resistant bacteria. The aim of this study was to identify and isolate bacterial pathogens that led to wound infection. Additionally, use the waste extract from Pomegranate peels to biologically synthesize ZnS nanoparticles, and then characterize those using UV-vis, FE-SEM, AFM, and XRD analyses. Moreover, we examined the synergistic action of antibiotics with ZnS nanoparticles against isolates that were multidrug resistant.

MATERIALS AND METHODS

Isolation and identification of clinical isolate

From December 2021 to March 2022, 79 samples were randomly taken from male and female patients at Medical City Hospital in Baghdad who had infected

wounds (burns, bedsores, and surgical wounds), specifically at the Ghazi Hariri Hospital for Specialized Surgery, Baghdad Teaching Hospital, Burns Specialist Hospital, and National Center for Educational Laboratories.

Every patient's pertinent data, including age (1-83 years), gender, and other details, were collected. The samples were brought into the lab for immediate macroscopical and microscopical analysis. The samples were grown on blood and MacConkey agar and kept at 37°C for 24 hours. The expanding colonies were subsequently developed on selective media. According to the identification scheme outlined by [16], after differentiating by relying on morphological and biochemical tests such the oxidase test, Catalase test, and IMVIC, API 20 E was then used to provide additional confirmation.

Antibiotic susceptibility testing by disk diffusion method

Use the McFarland standard solution to standardize the inoculum density for antimicrobial susceptibility tests. According to the requirements of the Clinical and Laboratory Standards Institute (CLSI), this test was conducted using the disk diffusion method on Muller-Hinton agar. In this test, the suspension was inoculated with sterile swabs after being streaked with 0.1 on a Mueller Hinton agar plate to compare it to the McFarland standard solution. After that, it was left at room temperature for five minutes. Each plate had five antimicrobial discs that were inserted using sterile forceps. The plate was then incubated for 18 to 24 hours at 37°C. Results were documented and compared to CLSI documentation's standard levels [4].

Preparation of Pomegranate peel extract

Pomegranate fruits were bought from a neighborhood shop in Baghdad, Iraq. To eliminate dust, peels were washed three times with double-distilled water. They were then allowed to dry for two weeks in the shade. As in Figure-1 peels weighing 50g were blended with blender and soaked in 500ml of double-distilled water for 72 hours. The mixture was then centrifuged for 10 minutes at 1500 rpm to remove biomaterials after being filtered using Whitman filter paper No. 1 to remove solid particles. The filtrate was stored at room temperature in a glass bottle with a tight lid for use in the manufacture of ZnS nanoparticles [27].

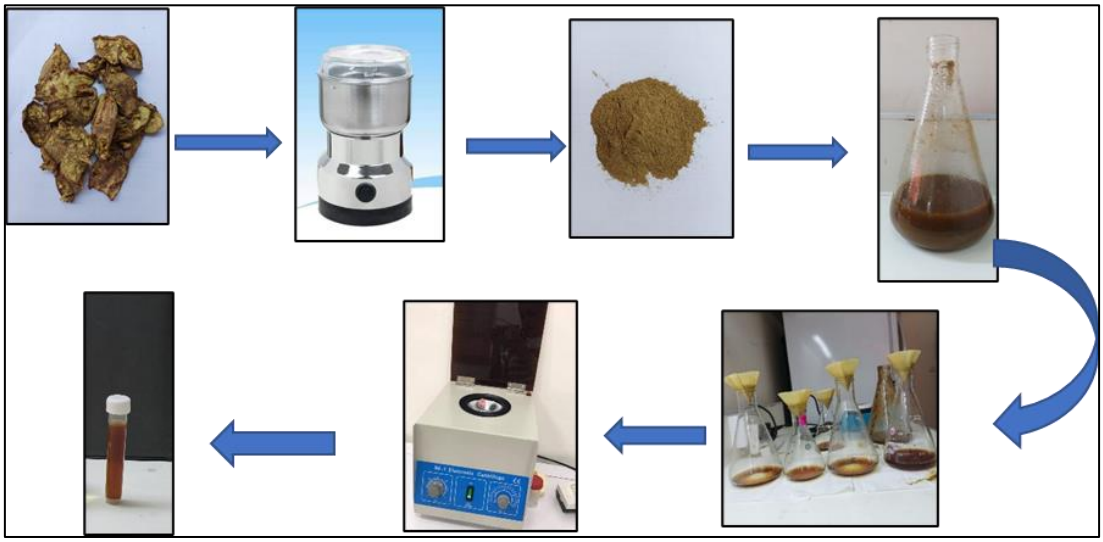

Fig 1. Preparation process for Pomegranate peel extract

Flavonoids Identification test

Several biochemical tests, including the Shinoda, Ferric Chloride, Lead acetate, Sodium hydroxide, were used to determine the presence of flavonoids in pomegranate peel extract [28].

Total flavonoid content

With a few minor modifications, the method outlined by [10] was used to determine the total flavonoid content. To 2 ml of dist. water, plant extracts (0.5 ml at 1 mg/ml) were added. Then 0.15 ml of 5 percent by weight $NaNO_2$ and 0.15 ml of 10 percent $AlCl_3$ were added and left to stand for 6 minutes each. The absorbance was measured at 510 nm after adding 2 ml of 4 percent w/v NaOH and 0.2 ml of distilled water. The mixture was left to stand for 15 minutes at room temperature. As a blank, distilled water was used. The calibration curve was generated using various catechin concentrations as the standard ($Y=0.0041 +0.0024$, $R2=0.9901$). The amount of flavonoid in 100 mg of dried extract was calculated as mg of catechin.

Preparation ZnS nanoparticles by using the extract of Pomegranate peel extract

Both sodium sulfide nonahydrate (99%, Na2S. 9H2O) and zinc acetate dihydrate (99%, Zn (CH3COO)2. 2H2O) were bought from Thomas Baker (India) and utilized without further purification. 2.4g of sodium sulphide nanohydrate was dissolved in 100 ml of double-distilled water using stirrer at room temperature. Then, 10 ml of pomegranate peel extract was added dropwise over a magnetic stirrer for 1 hour at 45 °C. As soon as the extract came into contact with sulfide ions, the mixture's color changed spontaneously to yellow. After then, 10 percent zinc acetate solution was gradually added to the pomegranate-sulfide combination. This caused homogeneous yellow-white suspended particles to develop, which was a sign that monodispersed zinc sulfide nanoparticles had formed. To remove any biological materials, the suspended zinc sulfide particles were centrifuged at 1700 rpm/min for 5 min. After that, they were thoroughly cleaned three times with deionized water. Following purification, zinc sulfide nanoparticles were dried in an incubator at 37 °C for the entire night.

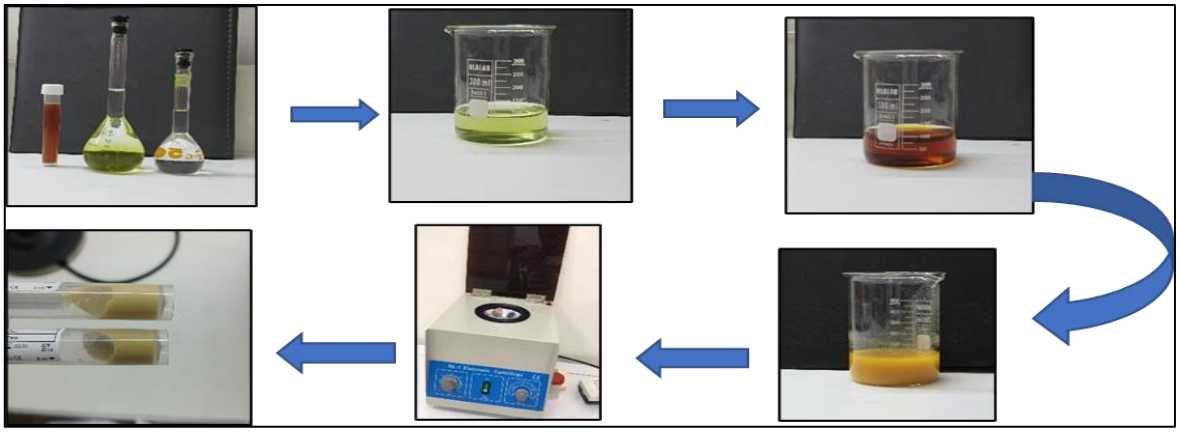

Fig 2. Synthesis of ZnS nanoparticles via Pomegranate peel extract

Characterization of synthesized ZnS nanoparticles

A UV-visible spectrometer was used to do UV-Vis analysis in order to find out how nanoparticles develop and stay stable. In order to learn more about the morphology and crystal structure of the produced particles, X-ray diffraction research was conducted. Symmetry, size, and shape are measured by XRD. The powdered form of synthesized nanoparticles was examined using an X-ray (°XRD 6000 SHIMADZU). Field emission scanning electron microscopy (FE-SEM) analysis with energy dispersive was used to examine the shape and microstructure of the produced ZnS nanoparticles. The pictures from the FE-SEM were captured using a (INSPECT F50) instrument**.**

Disk diffusion assay to evaluate synergistic effect

the disk diffusion method was used to examine the synergistic effects of green generated ZnS nanoparticles at different concentrations with various antibiotics for bactericidal activity against Staphylococcus aureus MDR isolates on Mueller Hinton agar. plates. Ciprofloxacin, azithromycin, tetracycline, gentamycin, and penicillin were the common antibiotic discs utilized. The inoculum was made by distributing on the plate after being diluted in 5 ml of NaCl and compared to a 0.5 McFarland standard. ZnS NPs at a final concentration (400, 200, 100, 50) µg/ml were added to the molten Mueller Hinton agar. The plates were then incubation at 37 ° C. The inhibitory zone was tested 24 hours later [7], as well as in comparison to Standard antibiotics that underwent antibiotic susceptibility testing.

RESULTS

Isolation and identification

Only 4 out of the 79 wound swab samples were negative for bacterial growth. Using biochemical tests, 75 isolates were found and identified. Like in Figure 3, 16 were Staphylococcus aureus, 50 were gram negative bacteria, and 9 were other gram-positive bacteria.

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 5.

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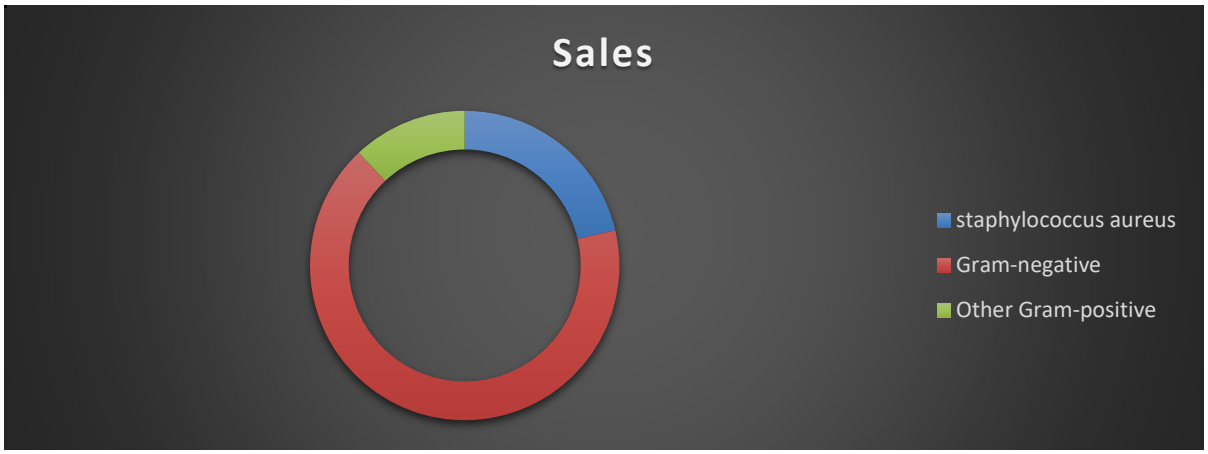

Fig 3. Distribution of bacterial isolates

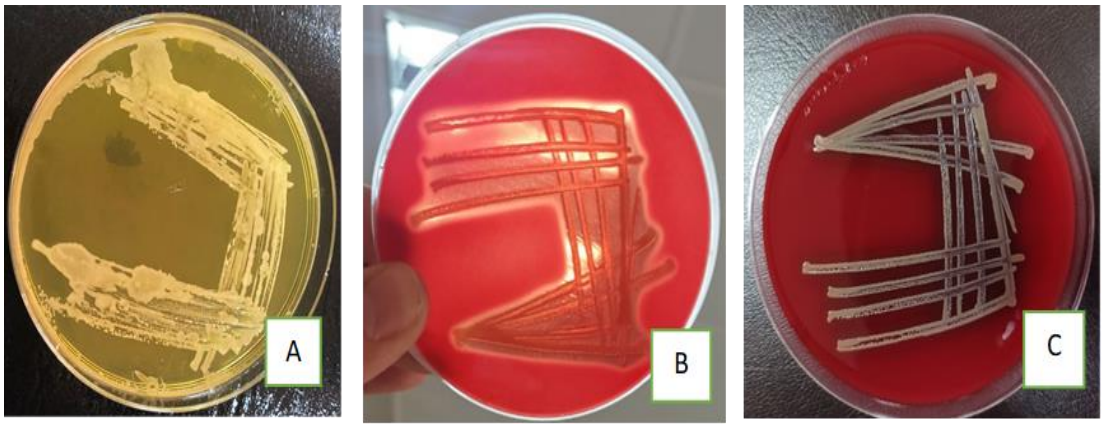

Fig 4. (A) S. aureus on Mannitol Salt Agar. (B) β hemolysis of S. aureus on blood agar (C) yellow color of S. aureus on blood agar

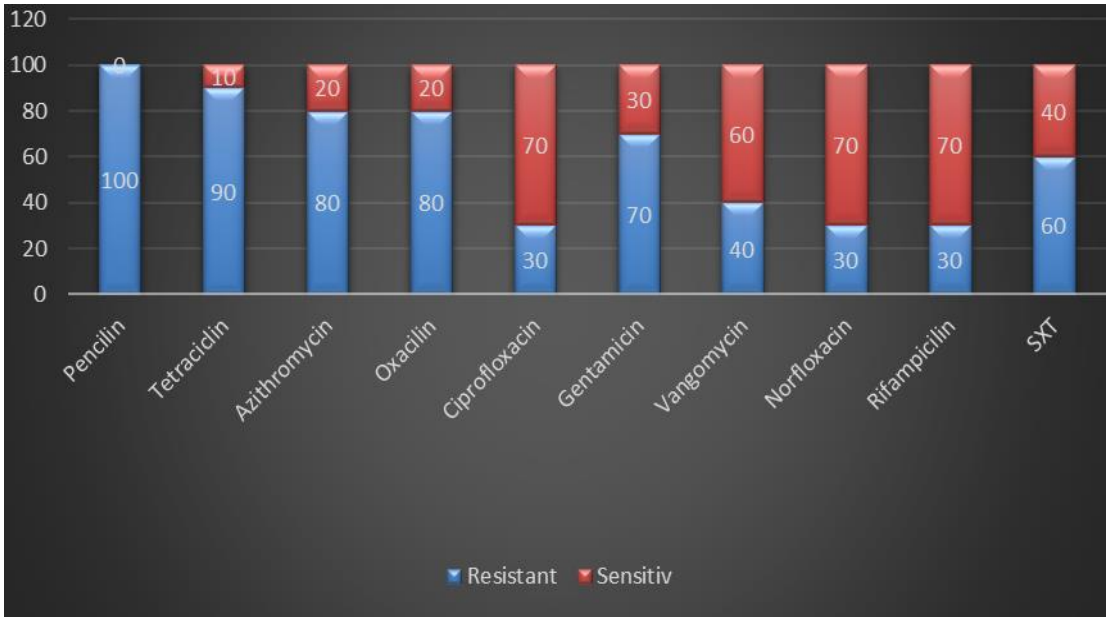

Fig 5. Antibiotics susceptibility against resistant bacteria

Pomegranate peel extract

Testing for flavonoids using phytochemicals

The tests produced positive results, as can be seen (Table 1) and Figure 6.

Table 1
The biochemical tests for Flavonoids Identification

Flavonoids Identification		
No	**biochemical tests**	**Result**
1	**Ferric Chloride test**	**positive**
2	**Shinoda test**	**positive**
3	**Lead acetate test**	**positive**
4	**Sodium hydroxide test**	**positive**

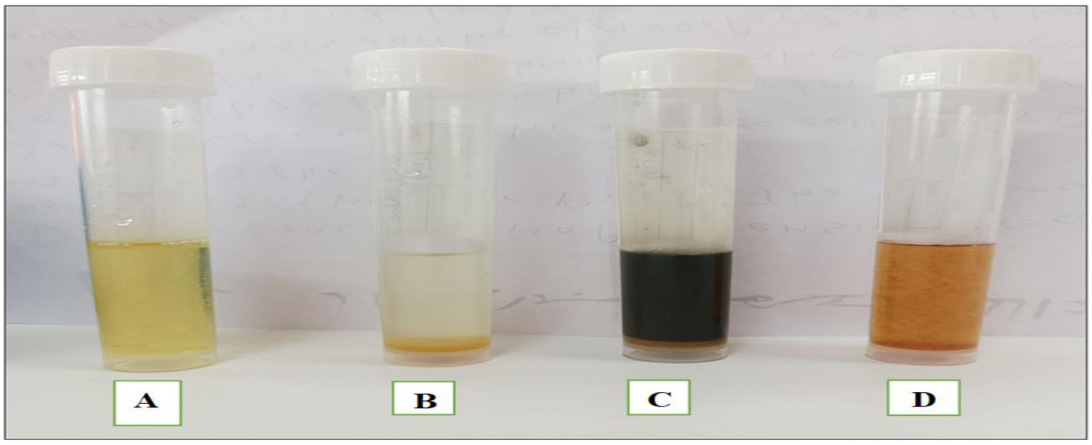

Fig 6. Phytochemical tests for the detection of flavonoid in Pomegranate peel extract (A) Sodium hydroxide test. (B) Lead acetate test. (C) Ferric Chloride test. (D) Shinoda test

Total flavonoid content

The total flavonoid concentration was 65.25 ± 0.037 mg catechin equivalent/100 mg dried extract, according to the calibration curve's calculation (R2 = 0.9901) (11).

Synthesis and Characterization of ZnS nanoparticles by Pomegranate peel extract

Visualization of color

In the current investigation, the biosynthesis of ZnS nanoparticles from pomegranate peel extract was detected by a distinct alteration in color and precipitate formation, with the help of phytochemicals like flavonoids, bioretention involved turning metal ions into metal nanoparticles.

Characterization of ZnS nanoparticles

ZnSNPs were identified as having a spherical shape by the acquired FE-SEM micrographs of nanoparticles, as shown in Figure 7. A homogenous and uniform distribution, and an average diameter under 40 nm.

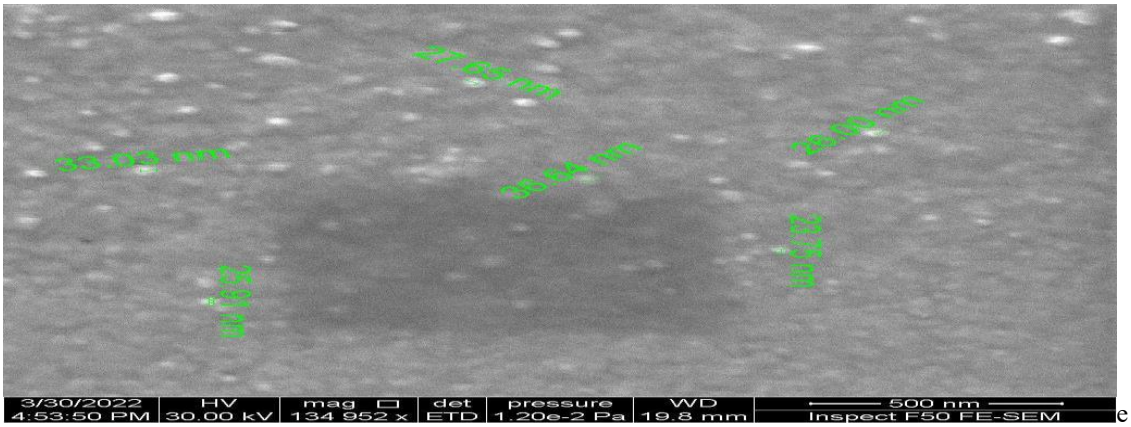

Fig 7. FE-SEM image of ZnS nanoparticles

Ultraviolet (UV) Spectra

Using a UV-Visible spectrometer, the optical characteristics of the nanoparticles were investigated. The absorbance of the sample in the nanoparticles is shown in Figure 8.The peak about 270 nm wavelength.

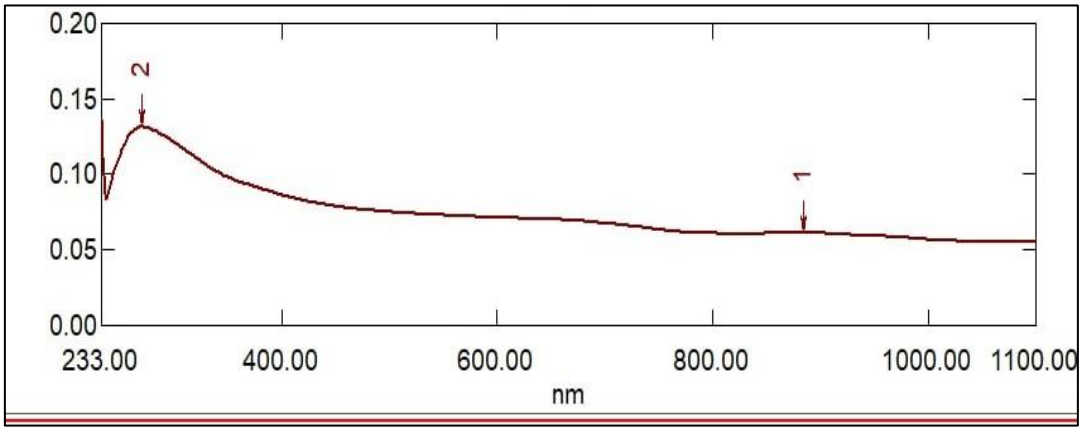

Fig 8. Absorbance spectrum of ZnS nanoparticles using UV-visible spectrometer

Atomic force microscope (AFM)

It was done by using an atomic force microscope (AFM) to detect the surface topography and morphology. AFM provides a two- and three-dimensional view of the atomically thin nanoparticle surfaces. The computed average particle diameter at the Nano scale is depicted in Figure 9. AFM was used to examine the ZnS NPs produced by Pomegranate peel extract. The outcome reveals that ZnS NPs were 20.10 nm in size on average.

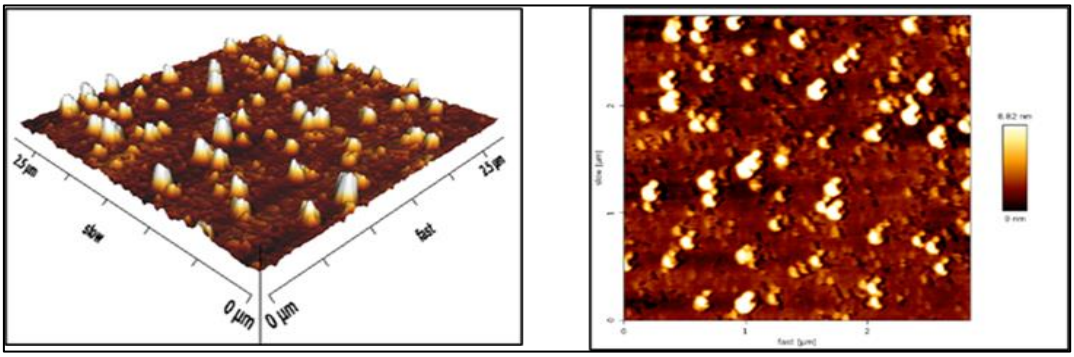

Fig 9. Atomic Force Microscopy illustrate 2D and 3D Topological of ZnS nanoparticles

X-ray Diffraction (XRD) analysis

The characteristic diffraction peaks of biosynthesized ZnS NPs are depicted in figure 10. XRD patterns at 27.12°, 48.19°, and 56.7°. These peaks were well matched to the (111), (220), and (311) Lattice parameters in the typical ZnS NPs diffraction data (JCPDS file no. 80-0020) of ZnS NPs was discovered to be a =1.6277 A °, confirming the spherical ZnS present, the size of the ZnS NPs determined by the Debye- Equation of Scherrer ($D = 0.94\lambda/d \cos\theta$). The ZnS NPs were 90 nm in size [31].

Antimicrobial activity Assay Synergistic effect of ZnSNPs with antibiotics by disk diffusion methods

This experiment used disk diffusion methods to examine the synergistic effects of ZnS NPs at deferent concentrations (400, 200, 100, 50, 25) µg/ml with deferent antibiotics (Ciprofloxacin, azithromycin, tetracycline, gentamycin, penicillin) against MDR S aureus. With increased ZnS NPs concentrations, the diameter of the inhibitory zone expands (Table 2).

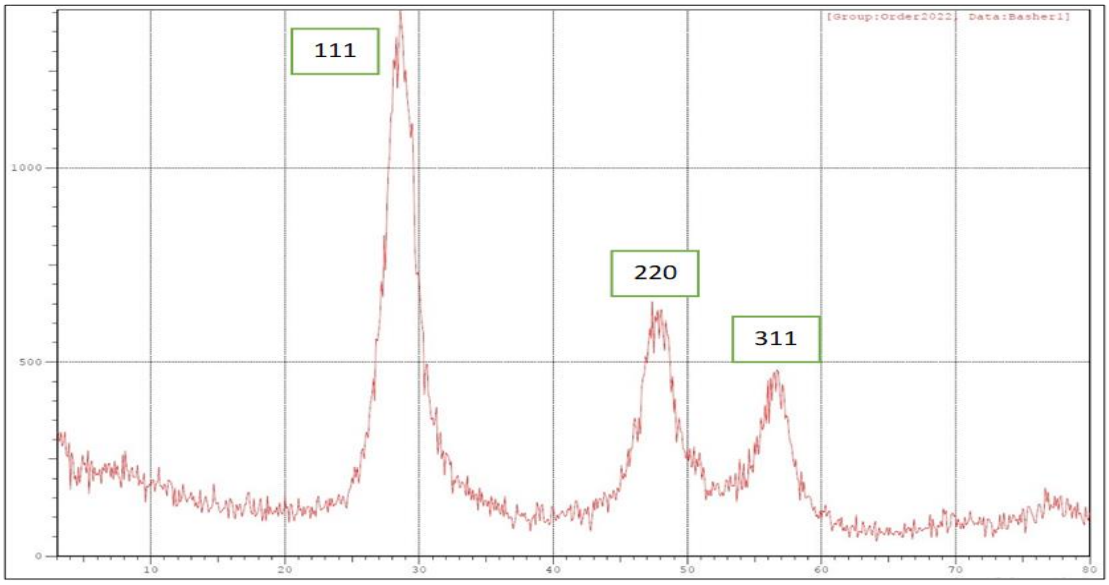

Fig 10. X-ray Diffraction (XRD) for biosynthesized ZnS nanoparticl

Table 2
The inhibition zone of different types of antibiotics with and without ZnS nanoparticles against multidrug resistance bacteria.

ZnS NP concentration µg/ml	Inhibition Zone of antibiotic (mm)									
	Ciprofloxacin 5 µg		Azithromycin 15 µg		Tetracycline 30 µg		Gentamycin 10 µg		Penicillin 10 units	
	without ZnS NP	with ZnS NP	without ZnS NP	with ZnS NP	without ZnS NP	with ZnS NP	without ZnS NP	with ZnS NP	without ZnS NP	with ZnS NP
400	26	38	R	32	R	26	20	30	15	30
200	26	36	R	30	R	23	20	23	15	24
100	26	35	R	30	R	12	20	20	15	23
50	26	30	R	12	R	10	20	20	15	23

DISCUSSION

Only 4 out of the 79 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 our experiment. The bacterial isolates tested positive for antibiotic resistance, (100%) Penicillin, (90%) Tetracycline, (80%) Azithromycin, (80%) Oxacillin, (30%) Ciprofloxacin, (70%) Gentamicin,

(40%) Vancomycin, (30%) Norfloxacin, (30%) Rifampicilin (60%) Trimethoprim/sulfamethoxazole (SXT). This result agreement with [6],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 [5].In the other hand The outcomes of Testing for flavonoids using phytochemicals produced positive results were consistent with [35], flavonoids bioretention involved turning metal ions into metal nanoparticles. For metal ions, flavonoids function as reducing agents [17]. However, the creation of nanoparticles is caused by the functional groups of flavonoids. In order to create nanoparticles [24], flavonoids are converted from enol to keto, which results in the reduction of metal ions [9].The chelating properties of flavonoids, such as

quercetin, which may chelate with the carbonyl, hydroxyl, and catechol groups in three different circumstances [12]. ZnSNPs were identified as having a spherical shape by the acquired FE-SEM micrographs of nanoparticles, a homogenous and uniform distribution, and an average diameter under 40 nm, which it is very similar to the result of the research [8] and it was between 25 nm to 50 nm. The outcome of Atomic force microscope (AFM) reveals that ZnS NPs were 20.10 nm in size on average, this outcome agrees with biological preparation of ZnS NPs in [14]. The combined action of ZnS NPs and antibiotics can be used to treat the development of resistant pathogenic bacteria due to the synergistic impact of ZnS nanoparticles and antibiotics on antibacterial activity. Additionally, it would reduce how much antibiotic was given out. The synergistic effect may be increased by the bonding reaction between ZnS NPs and antibiotics. This effect may be caused by either increasing the drug's bio-availability after conjugation in the bacterial cell membrane or by assimilatory effect of both components. The inhibition zone of the antibiotics has increased in the presence of ZnS nanoparticles. As a result, using ZnS NPs in conjunction with an antibiotic shown a synergistic impact. Previous research suggested that ZnS NPs might function in two different ways. The first approach is that they might promote the production of reactive oxygen species (ROS). Zn ions enhance the level of ROS generation including hydroxyl and singlet oxygen radicals. Additionally, many periplasmic enzymes and proteins that are crucial for sustaining regular physiological improvements in bacterial cells can be diminished by ROS. In contrast, the ZnS NPs- antibiotic can readily penetrate the cell membrane barrier and demonstrate its bioactivity [21,13]. This paper outlines a possible method for developing antibiotic compounds to combat bacteria with multiple drug resistance. To fight the threat posed by the emergence of new antibiotic resistance mechanisms in MDR bacteria, it may be helpful to design a new therapy.

CONCLUSION

Pomegranate peel, a byproduct of agriculture, was effectively used to create ZnS nanoparticles quickly and consistently, and it would be suitable for developing a biological technique for large - scale production. The focus of the study was on a potential antibiotic and ZnS NPs combination, which demonstrated improved antibacterial activity and was characterized as synergism. In this context, the synergistic antibacterial properties of ZnS nanoparticles with this antibiotic are viewed as an appealing alternative method to combat the rising prevalence of drug resistance. Such an approach is likely to have many potential applications in medical devices and microbial resistant systems. Since these antibiotics have limited effectiveness on resistant bacteria and have several side effects. We can reduce their concentration by mixing them with ZnS nanoparticles to lower adverse effects.

LIST OF REFERENCES

1. Andrade, J. C., M. F., Morais-Braga, G. M., Guedes, S. R., Tintino, M. A., Freitas, I. R., Menezes. Enhancement of the antibiotic activity of aminoglycosides by alpha-tocopherol and other cholesterol derivates. Biomedical Pharmacother. 2014 68, 1065–1069 22. https://doi.org/10.1016/j.biopha.2014.10.011
2. Baruah, J. M., S., Kalita and J., Narayan. Green chemistry synthesis of biocompatible ZnS quantum dots (QDs): their application as potential thin films and antibacterial agent. International Nano Letters. 2019 9(2), 149-159. https://doi.org/10.1007/s40089-019-0270-x
3. Brandelli, A., A. C., Ritter, and F. F., Veras. Antimicrobial Activities of Metal Nanoparticles. In Metal Nanoparticles in Pharma. 2017 (pp. 337-363). https://doi.org/10.1007/978-3-319-63790-7_15
4. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing, 24th informational supplement. 2022 34 (1), Pennsylvania USA.
5. Dizaj, S. M., F., Lotfipour, M., Barzegar-Jalali, M. H., Zarrintan, and K., Adibkia. Antimicrobial activity of the metals and metal oxide nanoparticles. Mater Sci Eng C Mater Biol Appl. 2014 44, 278-284. https://doi.org/10.1016/j.msec.2014.08.031
6. Duran, N., B.,Ozer, GG., Duran, Y., Onlen, C. Demir. Antibiotic resistance genes and susceptibility patterns in staphylococci. Indian Journal of Medical Research. 2012 135(3):389–96.
7. Fayaz, A. M., K., Balaji, M., Girilal, R., Yadav, P.T., Kalaichelvan, and R. Venketesan. Biogenic synthesis of silver nanoparticles and their synergistic effect with antibiotics: a study against gram-positive and gramnegative bacteria. Nanomedicine: Nanotechnology, Biology, and Medicine. 2010 61 (6), pp: 103–109. https://doi.org/10.1016/j.nano.2009.04.006
8. Hazra, C., D., Kundu, A., Chaudhari, and T. Jana. Biogenic synthesis, characterization, toxicity and photocatalysis of zinc sulfide nanoparticles using rhamnolipids from Pseudomonas aeruginosa BS01 as capping and stabilizing agent. Journal of Chemical

Technology and Biotechnology. 2013 88(6), 1039-1048. http://dx.doi.org/10.1002%2Fjctb.3934

9. Hussain, M., N. I., Raja, M., Iqbal, and S. Aslam. Applications of plant flavonoids in the green synthesis of colloidal silver nanoparticles and impacts on human health. Iranian Journal of Science and Technology, Transactions A: Science. 2019 43(3), 1381-1392. https://doi.org/10.1007/s40995-017-0431-6
10. Ibraheem, R. M., A. A., Mhawesh, and K. W. Abood. Estimation of the whole flavonoid, antioxidant, anti-bacterial challenge concerning viola odorata (banafsha) methanolic extract. The Iraqi Journal of Agricultural Science. 2018 49(4), 655. https://doi.org/10.36103/ijas.v49i4.75
11. Jaisinghani, R. N., S., Makhwana, and A. Kanojia. Study on antibacterial and flavonoid content of ethanolic extract of Punica granatum (pomegranate) peel. Microbiology research. 2018 9(1), 7480.47 https://doi.org/10.4081/mr.2018.7480
12. Król, A., V., Railean-Plugaru, P., Pomastowski, and B. Buszewski. Phytochemical investigation of Medicago sativa L. extract and its potential as a safe source for the synthesis of ZnO nanoparticles: The proposed mechanism of formation and antimicrobial activity. Phytochemistry Letters. 2019 31, 170-180. https://doi.org/10.1016/j.phytol.2019.04.009
13. Kumar, G. A., H. S. B., Naik, R., Viswanath, G., Krishnamurthy, and P. Walmik. Optical and antibacterial evaluation of biofunctionalized Cu:ZnS nanoparticles. Emergent Materials. 2020 3(6), 937-946. http://dx.doi.org/10.1007/s42247-020-00141-x
14. Kumar, M. S., M., Saroja, and M. Venkatachalam. Acalypha indica and Curcuma longa plant extracts mediated ZnS nanoparticles. Material Science Research India. 2019 16(2), 174-182. http://dx.doi.org/10.13005/msri/160210
15. Maeh, R. K., A. I., Jaaffar, and K. F., Al-Azawi. Preparation of Juniperus extract and detection of its antimicrobial and antioxidant activity. The Iraqi Journal of Agricultural Science. 2019 50(3), 1153-1161. http://dx.doi.org/10.36103/ijas.v50i4.759
16. Mahon, C.R., D.C. Lehman, and G. Manuselis. Textbook of Diagnostic Microbiology, 3rd edition, Elsevier. 2007 p. 508.
17. Mat Yusuf, S. N. A., C. N. A., Che Mood, N. H., Ahmad, D., Sandai, C. K., Lee, and V. Lim. Optimization of biogenic synthesis of silver nanoparticles from flavonoid-rich Clinacanthus nutans leaf and stem aqueous extracts. Royal Society open science. 2020 7(7), 200065.48 . https://doi.org/10.1098/rsos.200065
18. Mustafa H.N., and I. Al -Ogaidi. Efficacy of zinc sulfide- chitosan nanoparticles against bacterial diabetic wound infection. Iraqi journal of agricultural sciences. 2023 54 (1):1-17.
19. Nino-Martinez, N., M. F., Salas G. A., Orozco, Martinez-Castanon, F., Torres Mendez, and F. Ruiz. Molecular Mechanisms of Bacterial Resistance to Metal and Metal Oxide Nanoparticles. Int J Mol Sci. 2019 20(11). https://doi.org/10.3390/ijms20112808
20. O., Lazeeza. "Antioxidant activity of pomegranate". Iraqi journal of agricultural sciences. 2021. 52 (1):196-203. https://doi.org/10.36103/ijas.v52i1.1251
21. Panthi, G., R., Ranjit, S., Khadka, K. R., Gyawali, H.-Y., Kim, and M. Park. Characterization and antibacterial activity of rice grain-shaped ZnS nanoparticles immobilized inside the polymer electrospun nanofibers. Advanced Composites and Hybrid Materials. 2020 3(1), 8-15.https://link.springer.com/article/10.1007/s42114-020-00141-9
22. Pati, R., R. K., Mehta, S., Mohanty, A.,Padhi, M., Sengupta, B.,Vaseeharan, C., Goswami, and A. Sonawane. Topical application of zinc oxide nanoparticles reduces bacterial skin infection in mice and exhibits antibacterial activity by inducing oxidative stress response and cell membrane disintegration in macrophages. Nanomedicine. 2014 10(6), 1195-1208. https://doi.org/10.1016/j.nano.2014.02.012
23. Raghunath, A., and E. Perumal. Metal oxide nanoparticles as antimicrobial agents: a promise for the future. Int J Antimicrob Agents. 2017 49(2), 137-152.
24. Riaz, S., N., Fatima Rana, I., Hussain, T., Tanweer, A., Nawaz, F., Menaa, and S. Ali, M. Effect of flavonoid-coated gold nanoparticles on bacterial colonization in mice organs. Nanomaterials. 2020 10(9), 1769.49 . https://doi.org/10.3390%2Fijms222312890
25. Ruddaraju, L. K., S. V. N., Pammi, G. S., Guntuku, V. S., Padavala, and V. R. M. Kolapalli. A review on anti-bacterials to combat resistance: From ancient era of plants and metals to present and future perspectives of green nano technological combinations. Asian J Pharm Sci. 2020 15(1), 42-59. https://doi.org/10.1016/j.ajps.2019.03.002
26. Saif, S., A., Tahir, and Y. Chen. Green Synthesis of Iron Nanoparticles and Their Environmental Applications and Implications. Nanomaterials (Basel). 2016 6(11).
27. Salem, N. M., L. S., Albanna, and A. M. Awwad. Nano-structured zinc sulfide to enhance cucumis sativus (CUCUMBER) plant growth. ARPN J Agric

Biol Sci. 2017 12, 67-173. https://doi.org/10.3390%2Fnano6110209

28. Sarah N. L., and F. M. Zainab. Activity of marticaria chamomilla crude and total flavonoid extracts as anti-virulence factor for clinically isolated pseudomonas aeruginosa. Iraqi journal of agricultural sciences. 2023 54 (1):59-69.). https://doi.org/10.36103/ijas.v54i1.1676
29. Šavikin, K., J., Živković, A., Alimpić, G., Zdunić, T., Janković, S., Duletić-Laušević, and N. Menković. Activity guided fractionation of pomegranate extract and its antioxidant, antidiabetic and antineurodegenerative properties. Industrial Crops and Products. 2018 113, 142-149.
30. Singh, P., A., Garg, S., Pandit, V., Mokkapati, and I. Mijakovic. Antimicrobial Effects of Biogenic Nanoparticles. Nanomaterials (Basel). 2018 8(12). https://doi.org/10.3390%2Fnano8121009
31. S. M., Ibrahim, Dheeb, B. I., Al-dujayli, , I. M., Abbas, Q. A., Ali, A. H., Ramizy, A., ... & Hussain, A. F. Study the antifungal activity of ZnS: Mn nanoparticles against some isolated pathogenic fungi. In Journal of Physics. 2019 Feb. Conference Series (Vol. 1178, p. 012008). IOP Publishing. http://dx.doi.org/10.1088/1742-6596/1178/1/012008
32. Sorbiun, M.; E.; Shayegan Mehr, A.; Ramazani, A. Mashhadi Malekzadeh. Biosynthesis of metallic nanoparticles using plant extracts and evaluation of their antibacterial properties. Nanochem. Res. 2018 3, 1–16. https://doi.org/10.22036/ncr.2018.01.001
33. Tang, Z. X., and B. F. Lv. MgO nanoparticles as antibacterial agent: preparation and activity. Brazilian Journal of Chemical Engineering. 2014 31(3), 591-601. https://doi.org/10.1590/0104-6632.20140313s00002813
34. Vega-Jiménez, A.L.; A.R.; Vázquez-Olmos, E.; Acosta-Gío, M.A. Álvarez-Pérez. In-vitro antimicrobial activity evaluation of metal oxide nanoparticles. In Nanoemulsions: Properties, Fabrication and Application; Koh, K.S., Wong, V.L., Eds.; IntechOpen: Rijecka, Croatia. 2019 pp. 1–18. DOI: 10.5772/intechopen.84369
35. Vimalkumar, C. S., V. B., Hosagaudar, S. R., Suja, V., Vilash, N. M., Krishnakumar, and P. G. Latha. Comparative preliminary phytochemical analysis of ethanolic extracts of leaves of Olea dioica Roxb., infected with the rust fungus Zaghouania oleae (EJ Butler) Cummins and non-infected plants. Journal of Pharmacognosy and phytochemistry. 2014 3(4).
36. Wang, L., C., Hu, and L. Shao. The antimicrobial activity of nanoparticles: present situation and prospects for the future. Int J Nanomedicine. 2017 12, 1227-1249https://doi.org/10.2147/IJN.S121956