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Antibacterial and Cytotoxicity Effects of *Aloe Vera* Leaves Extract Gel and Zinc Oxide Nanoparticles (ZnO NPs) on Bacterial Skin Infections

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Abstract

The skin establishes an immunological barrier against bacterial infections. Despite the presence of several bacterial species on the skin, they typically lack the capacity to infect people. A bacterial skin infection can vary in size from little areas to extensive coverage and in severity from benign to life-threatening. Four identified isolates by VITEK-2 for *Staphylococcus* spp; S1, S1 Control, S2 and S2 Control were used in this study. S1, S1C, S2 and S2C were collected from alopecia disease assigned by S1, S2 and the healthy hair skin from the same patients (S1C and S2C). The ZnO NPs was green synthesized (GS- ZnO NPs) by aqueous extraction of *Aloe vera* leaves gel (AG) and characterized by UV-V is spectrometry, Atomic force microscope (AFM), Fourier transform infrared spectroscopy (FTIR), Scanning electron microscope (SEM) and XRD spectroscopy. Minimum inhibitory concentration (MIC) was determined for AG and GS-ZnO NPs, mixture of standard ZnO NPs and AG leaves extract in addition to standard ZnO NPs against *Staphylococcus* spp. (S1,S1C,S2 and S2C). Cytotoxicity was assessed by MTT assay against normal skin cell, Human dermal fibroblast (HdFn). Compared to the control group, the viability rate was drastically reduced at a dosage of 400 mg/mL. Results showed that the GS-ZnO NPs have the better MIC result (0.400 ±0.03, 0.650 ±0.07, 0.478 ±0.08 b, 0.632 ±0.08) on S1, S1C, S2 and S2C, respectively. The HdFn were slightly more affected by the cytotoxic effects of GS-ZnO NPs, at concentration of 400 mg/mL compared to the control group (100%) ($p < .05$). In conclusion, based on the outcomes of the microdilution experiment and the cytotoxicity impact of HdFn, GS-ZnO NPs displayed antibacterial activities against *Staphylococcus* spp., while also displaying cytotoxic effects on HdFn cells at 400 mg/ml.

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1. Introduction

Skin provides an immune barrier against microorganisms. The skin contains various bacterial species, however they rarely infect people. Bacterial skin infections can range from little to large and from benign to life-threatening [1]. Skin damage makes wounds susceptible to bacterial infection, slowing skin regeneration and healing. Skin infections caused by multidrug-resistant bacteria can kill people with significant burns and chronic illnesses like diabetes. Thus, a broad-spectrum drug that kills bacteria without antibiotics is in high demand [2]. The most common bacterial pathogens that cause skin infection are *Staphylococcus* and *Streptococcus* [3]. Complex etiology and pathophysiology characterize autoimmune alopecia. Every autoimmune disease depends on genetics. The collapse of immunological privilege in hair follicles causes scalp loss and alopecia [4]. The microbiota, a bacterial ecology in a specific body location, may influence the genesis of alopecia, as it does other autoimmune illnesses [5]. *Aloe vera* leaves Gel is believed to provide therapeutic effects. For decades, it has been utilized for a variety of ailments, including mild fever, wounds and burns, gastrointestinal disorders, diabetes, sexual vitality

and fertility issues, as well as cancer, immunological modulation, Acquired Immunodeficiency Syndrome (AIDS), and several skin infections[6].This plant exhibits antibacterial action against strains of *Staphylococcus aureus, Escherichia coli, Shigella flexneri, Helicobacter pylori,* and *Pseudomonas aeruginosa* [7].The inhibitory activity of *Aloe vera* Gel is linked to its phenolic chemicals and polysaccharides, which may interact with several bacterial target sites [8]. Extracts from AG exhibit advantageous diverse qualities, including enhancing blood flow in scar tissue, penetrating tissue, inducing tissue numbness, inhibiting bacterial, fungal, and viral proliferation, and promoting capillary expansion [9]. Zinc oxide nanoparticles (ZnONPs) are metal nanoparticles that have garnered significant interest due to their distinctive shape and antibacterial efficacy against both Gram-positive and Gram-negative bacteria [10, 11]. The antibacterial efficacy of ZnONPs is associated with their interactions with cell membrane constituents, including bonding with amino acids, membrane depolarization and the stimulation of reactive oxygen species (ROS) formation [12, 13]. Moreover, nanofabrication has challenges related to aggregation, potential cytotoxicity, and high material costs [14]. This study's goal was to determine the antibacterial activity and cytotoxicity effects of green synthesized ZnONPs by AG, as well as how these substances affected the bacterial species that were isolated from skin infections.

2. Materials and Methods

2.1. Specimens collection and preparation

One hundred eight specimens were collected and procced as mentioned in the Ahmed and Ahmed research [15].

2.2. Green synthesis of ZnONPs

Aleo vera leaves gel was prepared according to Ecer (2022) [16] ..

2.3. Preparation of ZnO Nanoparticle

ZnO Nanoparticle was prepared as mentioned by Nawar *et al.* (2024).

2.4. Characterization of ZnONPs

The Characterization of ZnO nanoparticles was done by UV–V spectroscopy, Atomic force microscopy (AFM), Fourier transform infrared spectroscopy (FTIR), and X-Ray Diffraction (XRD) which carried out in Dep. of Chemistry, College of Science, Al-Nahrain University.

2.5. Determination the MIC of AG and GS-ZnONPs, mixture of standard ZnONPs and AG leaves extract in addition to standard ZnONPs against Staph. aureus

Aloe vera leaves Gel and GS-ZnONPs, mixture of standard ZnONPs and AG leaves extract in addition to standard ZnONPs were tested for their MIC against *Staphylococcus* isolates. It was used the microdilution method as recommended by the CLSI [18]. A series of concentrations of each treatment were prepared in Mueller Hinton Broth(MHB) medium with a final volume of 180 µl in each well and in lines. The wells were inoculated with 20 µl of active isolates cultures in MHB medium for each treatment. Then the plates were incubated at 37°C for 24 hours. Growth was determined ELISA device in 650 nm

2.6. Cytotoxicity Test

The cytotoxic activity was evaluated on the normal skin cell line (HdFn) using a colorimetric test in biology Science department/ AL-Nahrain university according to the study of TAŞTAN *et al.* (2024) [9].

2.7. Statistical Analysis

Data from separately conducted experiments are presented as mean ± standard deviation (SD). The Statistical Package for the Social Sciences (SPSS) program (2019) was utilized to assess the impact of different groups on study parameters, namely the Minimum Inhibitory Concentration (MIC). The least significant difference (LSD) method was employed to compare means significantly in this investigation.

3. Results and Discussion

3.1. Aloe vera leaves gel extraction

From 1 kilo of Aloe vera, 480 gm of gel was extracted then put in mixer, then centrifuged and purified from impurities and used for different purpose.

3.2. Green synthesis of ZnO NPs

The ZnONPs was synthesized from 24g Zncl2, 0.06 g of ZnONPs were obtained then mixed with AG, the color of extract was transformed from colorless to the brown after adding the salt and nano formation after 24 h of shaking as shown in figure 1.

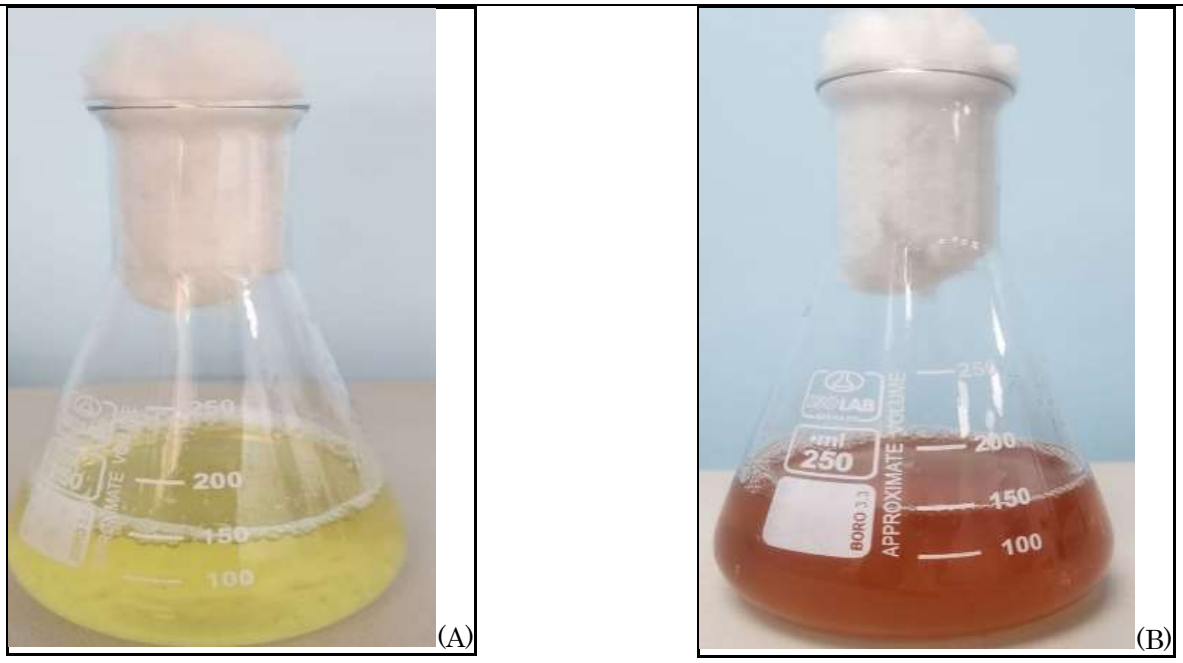
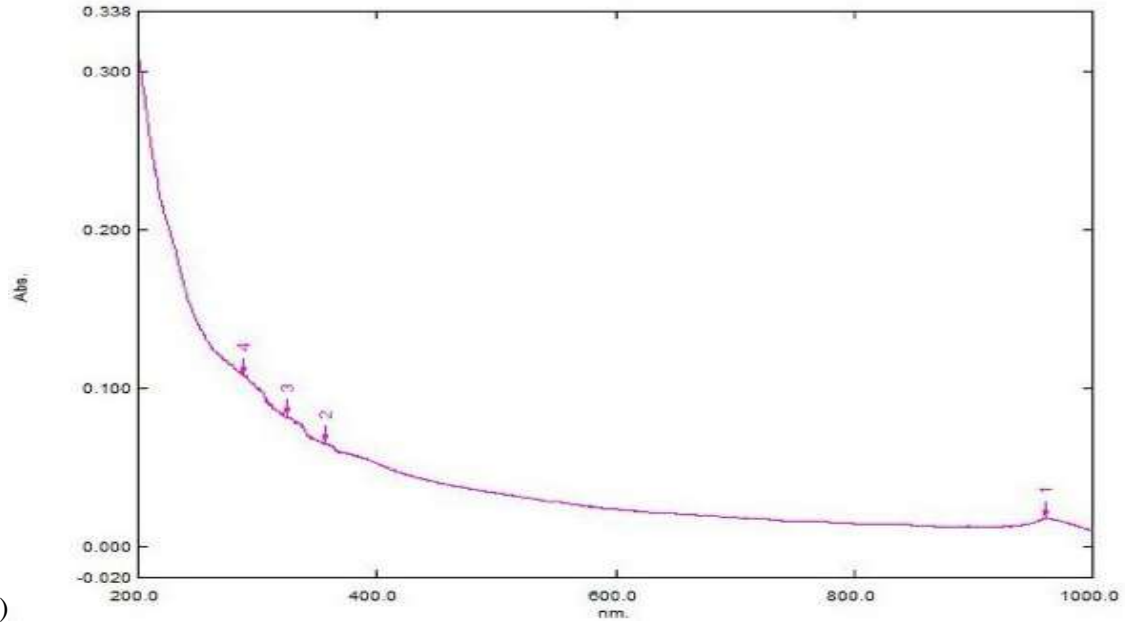


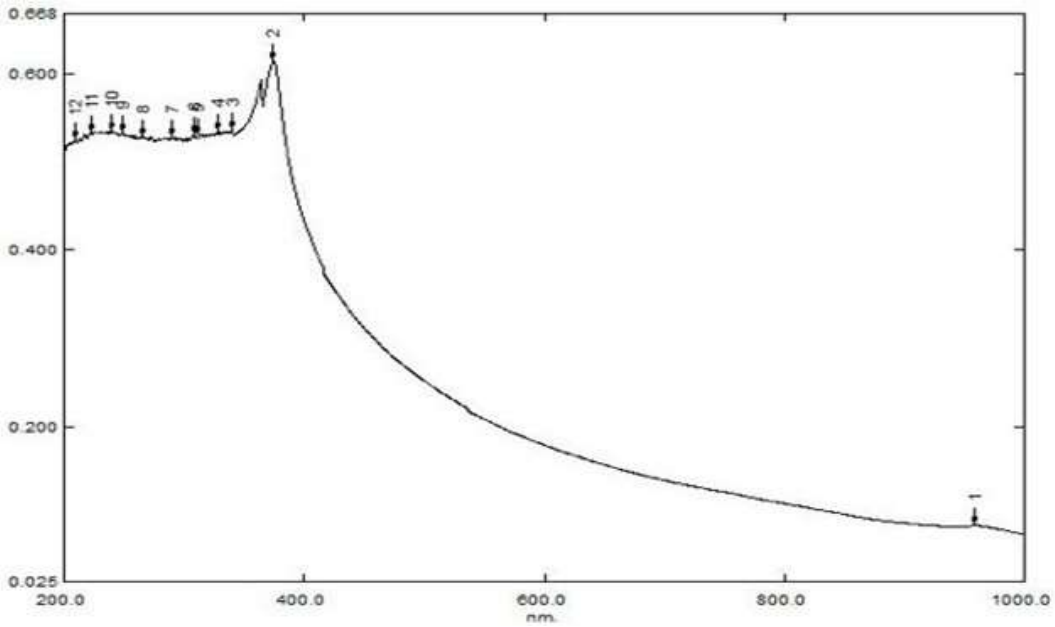
Figure1: Color change of nanoformation (ZnO NPs) (A): colorless extract (B): brown colored extract after 24 hour of adding salt.

3.3. Characterization of green synthesized ZnONPs and standard ZnO NPs

According to the study of WU et al. [19], The Characterization were carried out in Chemistry Department/Al-Naharain University. The UV-Vis absorption spectra of a ZnO NP can elucidate the absorption edges associated with the semiconductor band structure, figure (2). XRD examination identifies the crystalline structure of the biosynthesized ZnO particles. The XRD pattern presented in figure 3 demonstrates the formation of ZnO particles at diffracted intensities between 20° and 80°. In FTIR spectrum (Figure 4), the peak observed at 3737 cm^{-1} is attributed to the stretching vibration of the hydroxyl group and the peak appeared at 1621 cm^{-1} is attributed to the amino groups present in alcohol, phenol, and amines in the aloe vera extract involved in the NPs synthesis. AFM analysis of ZnONPs was performed, 3D image of the ZnO nanoparticles shows that the average size is 3000 nm. As average diameter were (62.45) for Standard ZnO NPs and (60.69) for GS-ZnO NP. Different magnification ranges were conducted to give an insight into the roughness and topography of nanoparticles. As shown in Figure 5. SEM images revealed that it was essentially spherical and uniform in appearance with the diameter ranging from 20µl to 500nm, (figure 6).

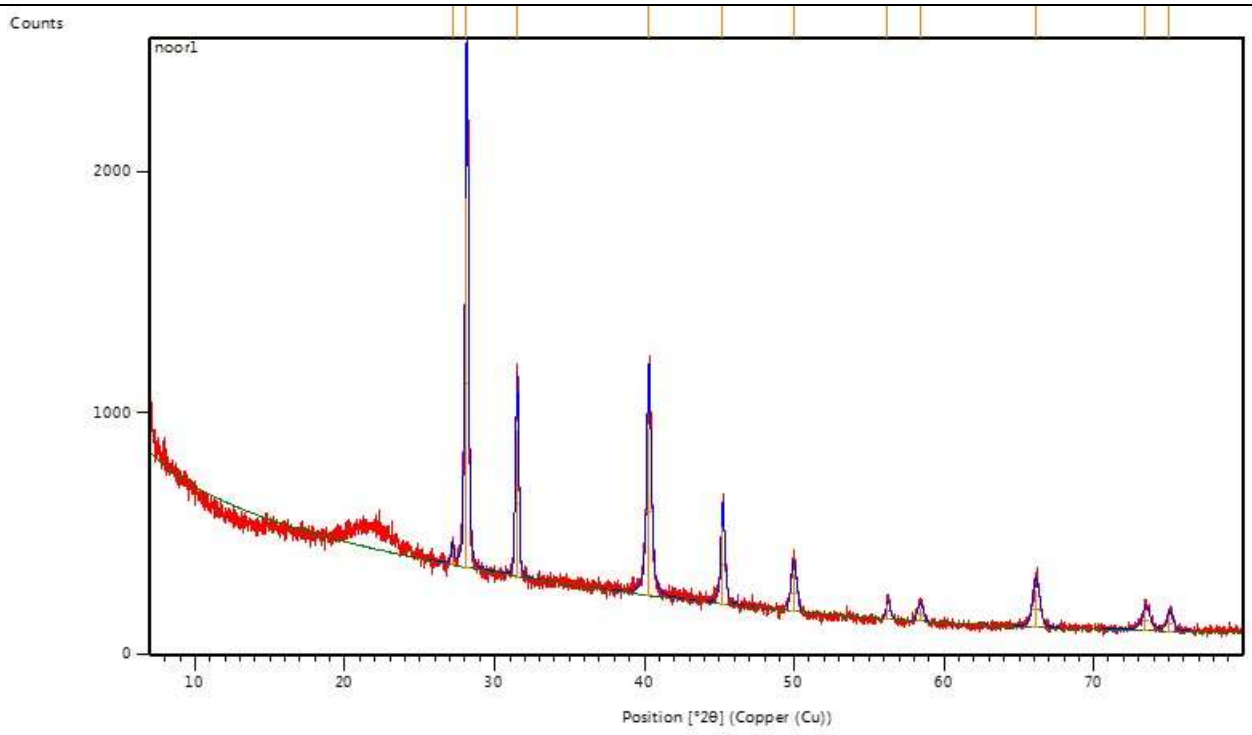


(A)

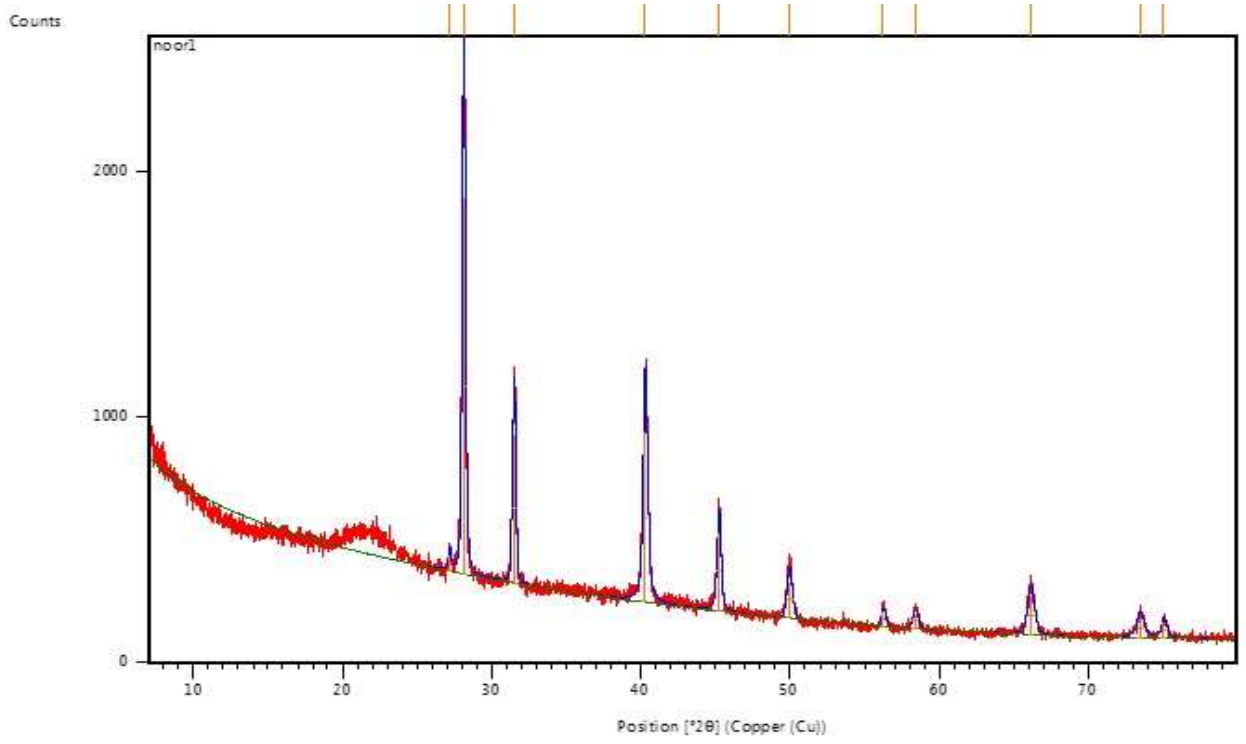


(B)

Figure 2: The UV-Vis absorption spectra of (A):Standard ZnONP (B): GS-ZnONP

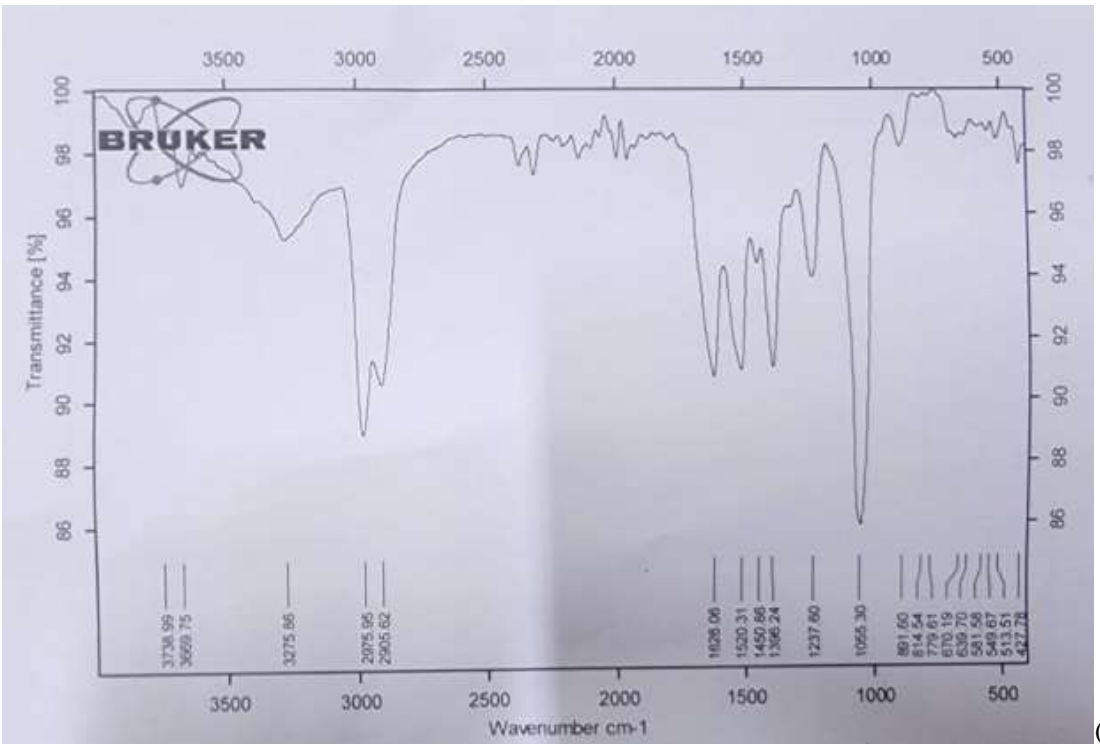


(A)

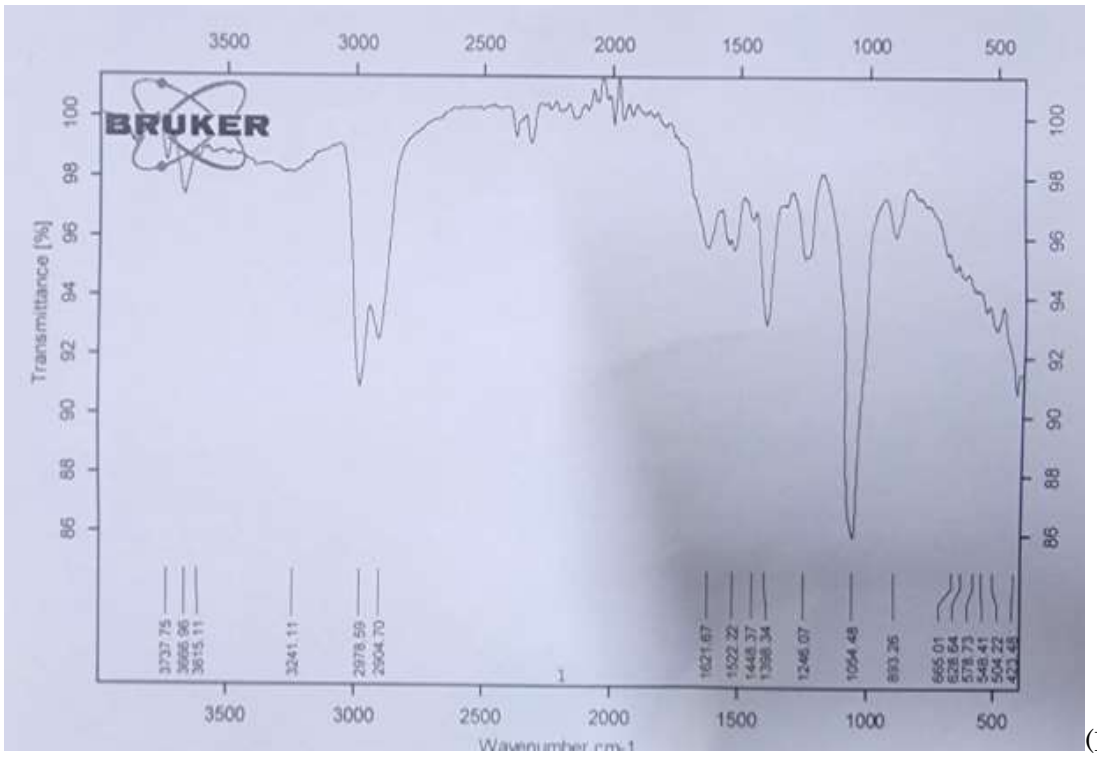


(B)

Figure 3: XRD pattern of A: Standard ZnONP B:GS-ZnONP

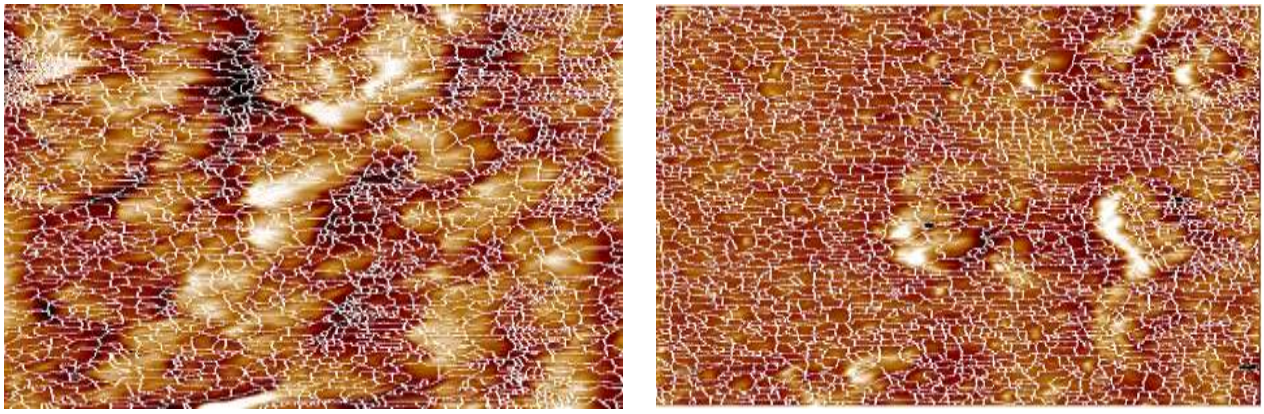


(A)



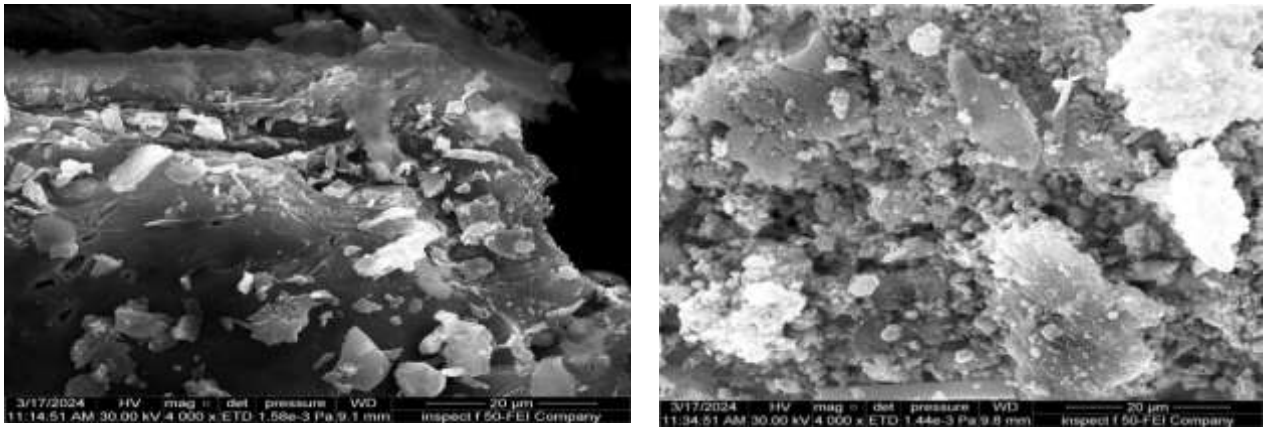
(B)

Figure 4: FTIR analysis of (A): Standard ZnO NPs (B):GS-ZnO NPs.



(A) (B)

Figure 5: AFM pattern of A: Standard ZnO NP B: GS-ZnO NPs



(A) (B)

Figure 6: SEM Images of A: Standard ZnO NP B: GS-ZnO NPs

3.4. Determination the MIC of different treatments against Staphylococcus spp.

The MIC of AG and GS-ZnONPs, mixture of standard ZnONPs and AG leaves extract in addition to standard ZnONPs was assessed using the microdilution method against *Staphylococcus* spp. Results showed that the GS-ZnONPs have the better MIC result (0.400 ±0.03,0.650 ±0.07 ,0.478 ±0.08 b, 0.632 ±0.08) on S1, S1C, S2 and S2C respectively. As showed in figures 7, 8, 9, 10 and 11.

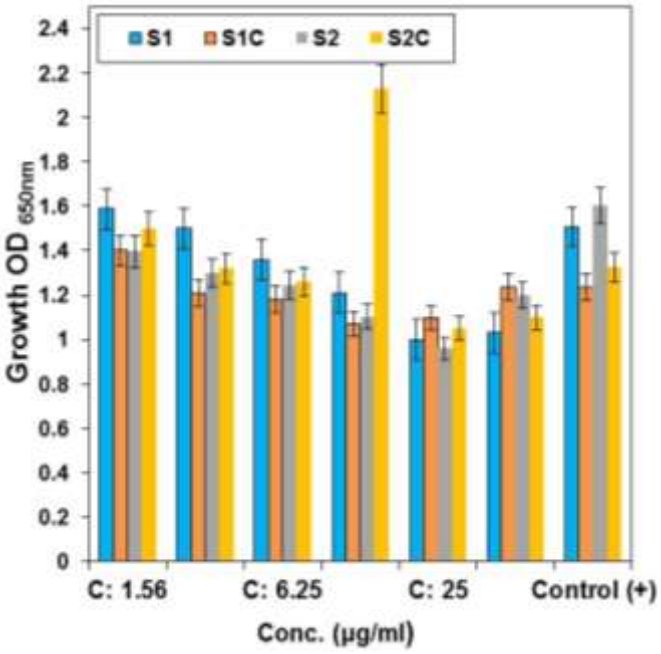


Figure 7: ZnO NPs (standard) and *Aloe Vera* leaves extract mixture for the growth *Staphylococcus* spp. Isolates.

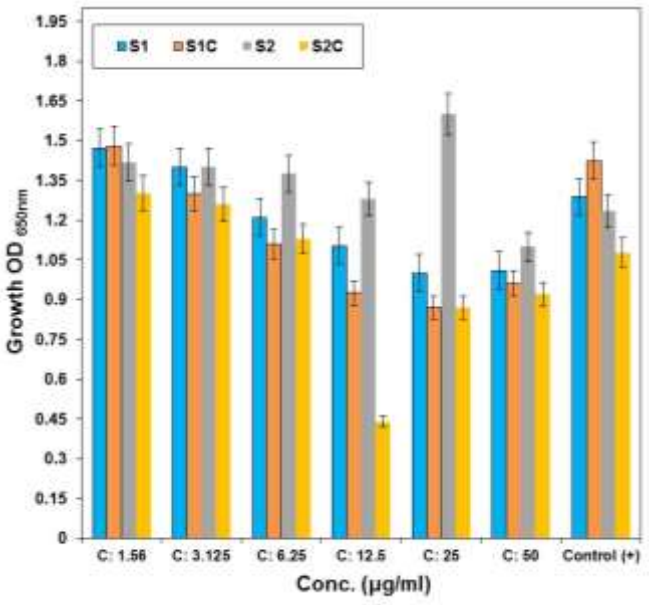


Figure 8: ZnO NPs (Standard ZnO NPs with BHI.

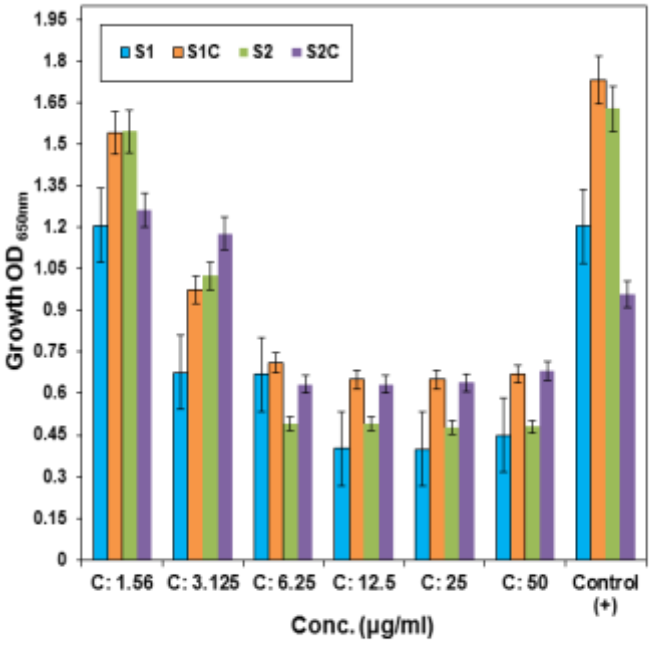


Figure 9: GS ZnO NPs (green synthesis) with *Aloe Vera* leaves extract.

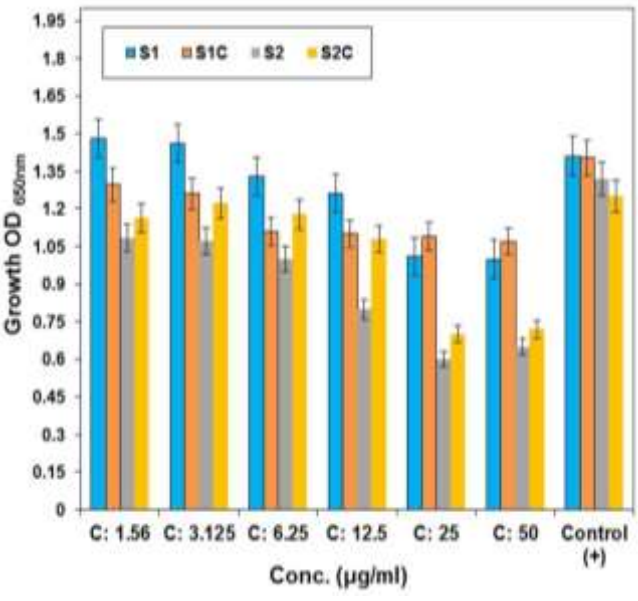


Figure 10: *Aloe vera* leaves extract.

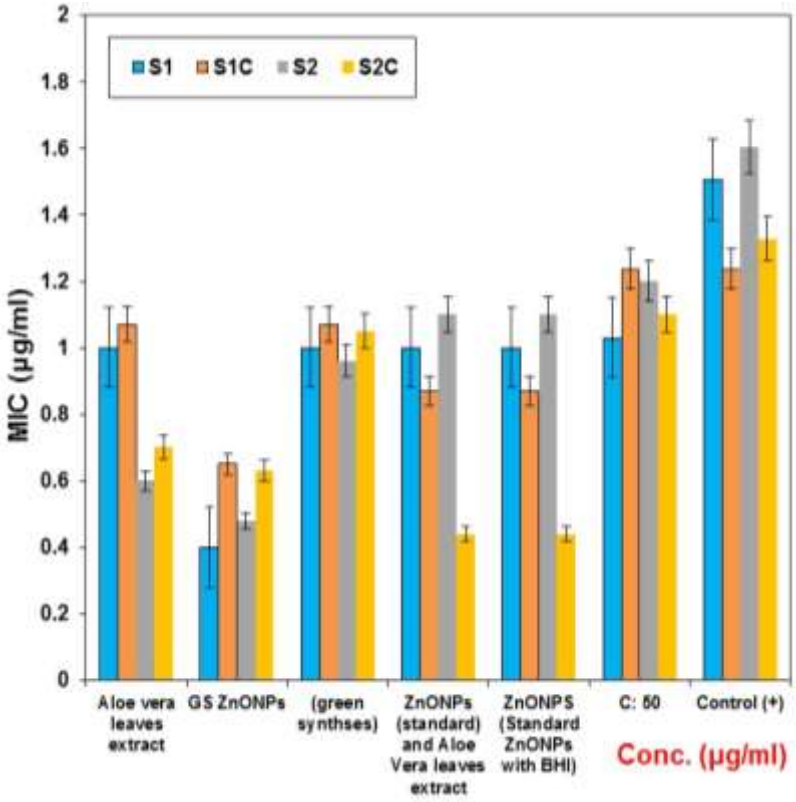


Figure 11: Comparison MIC of treatments on the growth of *Staphylococcus s*pp. isolates.

3.5. The cytotoxicity effects of ZnONPs, GS-ZnONPs and AG on normal skin cells (HdFn)

The cytotoxic characteristics were assessed using the MTT test. Table 1 presented the subsequent concentrations: Concentrations of 12.5–400 mg/mL of ZnONPs, ZnONPs/AG, and AG exhibited a negligible cytotoxic effect on HdFn relative to the control (100%) ($p < .05$). When compared to the untreated cells in the control group, the survival rate dropped dramatically at a dosage of 400 mg/mL. These results showed that the nanocomposites are biocompatible with the host and have low cytotoxicity. Moreover, at all concentrations, cell survival was much higher when AG was combined with standard ZnONPs than when each component was used alone. The results showed in figure 12.

Table 1: The cytotoxicity effects of ZnO NPs and ZnO NPs / AG and AG on HdFn.

Conc. %	Treatment					
	AG		ZnONPs		AG-ZnONPs	
	mean	SD	mean	SD	mean	SD
control	97.56933	0.2315	97.56933	0.2315	97.56933	0.2315
400	79.66833	1.75289	64.352	2.42491	80.36267	0.983923
200	84.105	0.65808	78.048	1.510898	85.03067	0.176647
100	89.73767	2.341721	90.008	2.483317	91.011	0.787514
50	93.51833	0.704121	92.901	0.334286	94.09733	0.400681
25	95.91033	1.049913	94.36733	0.176647	96.296	0.703874
12.5	96.68233	0.67819	95.679	0.240913	97.18367	0.788078
P-value	0.01		0.04		0.09	

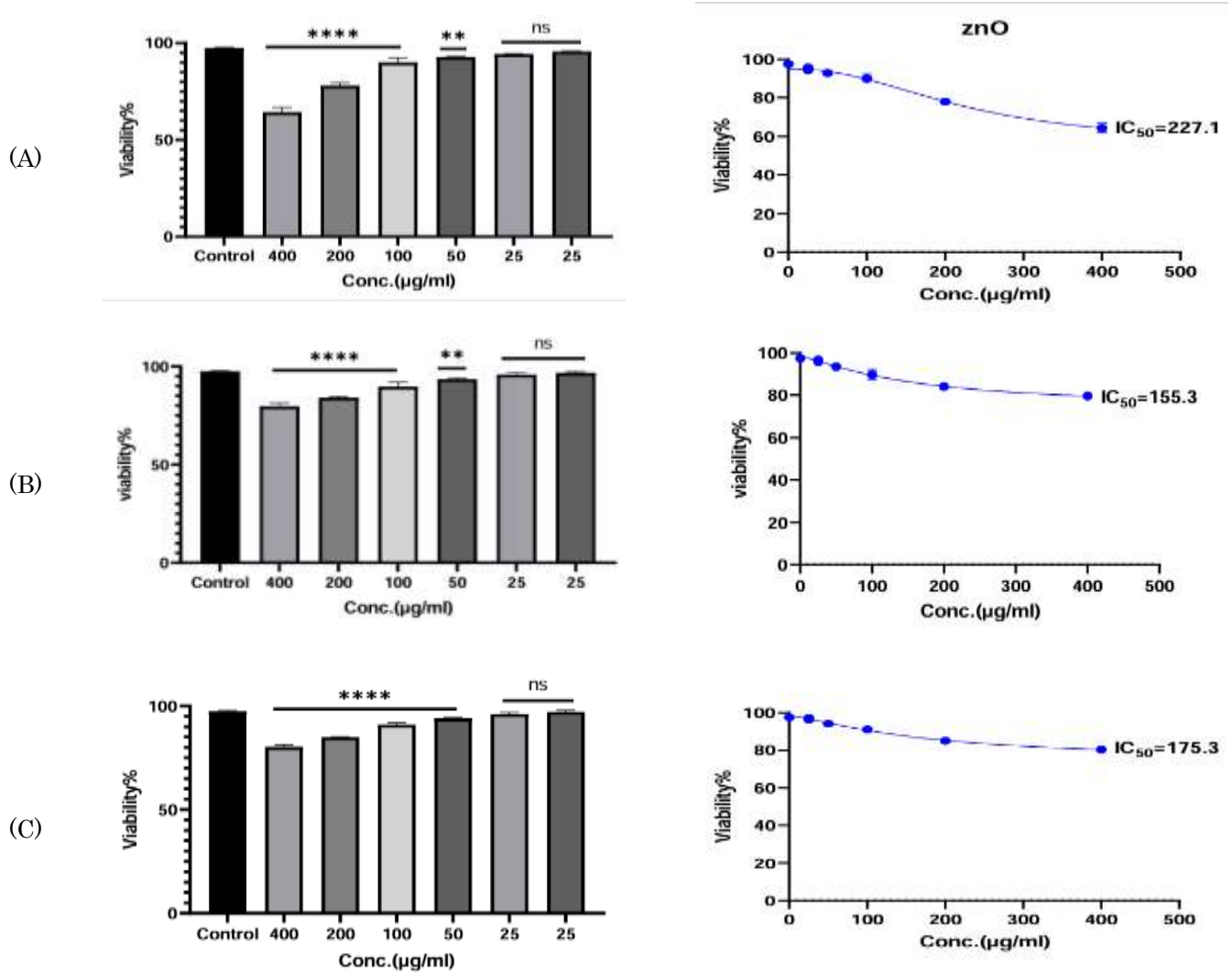


Figure 12: Effect of different concentrations of (A)- ZnO NPs (B)- AG leaves extract (C)- GS-ZnO NPs on the viability of cells.

4. Discussion

In the current study, the Standard and GS-ZnONPs were characterized by FTIR, AFM, UV-analysis, XRD and SEM. Zhang *et al.* (2006) investigate that AG may have multiple biological uses. Using the disc diffusion method, the antibacterial activity of AG was tested against different harmful bacteria. Perhaps providing a foundation for natural alternatives to prescription antibiotics, this study sheds light on the test substance's antibacterial capabilities. The results of this study demonstrate that aloe is effective against gram-positive bacteria. According to a Study conducted by Lawrence *et al.* (2009), AG has antibacterial capabilities that can successfully eliminate or greatly reduce the growth of *Staphylococcus* aureus [21]. The current study's findings are supported by a previous study by Naik *et al.* (2025) which found that AG has significant antibacterial activity against different bacterial strains that cause wound infections. The mean MIC of the extract against *Staphylococcus* spp was 94 ± 41.23 mg/ml and ($p < 0.00$) [22]. The research done by Arbab *et al.* (2021) demonstrated moderate sensitivity of AG against both Gram-positive and Gram-negative bacterial isolates. This finding indicates that AG can be utilized in conjunction with conventional antibiotics to combat prevalent skin infection pathogens [6]. A comparable experiment was conducted, revealing that the AG exhibited the most significant effect on *S. aureus, E. coli,*

Klebsiella pneumoniae, and Shigella [23]. This research used the MTT assay to determine whether AG and GS-ZnONPs, mixture of standard ZnONPs and AG leaves extract in addition to standard ZnONPs were cytotoxic to normal skin cells from HdFn volunteers. At 400 mg/mL, the survival rate was significantly lower than the untreated cell control group. Furthermore, prior tests demonstrated that ZnONPs exhibit considerable anti-biofilm efficacy against *Staphylococcus aureus* strains at sub-lethal doses [24]. The initial factor pertains to the size of ZnO nanoparticles, wherein a decrease in nanoparticle size correlates with an elevation in antibacterial activity [25]. The alternative method involves the release of Zn 2+ ions into the medium containing bacteria. Despite prior research indicating that AG extract has antibacterial properties [6, 26], ZnONP-AG shown differential antibacterial activity against Gram-positive and Gram-negative bacteria in a prior Study conducted by Ali *et al.* (2016) 27]. Nanomaterials possess distinct features relative to their bulk equivalents, which has intensified research on their synthesis, characterization, uses, and assessment, hence advancing scientific advancement and development in the agrifood sector [28]. As a result of Kumar *et al.*,[29], Phospholipids, lipoproteins, and lipopolysaccharides (LPS) make up the cell wall of Gram-negative bacteria therefore chemical transmission becomes more difficult. Inhibiting lipid peroxidation induced by reactive oxygen species generated by ZnO nanoparticles, the outer membrane's structural features lessen its susceptibility to ZnO. in Inhibiting bacterial growth, as demonstrated earlier by Esmaeili *et al.* [30], A. vera packing can decrease deterioration. Results like these prove that nanocomposites, and ZnONC/Zeo-AG in particular, are effective against bacteria and can stop their growth. Abd EL-Tawab *et al.* (2018)showed that increasing the powder content of ZnO nanoparticles improved their antibacterial activity in vitro against *Staphylococcus* spp [31] .

5. Conclusions

The isolated bacteria from infected area were pathogenic which differ from the bacteria that isolated from control area which appear as normal flora (not pathogenic). The present study proposed an eco-friendly, active nanocomposite comprising ZnO nanoparticles and *AG*. Based on the outcomes of the microdilution experiment and the cytotoxicity impact on normal skin cells, ZnO nanoparticles displayed best antibacterial activities against *Staphylococcus* spp. which can used in therapeutic purposes instead of antibiotics. Also GS-ZnONPS displaying high cytotoxic effects on HdFn cells. The GS-ZnONPs have the better MIC result on isolated bacteria.

Conflict of Interest: The author confirms that there are no conflicts of interest.

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