



Cytotoxicity Effect and Antibacterial Activity of Al₂O₃ Nanoparticles Activity against *Streptococcus Pyogenes* and *Proteus Vulgaris*

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Abstract

Aluminum oxide, often known as Al₂O₃, is a chemical compound of aluminum and oxygen with the formula Al₂O₃. It's the most common of many aluminum oxides, known as aluminum (III) oxide. The study investigates the cytotoxicity and antibacterial effects of Aluminum oxide nanoparticles (Al₂O₃-NPs) in different cells and bacteria. Different characterization methods such as dynamic light scattering (DLS), field emission scanning electron microscopy (FESEM), X-ray diffraction (XRD), and Fourier transform infrared spectroscopy (FTIR) have been used to evaluate morphologies and physicochemical properties of Al₂O₃-NPs. MTT technique is used for determining NPs cytotoxicity. The size distribution of Al₂O₃-NPs was 68 ± 12 nm in diameter, while the zeta potential was (-36 ± 10 mV). There is no toxicity by using the MTT assay, as well as showed antibacterial activity was formed at 200 µg/mL, while the higher antibacterial activity was occurring at (18 ± 0.2) and (17 ± 0.1) for *Proteus Vulgaris* and *Streptococcus pyogenes*, respectively. The findings confirmed that the Al₂O₃-NPs have small dimensions, high stability, and increased antibacterial activity.

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1. Introduction

Al₂O₃-NPs are white, odorless crystalline powder. These NPs are water-insoluble in both physical and chemical conditions, and their dimensions vary according to the method of preparation. Different crystalline modifications are generated and utilized broadly in a wide range of products medicinal, domestic, and manufactured products. The prevalence of microbial infections poses a significant threat to global public health. Antibiotics are a standard treatment for treating bacterial infections due to their cost-efficacy and positive effect. However, numerous studies have shown clearly that extensive antibiotic use has contributed to the development of multidrug-resistant bacterial strains [1,2]. More recently, metallic nanomaterials have become highly attractive and rapidly emerging materials in different scientific fields [3-7]. The significant interest of nanomaterial research and development, particularly NPs, is due to their attractive characteristics, revealed by their small size, unusual high surface area activity offering excellent catalytic, optical, and electrical properties [8-10]. Therefore, Metallic NPs have been active in comprehensive research, methods, and advanced micro-and nanotechnology

applications [1,11-16]. Due to their unique physicochemical properties in biological applications, metal oxide NPs like iron oxide NPs (Fe_3O_4), manganese oxide NPs (MgO), titanium dioxide NPs (TiO_2), zinc oxide NPs (ZnO). These NPs used widely during the last years. Al_2O_3 -NPs are a metallic NPs that has been used as an antimicrobial agent in the previous ten years. This NPs is used in medicine due to it has minimal toxicity [17]. Al_2O_3 -NPs have good antimicrobial activity against a broad spectrum of pathogenic bacteria, even in low doses. Simultaneously, they may be caused complete growth inhibition of bacteria at only a few $\mu\text{g/ml}$ concentrations. These NPs have been proven valuable agents for inhibiting antibiotic-resistant bacteria and developing resistance strains [18]. In this work, the in vitro study of Al_2O_3 -NPs was performed by antibacterial activity and cytotoxicity effect to determine the effectiveness of these NPs on some specific colonies of *S. pyogenes* and *P. Vulgaris*. Characterization of Al_2O_3 -NPs was studied using FESEM, FTIR, DLS, and XRD.

2. Materials and methods

Al_2O_3 -NPs (Mw of 136.29) were purchased from skyspring Nanomaterials, Inc, USA. Muller Hinton media was providing by Himedia, India. Aquacel®Ag is provided by ConvaTec Company, England. Dulbecco's Modified Eagle Medium (DMEM), MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) powder, Phosphate-Buffered Saline (PBS), Fetal Bovine Serum (FBS) were purchased from Sigma Aldrich (Sydney, Australia).

2.1. Characterization of Al_2O_3 -NPs

The shape, size, and morphological properties of Al_2O_3 -NPs characterized using FESEM (Hitachi S-4800 II, Japan) with 20kV and the TEM (Hitachi H-7650, Tokyo, Japan) at an acceleration voltage of 80 kV. The hydrodynamic size and zeta potential of the Al_2O_3 -NPs were measured using DLS (Horiba SZ-100 nanoparticle analyzer). For XRD analysis, the $\text{CuK}\alpha$ ($\alpha = 1.54056 \text{ \AA}$) in Bragg-Brentano were produced using a Phillips X'pert 1710 diffractometer. The voltage used was 40 kV, and the current intensity was 25 mA. The FTIR analysis was performed in Shimadzu Corporation, Japan. For this purpose, the prepared samples were analyzed over a wavelength range of 400–4000 cm^{-1} to determine the specimens 'functional groups.

2.2. MTT assay

To measure the cytotoxic effect and biocompatibility of prepared Al_2O_3 -NPs, (MTT) assay was used following instruction from the manufacturer. Vero cell culture was incubated at 37°C in 5% CO_2 . The extracted solutions are filtered by filters (0.22 μM , sigma company, united states American). Preparation of the DMEM is performed at several dilutions (50, 100, 150, and 200 $\mu\text{g/ml}$) of the extract. The Vero cells (ATCC® CCL-81™) (1×10^4 cells/well) were kept for one day in 96 wells at 37°C . Afterward, the cells were treated with the extract solutions with several dilution ratios for two days. Then, add the MTT solution (20 μl) to all the wells and hold for 4 hours. DMSO (100 μl) was added for the dissolving of the formazan. At the wavelength of 590, the O.D. was determined for each sample [18].

2.3. Antibacterial activity

Kirby-Bauer used the disk diffusion method to test antibacterial activity [19] was considered a suitable method for evaluating the antibacterial activity of Al_2O_3 -NPs. A sterile inoculating loop was briefly collected from four or five bacterial colonies and suspended in 2ml of sterilized PBS. The studied bacteria, including *S. Pyogenes* and *P. Vulgaris*. The bacteria suspension turbidity has been modified to 0.5 McFarland level by diluting with sterile PBS. Sterile swabs have been inserted into the inoculum channels. Muller–Hinton agar plates inoculated with bacteria by streaking swabs. Dissolve 0.1 mg Al_2O_3 -NPs powder in 1ml of distilled water to disperse Al_2O_3 -NPs suspension. The suspension has been sonicated 10 min before use. A 40 μl of Al_2O_3 -NPs suspension, distilled water (as negative control), and HCl (as positive control) have been impregnated into the standard antibiotic disk. Lastly, all disks were used to evaluate antibacterial activity in Mueller-Hinton (Merck, Germany) against two types of bacterial strains. Impregnated disks were placed with sterile forceps on the surface of the agar. Plates were incubated for 24 h at 37°C to control antibacterial activity; the activity (inhibition zone) was measured by millimeters, the tests were done three times.

2.4. Statistical analysis

The mean \pm standard division (S.D.) was used for the statistical analysis of our results. Excel was also used to evaluate statistical significance. Results $p < 0.05$ and $p < 0.01$ are defined as the criteria for statistical significance.

3. Results and Discussion 3.1 Characterization of Al₂O₃-NPs

To evaluate the size and morphology characteristics of the Al₂O₃-NPs, FESEM and HRTEM were performed. Both FESEM and HRTEM images presented that synthesized Al₂O₃-NPs have a spherical shape with a nanoscale size and notable dispersion ratio (Figure 1). For the XRD (figure 2) test, the Cu – Ka-wavelength ($\lambda = 1.5405 \text{ \AA}$) was used via a Phillips diffractometer using powder samples of Al₂O₃-NPs. The voltage used was 35 kV, and the current intensity was 30 mA.

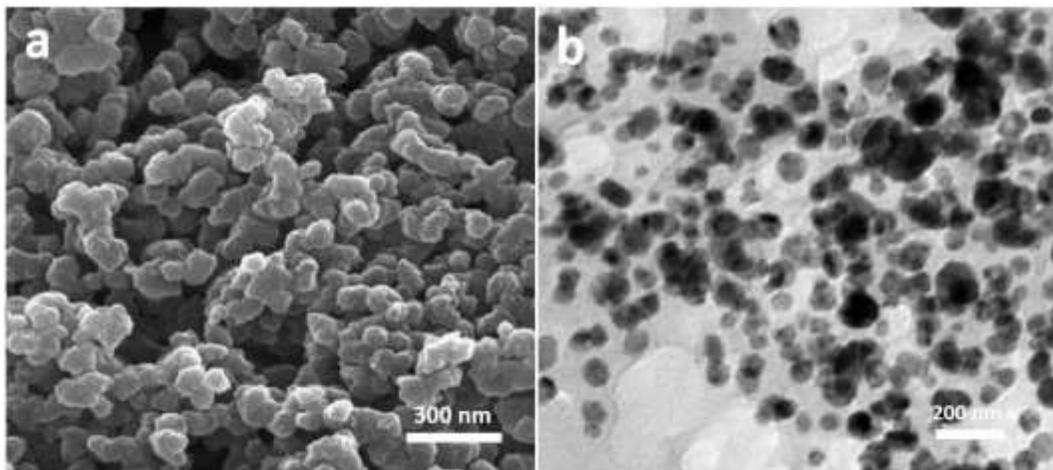


Figure 1: SEM (a) and TEM (b) images of synthesized gamma-alumina.

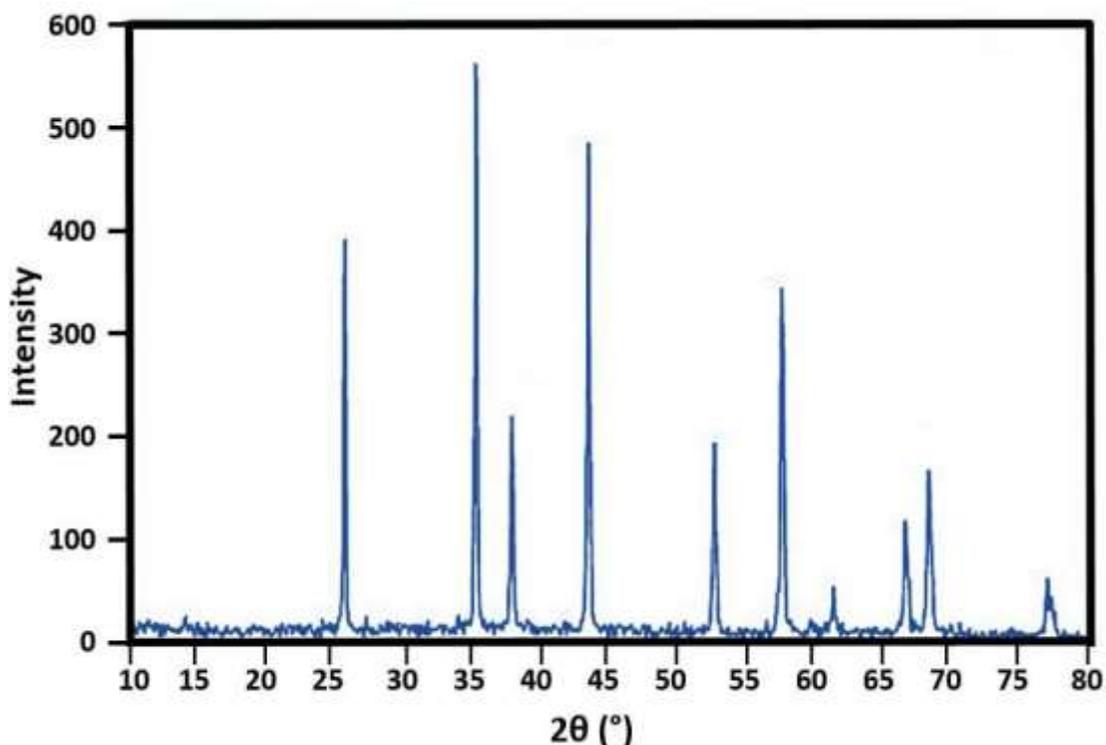


Figure 2: XRD pattern of synthesized gamma-alumina.

The DLS analysis was done to study the hydrodynamic diameter and charge of Al₂O₃-NPs (Figure 3). DLS showed small bodies with a hydrodynamic Al₂O₃-NPs of $68 \pm 12 \text{ nm}$ and $-36 \pm 10 \text{ mV}$ in diameter and charge, respectively. The study on prepared suspension also confirms the general zeta potential criteria negatively for improved stability. Previous studies confirmed that the zeta assay was used to determine the cell interactions,

cell diagnosis, and normal and cancer-cell effect therapeutics had been measured [20-22]. In the FTIR experiment, the Al-OH vibrations were in the vibrational frequencies of 1551 and 1615 cm^{-1} . This analysis also found that the absorption in the frequencies of 642 cm^{-1} and 970 cm^{-1} increased, which is related to the sites of octahedral AlO₆ and tetrahedral AlO₄, suggesting the phase change into the state α -alumina in the 4% dopant [20].

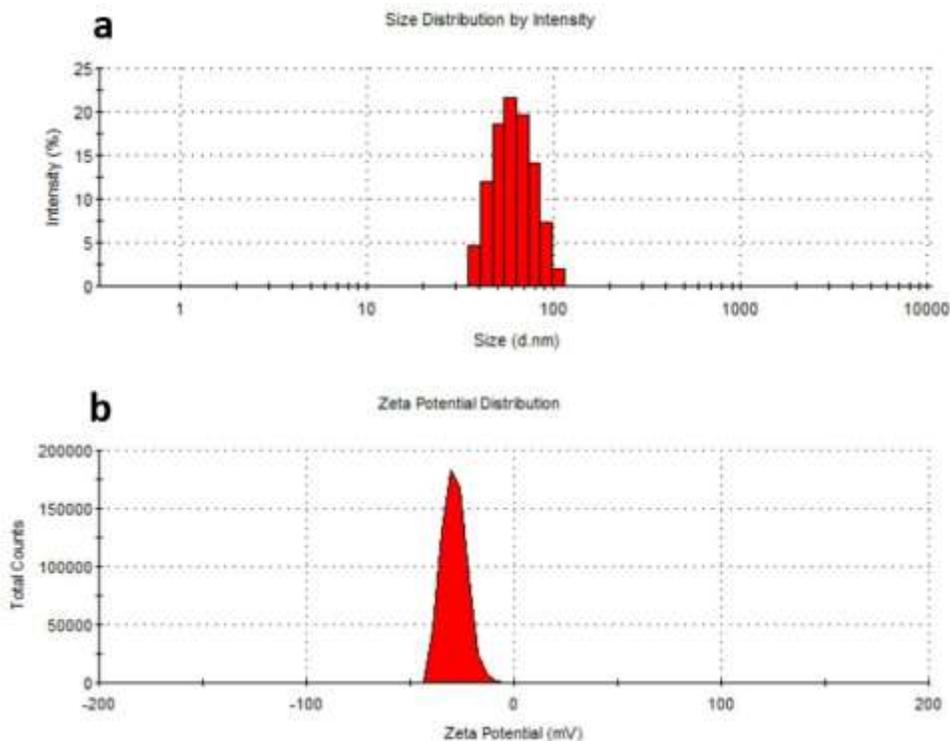


Figure 3: Size distribution and zeta potential of Al₂O₃-NPs.

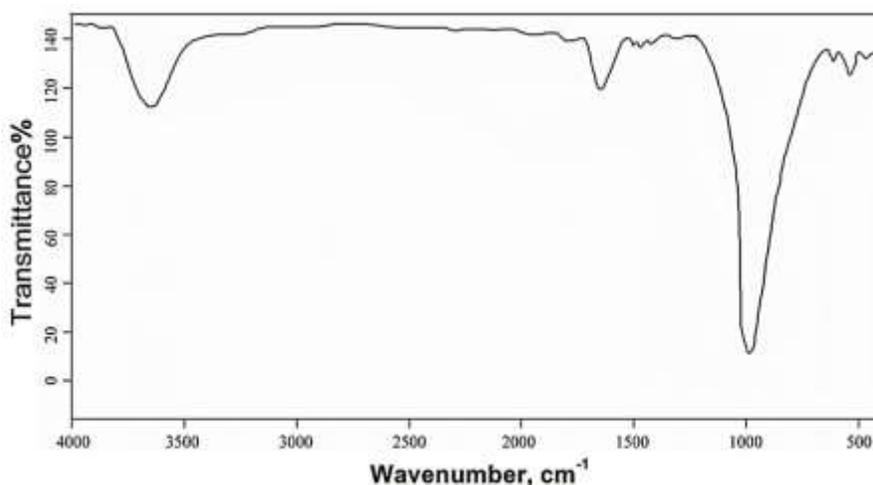


Figure 4: FTIR analysis was used to determine the functional groups of Al₂O₃-NPs.

3.1. MTT results

The MTT assay was used to evaluate the viability/proliferation of Al₂O₃-NPs at various concentrations, 50 mg/ml, 100 mg/ml, 150 mg/ml, and 200 mg/ml on Vero cell lines for 24 h (Figure5). Compared to the control group (without Al₂O₃-NPs), the samples' did not show any cytotoxicity at mentioned concentrations for 24 h.

Al₂O₃-NPs is induced endothelial leakiness and improves nanomedicine's biodistribution to target sites [23-25]. Several studies show that when the endothelial cells were exposed to Al₂O₃-NPs, it will cause leakiness of the endothelial cells and interfere with endothelial cells' adherens Al₂O₃-NPs and vascular endothelial cadherin. The leakiness of the endothelial cells is attributed to the toxic effects of inorganic NPs in the cells [26-28].

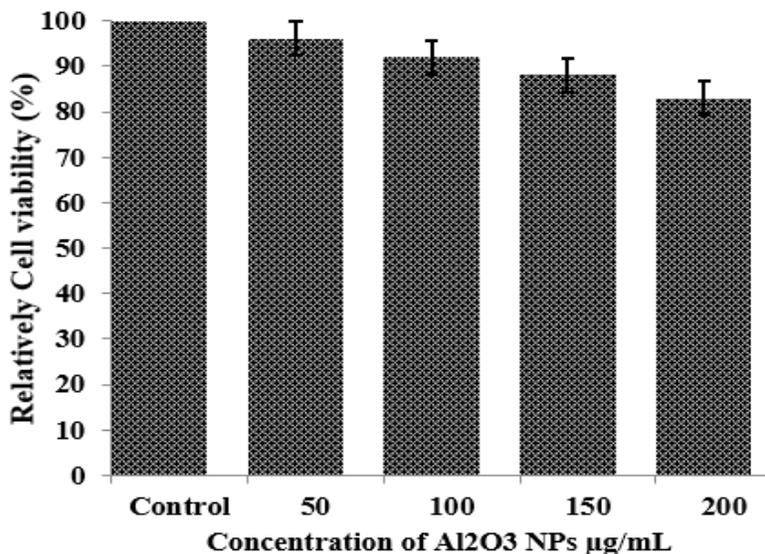


Figure 5: Showed the Vero cell lines treated with several doses of Al₂O₃-NPs for one day. Mean \pm standard error was expressed for the data from three replications.

3.2. Antibacterial activity

The synthesized Al₂O₃-NPs were used for antibiotic susceptibility at five concentrations (25, 50, 100, 150, and 200 µg/ml), as shown in Figure 6. The figure exhibits the inhibition zone Al₂O₃-NPs against tested bacteria. Figure 7 shows an exemplary diffusion assay that Al₂O₃-NPs were more efficient as antibacterial agents on *S. pyogenes* and *P. Vulgaris*. It showed antibacterial activity was formed at 200 µg/mL, while the higher antibacterial activity was occurring at (18 ± 02) and (17 ± 01) for *P. Vulgaris* and *S. pyogenes*, respectively. The reduction of antibacterial activity may be due to the calcination of Al₂O₃-NPs, which caused an increase in particle size. Al₂O₃-NPs exhibited an interesting antibacterial decrease due to the enhancement of the specific surface area. The nature of Al₂O₃-NPs can explain this phenomenon, and one of the main mechanisms of its action is the production of reactive oxygen species (ROS) on its surface during the photocatalysis process when it is exposed to light at a suitable wavelength, which leads to phospholipid peroxidation and, finally cell death [29-33]. This study was carried out to strengthen further the physical-chemical properties of Al₂O₃-NPs by antibacterial activity. With a specific surface area, the crystalline category of our Al₂O₃-NPs, assesses the negative zeta potential of -36 ± 10 mV, besides the 68 ± 12 nm average diameter in size. Many studies have been made to understand biomaterials biocompatibility effect on cells for produce osteointegration during the recent decades, such as prevailing environment, like adhesion, cell viability, and proliferation. The physicochemical properties like shape, size, phase purity, surface area, surface topography, particle concentration, crystalline structure, and surface charging greatly increase cell viability. However, Shi et al. [34]. have reported the potential side effects and beneficial activity of the conventional Al₂O₃-NPs. A profuse and overdoses, form, size, composition, crystalline properties, added functional groups, solubility, and the manufacture of impurities of such a heterogeneous nature was applied on many cell lines like lung cells, mucosal cells, cardiovascular cells, nerve cells, skin cells, reproductive cells, and kidney cells to study the activity of the titanium.

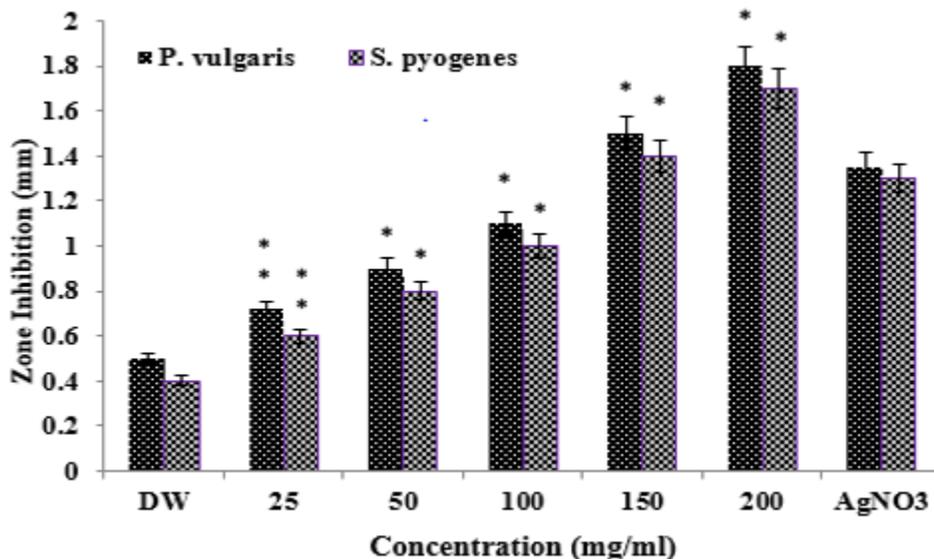


Figure 6: Antibacterial activity of Al_2O_3 -NPs against *P.vulgaris* and *S.pyogenes*. mean \pm SD are presented for data for *p.vulgaris* and AgNO_3 NPs at $p < 0.01$ and $p < 0.05$.

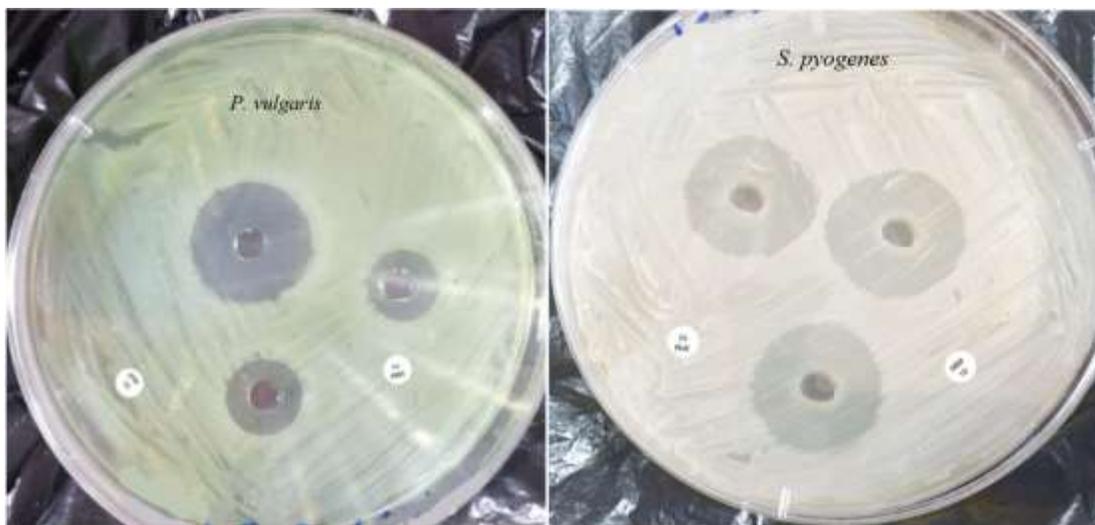


Figure 7: Well diffusion assay exhibiting the impact of Al_2O_3 -NPs as antibacterial agents against *S.pyogenes* and *P.vulgaris*.

Al_2O_3 -NPs stuck in a suspension on bacterial surfaces and resulting in Al_2O_3 -NPs being adsorbed on the surface of bacteria [35] that could lead in combination with a photocatalytic oxidation reaction to inactivating bacteria [36]. There are several possible mechanisms to explain bacteria's impact on Al_2O_3 -NPs [37]. Not all antibiotics have synergistic antimicrobial effects when used with Al_2O_3 -NPs since one study showed the lack of increased antibacterial activity using CEF antibiotics in combination with Al_2O_3 -NPs. Compared to the CEF antibiotic combined with Al_2O_3 -NPs, the CEZ antibiotic showed an excellent synergistic impact [38].

4. Conclusions

This work studied the cytotoxicity and antibacterial effects of Aluminium Al_2O_3 -NPs in different cells and bacteria. Different characterization methods such as DLS, FESEM, XRD, and FTIR, confirmed the morphologies and physicochemical properties of Al_2O_3 -NPs. The size distribution and zeta potential of Al_2O_3 -

NPs has been proved the suitability of this nanoparticle for antibacterial and cytotoxic studies. There is no toxicity by using the MTT assay, as well as showed antibacterial activity was formed at 200 $\mu\text{g/mL}$, while the higher antibacterial activity was occurring for *Proteus Vulgaris* and *Streptococcus pyogenes*, respectively. The findings confirmed that the Al_2O_3 -NPs have small dimensions, high stability, and increased antibacterial activity.

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