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Investigation of the Impact of Environment Friendly Alumina Nanoparticles on Bacterial Activity

Taqwa Gh. Hamad, Taghried Ali Salman*

Authors' Affiliation:
Department of Chemistry,
College of Science, Al-Nahrain
University, Jadriya, Baghdad -
Iraq

***Corresponding Author:**
Taghried Ali Salman
Email:
dr.tag_s@yahoo.com

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Editorial Note:
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Abstract

Background: *Staphylococcus aureus* is a significant nosocomial pathogen and frequently causes postoperative wound infections, which is a serious health issue in hospitals. *Staphylococcus aureus* has become increasingly drug resistant, and most of its strains have been shown to be resistant to practically all antibiotics. Numerous medical applications of aluminum oxide have been investigated, and this research indicates that the growth of *Staphylococcus aureus* may be inhibited in the presence of aluminum oxide nanoparticles.

Methods: The study was conducted from March 2023 to the end of July 2023 at Al-Aziziya Hospital in Iraq. Using spectrophotometry on microtiter plates, biofilms were created in vitro. Single isolated organisms of *Staphylococcus aureus* were examined for susceptibility to aluminum oxide (Al_2O_3) nanoparticles using the disk diffusion method. Furthermore, Prepared aluminum oxide nanoparticles were characterized using various techniques.

Results: The results show that growth of *Staphylococcus aureus* was strongly inhibited after 24 hours at 0.06125, 0.1225, and 0.245 M; at 0.490 M, no effect was observed (per Table 1). On the other hand, X-ray diffraction analysis, based on Scherrer equation estimation, revealed an average crystallite size of ~35 nm for the aluminum oxide nanoparticles. The FT-IR spectrum displayed prominent peaks at 615 cm^{-1} and 636 cm^{-1} , corresponding to the stretching vibrations of aluminum oxide. The EDX measurements detected Al and O peaks, consistent with the formation of Al_2O_3 .

Conclusions: The antimicrobial assay demonstrated that the aluminum oxide nanoparticles exhibited significant antibacterial activity against *Staphylococcus aureus*. At a concentration of 0.06125 M of Al_2O_3 , *Staphylococcus aureus* showed maximum inhibitory effect.



Introduction

The production and characterization of nanoparticles have attracted significant scientific interest due to their unique features and potential applications, which differ from their bulk counterparts [1-5]. In the field of nano-biotechnology, there is currently active research focused on plant-mediated green chemistry [6, 7]. When regulated nanoparticles are taken up by plants, they can produce more stable nanoparticles with higher yields. Plant biosynthesis of nanoparticles requires careful consideration of factors such as temperature, pH, reaction time, plant extract quantity, and concentration of metal ions [8]. The market for nanoparticles (NPs) is projected to grow by 2025, particularly in the field of biomedical research focusing on metal oxide NPs [9]. The number of publications and patents related to the use of metal oxide NPs as medicinal agents and antibacterial agents has increased significantly in recent years [10]. There has been considerable interest in utilizing NPs as biosensors, for oncological diagnosis and treatment, and for drug delivery purposes [11, 12]. Metal oxide nanoparticles, including zinc oxide, iron oxide, titanium dioxide, silver oxide, and copper oxide, have demonstrated antibacterial properties, addressing bacterial infections, including antibiotic-resistant strains [13, 14, 15, 16]. Aluminum oxide nanoparticles (Al_2O_3 NPs), being the second interesting contender, have attracted attention due to their low cytotoxicity resulting from the inert nature of aluminum oxide [22, 23]. However, further research is needed to definitively establish their antibacterial activities. Due to its special qualities, such as strong mechanical strength, a large surface area to volume ratio, high hardness, and outstanding chemical stability, nanosized aluminum oxide (Al_2O_3) is widely employed in a variety of industries [24, 25]. Al_2O_3 NPs find applications as catalysts, adsorbents, components in concrete mixtures, tribological additives in lubricating liquids, raw materials for ceramics, and in industries such as cosmetics, textiles, and microelectronics [26, 27, 28, 29, 30, 31]. Al_2O_3 NPs also hold potential in biomedicine, particularly as antibacterial agents [32, 33]. However, the mechanisms by which these nanoparticles affect microbial growth and development are not yet fully understood.

Typically, the biogenesis of nanoparticles happens when a plant's water-soluble extract goes through the bio reduction process with a water-soluble solution of the right metal salt. Fresh pomegranate leaf material was pro About 20–30 g of leaves was crushed with a mortar and pestle, and the resultant extract was filtered through muslin cloth. The extract was then maintained at room temperature for additional analysis [32]. The leaf extract contains flavonoids and phenolic acids with a variety of functional groups, which can act

as reducing and stabilizing agents to produce nanoparticles.

Aluminum chloride was the primary chemical used to make aluminum oxide nanoparticles. The obtained extract was mixed with a saturated solution of aluminum chloride and allowed to sit for two hours in a magnetic stirrer (REMI, Laboratory Instruments) to ensure that the solutions were properly mixed. After stirring, the pH was measured to determine whether the extract's phytochemicals were reacting with the metal ions to form metal oxide nanoparticles. To settle the unused extract, the reaction mixture was next centrifuged at 2500 rpm for 20 minutes using a cooling centrifuge (REMI C-24 Plus). The resulting supernatant was then once more ultra-centrifuged at 14,000 rpm for 30 minutes. Al_2O_3 NPs were produced by discarding the resultant supernatant and repeatedly washing the pellets in water and ethanol before drying them at 60 °C in a hot air oven as shown in Figure 1.

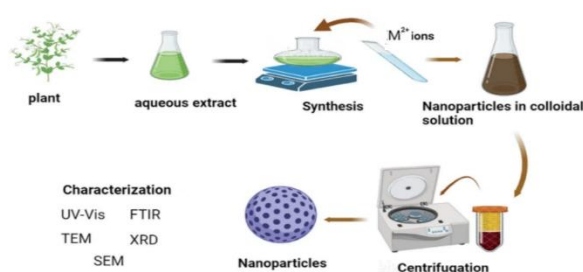


Figure 1: Green Synthesis of Metal oxide Nanoparticles.

In the present study, aluminum oxide nanoparticles (Al_2O_3 NPs) loaded with green material were created using a straightforward biological reduction technique. The produced Al_2O_3 NPs were characterized using a variety of widely used analytical and imaging techniques. Investigated was the nanoparticles' effectiveness as antibacterial against *Staphylococcus aureus*.

Methods

Plant extract preparation

A plant's aqueous extract is typically bio-reduced with an appropriate metal salt solution as part of the biogenesis of nanoparticles. Fresh pomegranate leaves for this investigation were procured from a farm and properly cleaned with distilled water to remove any impurities. The leaves were then around 20–30 grams in weight, and after being ground with a mortar and pestle, 100 mL of distilled water were added to the ground leaf material. The extract was then kept at room temperature for upcoming tests. The extract of the leaves contains phenolic acids and flavonoids with a variety of functional groups that can act as both

reducing and stabilizing agents, aiding the creation of nanoparticles.

Synthesis of Alumina Nanoparticles

The primary chemical used in the synthesis of aluminum oxide nanoparticles was aluminum chloride from Merck, India. To ensure proper mixing of the solutions, the prepared plant extract was added to a saturated solution of aluminum chloride and stirred using a magnetic stirrer (REMI, Laboratory Instruments, India) for two hours. The pH of the mixture was monitored during the stirring phase to observe any changes, suggesting the occurrence of an interaction between the phytochemicals in the extract and the metal ions, leading to the creation of metal oxide nanoparticles. The resultant mixture was then treated to centrifugation at 2500 rpm for 20 minutes using a cooling centrifuge (REMI C-24 Plus) to separate the unused extract. The gained solution underwent additional processing by ultra-centrifugation for 30 minutes at 14,000 rpm. The pellets were then rinsed repeatedly with water and ethanol after the supernatant was removed. Aluminum oxide nanoparticles (Al_2O_3 NPs) were then produced by drying the pellets in a hot air oven at 60 °C. A suspension was prepared by dispersing 0.5 g of nanoparticle powder in 10 mL of distilled water. Four test concentrations were prepared from this suspension and applied onto plates for bacterial growth.

Nanoparticles Characterization

A Shimadzu UV-1800 spectrophotometer was used to measure the ultraviolet spectrum at room temperature. A Shimadzu FTIR-8400S spectrometer was used to obtain the Fourier transform infrared (FTIR) spectrum. The plant extract containing aluminum oxide nanoparticles (Al_2O_3 NPs) was dried at 60 °C for one hour before being combined with the proper quantity of KBr for FT-IR analysis. To verify the biogenesis of Al_2O_3 NPs, X-ray diffraction (XRD) patterns were acquired using a Shimadzu XRD-6000 diffractometer. Using AA300 Angstrom AFM Atomic Force microscopy, the morphology and surface properties of the aluminum oxide nanoparticles were investigated. The SEM S-4160 electron microscope was also used to investigate aliquots of the plant extract filters containing aluminum oxide nanoparticles.

Antibacterial Activity Studies

The antibacterial efficacy of aluminum oxide nanoparticles against *Staphylococcus aureus* was evaluated using the disk diffusion method. In preparation for upcoming tests, the bacterial strain *Staphylococcus aureus* (KACC, South Korea) was used as the test organism. It was then kept on nutrient agar slants at 4 °C. The plate approach was used to inoculate

the bacterial cultures. Brain heart infusion (BHI) broth was incubated at 37°C for 24 hours in the case of cultures. After that, Mueller-Hinton agar plates were used to cultivate pathogenic bacteria. A sterile paper disk with a diameter of 5 mm was used to prepare each plate. Different concentrations of the manufactured aluminum oxide nanoparticles were added to the disks, with the plant extract serving as a control. The plates were then incubated for 24 hours at 37°C, and the inhibitory zones were measured and noted. In addition to the plant extract, a negative control (blank disk with solvent only) and a positive control (standard antibiotic disk, e.g., gentamicin) were included. Each test was performed in triplicate (n=3), and inhibition zones were recorded as mean $\pm$ standard deviation (mm). The assay was conducted following Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results

Synthesized Aluminum Oxide Nanoparticles Characterization

FT-IR spectroscopy is a useful method for determining the amount of infrared light absorbed by a sample and displaying the results as a wavelength spectrum. Correlating the absorption bands (vibrational bands) with the chemical constituents present in the sample is necessary for the interpretation of the IR spectra [28]. The FTIR spectra for artificially created aluminum oxide nanoparticles is shown in Figure 2.

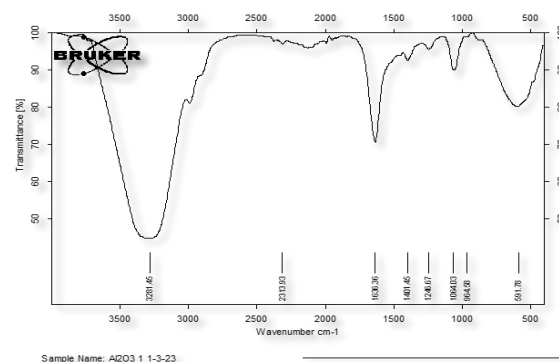


Figure 2: FT-IR spectra for aluminum oxide nanoparticles.

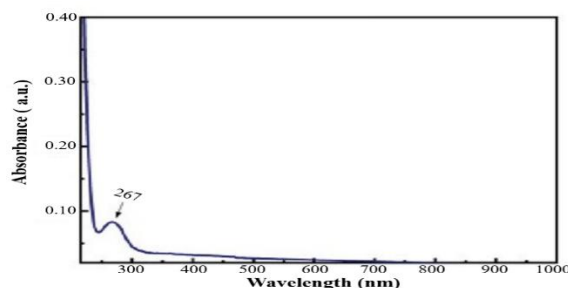


Figure 3: UV-visible spectra of Al_2O_3 synthesis using Pomegranate leaves extract.

The UV-vis absorption spectrum of the produced nanoparticles was examined to estimate their optical energy gap. A noticeable absorption peak (λ_{max}) at 267 nm in the UV area was visible in the recorded spectrum, which supports the creation of aluminum oxide (Al_2O_3) particles as shown in Figure 3.

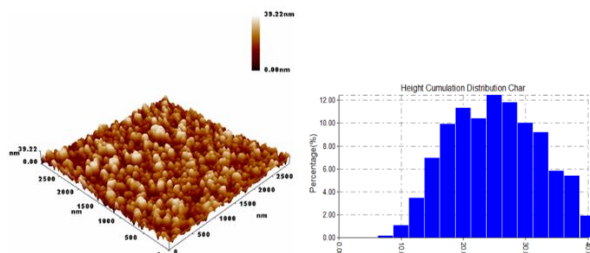


Figure 4: AFM images of Al_2O_3 synthesis using Pomegranate leaves extract.

AFM (Atomic Force Microscopy) analysis is employed to investigate the topography and surface characteristics of materials. It enables the generation of three-dimensional images that provide microscopic resolution of a nanoparticle's surface. In this study, AFM was utilized to examine the surface details of aluminum oxide nanoparticles (Al_2O_3 NPs) synthesized using pomegranate leaf extract. Figure 4 presents a representative three-dimensional image illustrating the surface topography of the Al_2O_3 NPs. The size, shape, and distribution of aluminum oxide nanoparticles synthesized using pomegranate leaf extract were examined using a scanning electron microscope (SEM). Figure 5 shows the results of the SEM investigation. The larger sizes observed in SEM images likely represent agglomerates, whereas AFM captured the nanoscale primary particle size.

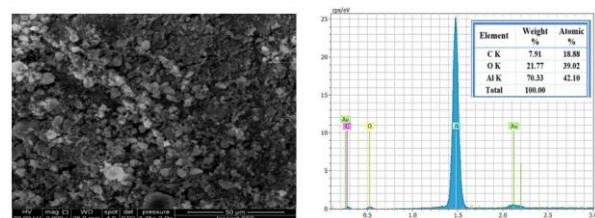


Figure 5: SEM and EDX images of Al_2O_3 synthesis using Pomegranate leaves extract.

Figure 6 displays the aluminum oxide (Al_2O_3) nanoparticles' diffraction peaks. The synthesized Al_2O_3 NPs' XRD (X-ray diffraction) pattern exhibits a monoclinic structure and theta (θ) phase. The relative intensities of the observed diffraction peaks and the published values (JCPDS # 35-0121) are very similar to each other. The powder XRD pattern of Al_2O_3 nanoparticles exhibits diffraction peaks at specific 2θ angles: 18.96° , 30.08° , 38.57° , 40.98° , 44.74° , 47.49° ,

54.94° , 62.25° , 63.25° , and 75.46° , which correspond to the (102), (200), (104), (006), (015), (304), (311), (313), (306), and (107) crystallographic planes, respectively. Applying the Scherrer equation $D = 0.9 \lambda / (\beta \cos \theta)$, where D is the crystallite size, λ is the X-ray wavelength, β is the full width at half maximum (in radians), and θ is the Bragg angle, the size of the Al_2O_3 nanoparticles was calculated [26]. Based on this calculation, the average crystallite size was estimated to be ~ 35 nm.

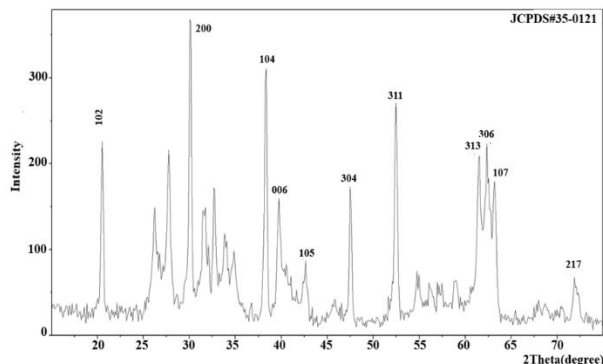


Figure 6: XRD Diffraction Pattern of Al_2O_3 Nanoparticles.

The experimental results demonstrated that Al_2O_3 nanoparticles synthesized using a green plant-mediated method exhibited antibacterial activity, strongest at lower concentrations (0.06125–0.1225 M). The results showed, as indicated in Table 1, that Al_2O_3 nanoparticles exhibited inhibition at 0.06125 M and 0.12250 M ('Kill'), with reduced effect at 0.24500 M ('Low kill') and no effect at 0.49000 M. These outcomes are qualitative; inhibition zones were categorized as 'Kill', 'Low kill', or 'No effect' rather than reported as exact diameters.

Conc./ M	0.49000	0.24500	0.12250	0.06125
<i>Staphylococcus aureus</i>	No effect	Low kill	Kill	Kill

Table 1: Effect of different Al_2O_3 nanoparticle concentrations against *Staphylococcus aureus*. 'Kill' = complete inhibition; 'Low kill' = partial inhibition; 'No effect' = absence of inhibition. Exact inhibition zone diameters were not recorded.

Discussion

In this study, the stretching vibrations of aluminum oxide are thought to be responsible for the peaks seen at 615 cm^{-1} and 636 cm^{-1} as shown in figure 2. The sulfate ion's triply degenerate vibrational mode is associated with the peak at 1127 . Furthermore, the water molecule's bending and stretching vibration modes are connected to the maxima at 1646 and 3526 , respectively [30]. The reported absorption peak of Al_2O_3 nanoparticles at 267 nm agrees with earlier research [7, 8] Al_2O_3 nanoparticles were shown to have a significant absorption peak between 200 and 280 nm

(258 nm) in which is consistent with description of a peak at 267 nm. Together, these results confirm that Al_2O_3 nanoparticles were successfully formed in the current investigation, as shown by the significant absorption peak seen in the UV-vis spectrum at 267 nm. AFM image for synthesized nanoparticles reveals the nano-scale particle diameter, maintaining a 29.23 nm average size. The AFM analysis provides valuable insights into the morphology and structure of the synthesized Al_2O_3 NPs, allowing for a better understanding of their physical properties and potential applications. The difference between AFM and SEM observations suggests that while AFM measured the primary nanoparticle size (~29 nm), SEM revealed larger agglomerates formed by clustering of these nanoparticles (0.5–0.9 μm). This information provides valuable insights into the physical characteristics of the synthesized Al_2O_3 nanoparticles and contributes to our understanding of their potential applications. Additionally, X-ray diffraction data, analyzed using the Scherrer equation, indicated an estimated average crystallite size of ~35 nm. Additionally, the calculated cell parameters of $a = 0.5681$ nm, $b = 0.2890$ nm, and $c = 1.1776$ nm nearly match the values that were previously reported. Al_2O_3 nanoparticles created by the bio reduction were measured using X-ray diffraction and their estimated size was found to be using the Scherrer equation ($D = 0.9 \lambda / (\beta \cos \theta)$). On the other hand, antibacterial behavior presented in table 1, revealed that the size of the inhibitory zone provides a clue as to how susceptible microorganisms are. These findings show how well Al_2O_3 nanoparticles work to stop the spread of harmful germs and point to possible uses for them as antibacterial agents.

In this study, aluminum oxide (Al_2O_3) nanoparticles with improved stability were created using a simple, low-cost green chemical process. This one-step technology is ideal for large-scale production since it does away with the complicated steps required by other organic techniques that use fungi and bacteria. By using XRD analysis, it was determined that the crystallite size of the described nanoparticles was 35 nm. The presence of aluminum oxide in the produced nanoparticles was confirmed by the FT-IR spectra and the EDX tests. It is interesting to note that the Al_2O_3 nanoparticles showed antibacterial activity that was more pronounced at lower concentrations but decreased with higher doses, consistent with the qualitative outcomes in Table 1. According to these results, Al_2O_3 nanoparticles could be used as antibacterial agents to control a variety of harmful organisms.

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Author Contributions

Taqwa Ghalib: writing-original draft, Data curation
Taghried Salman: Supervision, methodology, Writing-Review and editing.

Competing Interests

The authors declared that there were no conflicts of interest.

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