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Microwave Mediated Fast Synthesis of Silver Nanoparticles and Investigation of Their Antibacterial Activities for Gram-Positive and Gram-Negative Microorganisms

Faheem Ahmed ^{1,*}, Suliman Yousef AlOmar ², Fadwa Albalawi ³, Nishat Arshi ⁴, Sourabh Dwivedi ⁵, Shalendra Kumar ^{1,6}, Nagih Mohammed Shaalan ¹ and Naushad Ahmad ⁷

¹ Department of Physics, College of Science, King Faisal University, P.O. Box-400, Al-Ahsa 31982, Saudi Arabia; sjagdish@kfu.edu.sa (S.K.); nmohammed@kfu.edu.sa (N.M.S.)

² Doping Research Chair, Zoology Department, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; syalomar@ksu.edu.sa

³ Department of Zoology, King Saud University, Riyadh 11451, Saudi Arabia; fadwa_saad@hotmail.com

⁴ Department of Basic Sciences, Preparatory Year Deanship, King Faisal University, P.O. Box-400, Al-Ahsa 31982, Saudi Arabia; nshastri@kfu.edu.sa

⁵ Department of Applied Physics, Aligarh Muslim University, Aligarh 202002, India; sourabh25june@gmail.com

⁶ Department of Physics, School of Engineering, University of Petroleum & Energy Studies, Dehradun 248007, India

⁷ Department of Chemistry, College of Science, King Saud University, Riyadh 11451, Saudi Arabia; anaushad@ksu.edu.sa

* Correspondence: fahmed@kfu.edu.sa; Tel./Fax: +966-013589-6900



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Abstract: Herein, a simple one-step microwave irradiation technique has been used to synthesize the silver nanoparticles using silver nitrate (AgNO₃) solution and cetyltrimethyl ammonium bromide (CTAB) as a stabilizing agent. The crystals of as-prepared nanoparticles were studied using X-ray diffraction (XRD) and a selected area electron diffraction (SAED) pattern, confirming the single-phase face-centered cubic structure. The optical property measured using UV-Vis spectroscopy shows an absorption maximum at 420 nm, which also confirms the formation of silver nanoparticles. Transmission electron microscopy (TEM) analysis revealed that the silver nanoparticles have a spherical shape with an average diameter of ~6 nm. The antibacterial properties of silver nanoparticles were investigated using both Gram-positive and Gram-negative microorganisms, such as *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. *Klebsiella pneumoniae*, and *Candida albicans*. Results showed a highest zone of inhibition of about 35 mm against *P. aeruginosa* as compared with *E. coli* (21 mm), *S. aureus* (30 mm), *K. pneumoniae* (28 mm), and *C. albicans* (29 mm). These studies suggested that silver nanoparticles prepared by this fast and effective method might be developed as antibacterial agents against an extensive range of microorganisms to control and stop the spreading and persistence of bacterial infections.

Keywords: silver nanoparticle; XRD; TEM; antibacterial; UV-Vis spectroscopy

1. Introduction

In the last few years, various attempts have been made to advance safe, more effective treatment protocols to overcome the challenges of antibiotic resistance. It is observed that the microorganisms are responsible for creating microbial pollution and contamination, which results in many problems in manufacturing and other vital fields, such as degradation and infection. Therefore, to overcome such problems, various research groups have studied new pasteurization and antibacterial techniques and tried to develop new, effective antibacterial reagents [1–3]. However, advancements in the field of nanotechnology attracted the attention of researchers in the growth of the pharmaceutical field. Since

nanomaterials have a large surface area and high reactivity compared with bulk solids, as a result, nanoparticles display extraordinary physical, chemical, and biological properties.

A large amount of research has been reported on antibacterial activity using various nanoparticles like silica/iron oxide, silica, ferrites, ZnO, CuO, and metal nanoparticles [4–8]. Among diverse metal nanoparticles, silver nanoparticles are very encouraging because of their various unique properties, such as catalysis, electrochemical, and antimicrobial. It is also observed that silver nanoparticles exhibit high antimicrobial activity against various viruses and bacterias, such as Gram-positive and negative bacteria, fungi, protozoa, and certain viruses. Recently, it has been reported that silver nanoparticles have shown antitumor activity in many cancerous cell lines [9]. These excellent properties and several biomedical applications of silver nanoparticles make their use effective in different applications, such as antibacterial agents in apparel, diagnostic applicative biological tags, and biosensors, cosmetics, footwear, wound dressings, paints, and so forth [10]. However, it has been observed that silver nanoparticles display superior properties as compared with bulk material. These silver nanoparticles easily interact with other particles and improve antibacterial activity. According to the Mie summation, where particles' diameter is <20 nm, absorption in a UV range only depends on the dipole term [11]. Moreover, there are many reports which explain the antibacterial action of silver nanoparticles. Gu et al. [12] reported that vancomycin-covered silver and gold nanoparticles show better antibacterial activity as compared to antibiotics. Nida et al. showed the synthesis of stable silver-substituted hydroxyapatite nanoparticles with controlled morphology and particle sizes which was achieved by using CTAB as a surfactant. Their results revealed that these nanoparticles demonstrated anti-bacterial activity against *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli* [13]. Hence, these results suggest that the synthesis of stable silver nanoparticles is a subject of great interest.

There are various methods that are used for the synthesis of silver nanoparticles, such as chemical reduction [14], electrochemical methods [15], radiation [16], laser ablation [17], photochemical methods [18], sonochemical methods [19], and sputtering [20]. Among these methods, the most popular one is the chemical reduction of silver salt in the presence of any stabilizing agent. Utilization of nontoxic chemicals and renewable materials has attracted significant attention due to their benefit in reducing environmental risk. However, the major challenge is to prepare nanoparticles of high purity and low polydispersity. Recently, microwave irradiation has been considered to be an effective and distinct heating method for the preparation of silver nanoparticles, and due to its exceptional features, such as short reaction time, quick volumetric heating, energy saving, and high reaction rate, it has attracted researchers' interest [13,21].

Herein, we have reported on the easy, rapid, and cost-effective synthesis of spherical and monodispersed silver nanoparticles by the microwave irradiation method within 3 min of irradiation time which has shown excellent antibacterial efficiency against both gram-positive and gram-negative bacteria. In particular, silver nanoparticles showed higher antibacterial activity for *P. aeruginosa* than that of *E. coli*, *S. aureus*, *K. pneumoniae*, and *C. albicans*.

2. Experimental Details

E. coli, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *C. albicans* were acquired from the 'JNMC', Aligarh Muslim University, India. All the chemicals used in this study were AR-grade materials and used without further purification. Silver nitrate (AgNO₃, 99%), oxalic acid (99.8%), and cetyltrimethyl ammonium bromide (CTAB, 99.5%) were procured from Sigma Aldrich, Missouri, United States. De-ionized water was used for aqueous solutions. For synthesis of silver nanoparticles, 10 mL of 5 mM AgNO₃, 0.2 gm of oxalic acid, and 0.2 gm of CTAB were mixed to get an aqueous solution. Further, the solution was stirred for 1 min and then kept in a microwave oven (SAMSUNG, Seoul, South Korea; 800 W) for heating at 100% power of 800 W for 3 min. Upon microwave irradiation, the colorless solution immediately turned into a characteristic pale-yellow color, repre-

senting successful synthesis of silver nanoparticles. The benefit of microwave-mediated synthesis over conventional heating is the enriched kinetics of the reaction mostly by one or two orders of magnitude, due to fast initial heating and the generation of localized high-temperature zones at reaction sites [22]. The as-obtained product was used for the characterization.

The XRD pattern of as-synthesized silver nanoparticles was acquired using an X'pert MPD 3040 X-ray powder diffractometer using $\text{CuK}\alpha$ radiation ($\lambda = 1.5418 \text{ \AA}$). Optical properties were measured by a UV-Vis Spectrophotometer (Agilent 8453, Santa Clara, CA, USA) with a 1 cm quartz cell.

Field emission transmission electron microscopy (FETEM; JEOL, JEM-2100F, Tokyo, Japan) was used to study the surface morphology and selected area electron diffraction (SAED) of the synthesized silver nanoparticles. In order to measure the TEM measurements, a small drop of nanoparticle solution was cast onto a carbon-coated copper grid and then dried in air before moving it to the microscope. Image J' software was used to determine the average particle size and the size distribution.

A variety of microorganisms, including *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC 25922), *K. pneumonia* (ATCC 700603), *P. aeruginosa* (ATCC 27853), and *C. albicans* (ATCC 10213) were used to study the antibacterial activity of silver nanoparticles. The standard agar-well diffusion method using standard strains of *E. coli*, *S. aureus*, and *P. aeruginosa* was used for silver nanoparticles' antibacterial activity. The bacterial strain used here was grown in Luria Bertani (LB) Broth at 37°C overnight up to a turbidity of 0.5 Mac Farland standard (10^8 CFU perml) [23]. About 50 μL of silver nanoparticle suspension was used to inoculate the petridish filled with LB agar. Wells were punched in the agar plates and filled with the nanoparticle solutions, and the LB agar plates were incubated overnight at 37°C . The bacteria-free water extract alone and deionized Milli-Q water were used as controls. Samples treated with nanoparticles were spread on nutrient agar plates and after incubation at 37°C for 24 h, the zones of inhibition were observed only in the presence of silver nanoparticles and documented.

3. Results and Discussion

The color transformation of the solution during synthesis clearly shows the formation of silver nanoparticles. The colorless silver nitrate solution changes to yellow at 3 min microwave irradiation. Figure 1 highlights the UV-Vis absorption spectrum of CTAB stabilized silver nanoparticles. It can be clearly seen from the spectrum that the absorption maxima occurred at 420 nm. The absorption peak observed at 420 nm infers materialization of the silver nanoparticles, which is accredited to the Mie-scattering by silver metal [24].

The typical XRD pattern of the as-prepared silver nanoparticles is shown in Figure 2. The XRD pattern of silver nanoparticles revealed a single-phase nature with a face-centered cubic (FCC) structure. No secondary phase was detected within the detection limit of the XRD, and the high intensity of peaks confirmed the high crystalline nature of as-prepared silver nanoparticles. The XRD spectrum of silver nanoparticles point out characteristic peaks of silver which are allocated to the (111), (200), (220), and (311) planes of the face-centered cubic structure (FCC) (JCPDS 01-1167) [25,26].

The transmission electron microscopic measurement was conducted to study the size and morphology of the synthesized silver nanoparticles. The TEM micrograph of the synthesized silver nanoparticles is shown in Figure 3, which reflects that the nanoparticles are uniformly distributed and spherical in shape with an average diameter of ~ 6 nm. The diameter of the particles was measured using Image J software. The particles' size distribution histogram is plotted by using ~ 100 randomly selected nanoparticles from the TEM micrograph. The lower inset of Figure 3 shows the particle-size distribution plot of the synthesized silver nanoparticles. From the TEM micrograph, it can be seen that microwave synthesis gives highly monodisperse silver nanoparticles, with particle sizes ranging from 4.5 to 8.5 nm and an average size of 6.1 nm. The crystalline nature of the silver nanoparticles was further revealed by the selected area electron diffraction (SAED)

pattern. The typical SAED pattern of silver nanoparticles is shown in the upper inset of Figure 3. The diffraction rings are clearly visible, and these can be indexed to the reflections of face-centered cubic (FCC) silver nanoparticles [27,28].

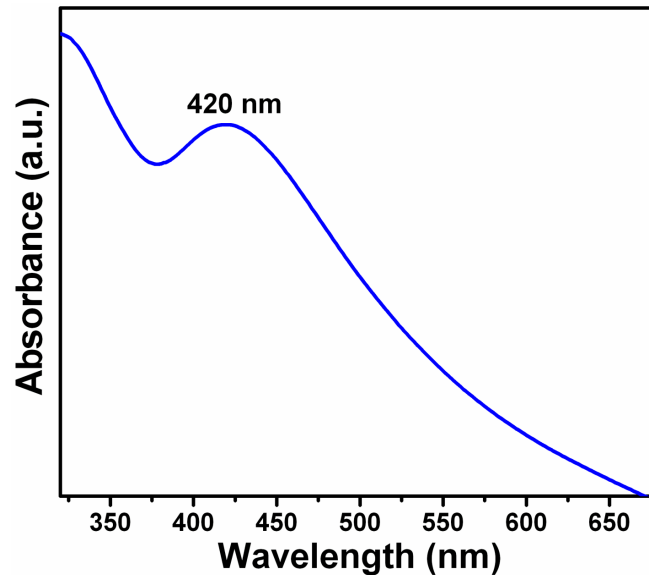


Figure 1. UV-Vis absorption spectrum of silver nanoparticles.

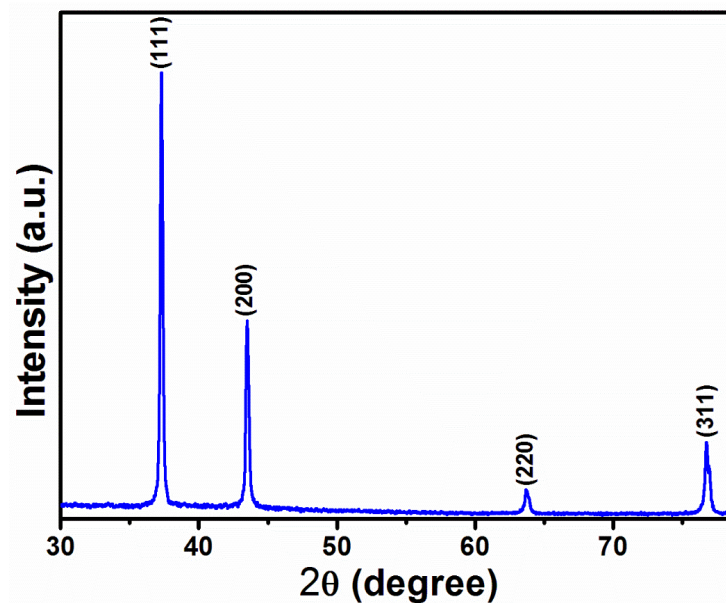


Figure 2. XRD pattern of silver nanoparticles.

The reduction of Ag^+ to Ag^0 within 3 min of irradiation shows the successful formation of nanoparticles using the microwave irradiation method. The microwave irradiation utilizes the internal heat generated within the matrix and gives a complete reduction within a short duration of time. All these results give a clear suggestion of the highly crystalline nature of the silver nanoparticles synthesized by this method.

It is observed that as-synthesized silver nanoparticles revealed the broad spectrum of antimicrobial activities against Gram-negative and Gram-positive bacteria. The silver nanoparticles' exposure to three different test microorganisms, such as *E. coli*, *S. aureus*, and *P. aeruginosa* resulted in the formation of the zones of inhibition. The zones of inhibition studied in the silver nanoparticle solutions were 21, 30, 28, 35, and 29 mm for *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *C. albicans*, respectively, as shown in Figure 4.

It is evident from Figure 4 that the largest zone of inhibition was observed for silver nanoparticles toward *P. aeruginosa*, while keeping the concentration of nanoparticles fixed. The efficient antimicrobial property of silver nanoparticles is because of their extremely large surface area, which delivers better contact with the microorganisms.

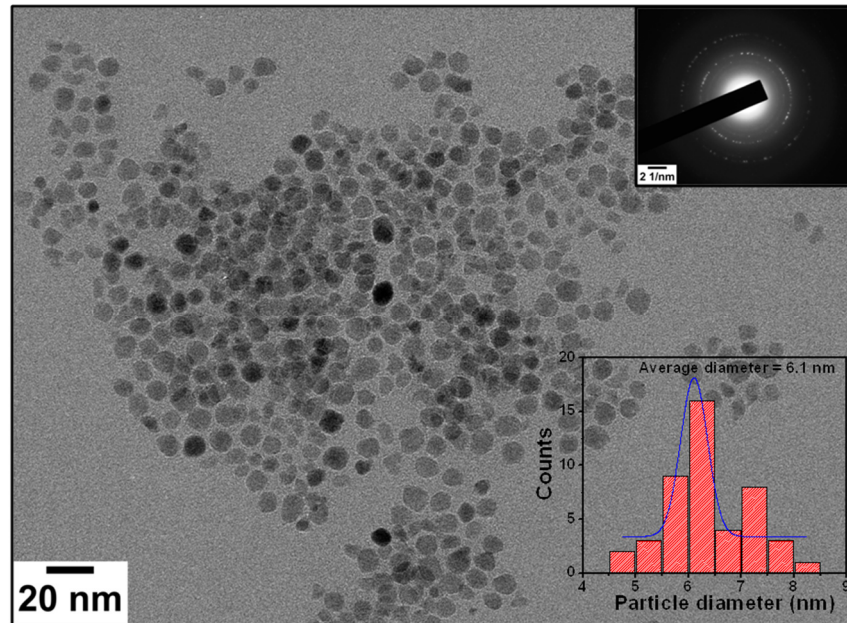


Figure 3. TEM image of silver nanoparticles. The upper inset shows the corresponding SAED pattern, and the lower inset shows the particle size distribution plot.

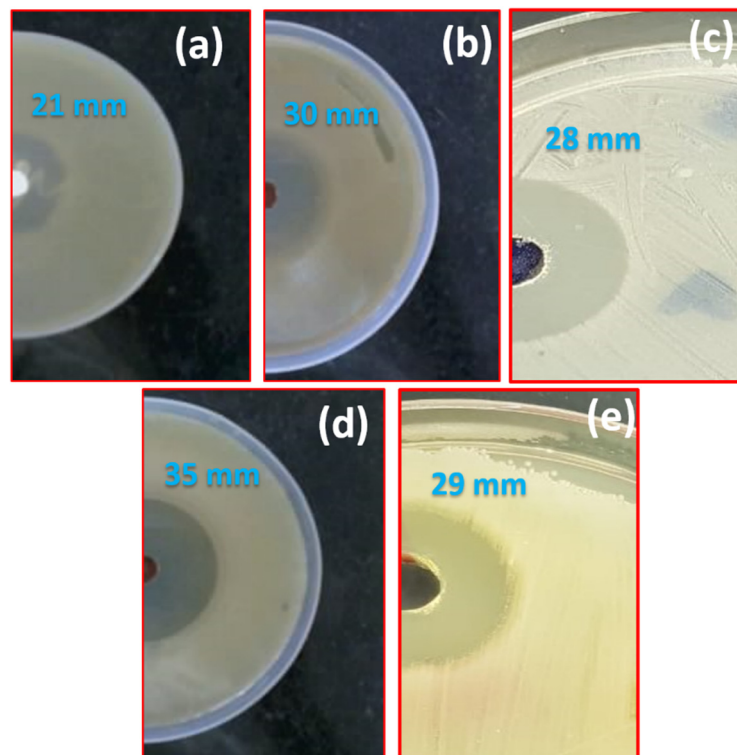


Figure 4. Zone of inhibition obtained with silver nanoparticles for (a) *E. coli*, (b) *S. aureus*, and (c) *K. pneumoniae*, (d) *P. aeruginosa*, and (e) *C. albicans*.

The optical density (OD), which is used to measure the turbidity, is measured using a spectrophotometer. The bacterial inoculum is introduced into a liquid suspension of bacteria in a nutrient broth. It is observed that when the bacterial population in the suspension develops, the turbidity (optical density) of the suspension shall increase. The higher value of turbidity infers that the bacterial population is greater. It is found that the turbidity (optical density) of the solution reduces after introduction of an antimicrobial agent. This may happen due to the killing of the bacteria by an antimicrobial agent. This method delivers a fast and convenient approach of testing the anti-microbial properties of liquid formulations. Figure 5 displays growth curves of the *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *C. albicans* strains in LB medium inoculated with 10^8 CFU of bacteria in the presence of silver nanoparticles. The highest concentration of the silver nanoparticles (50 μ L) was found to strongly inhibit the growth of all the bacterial test strains.

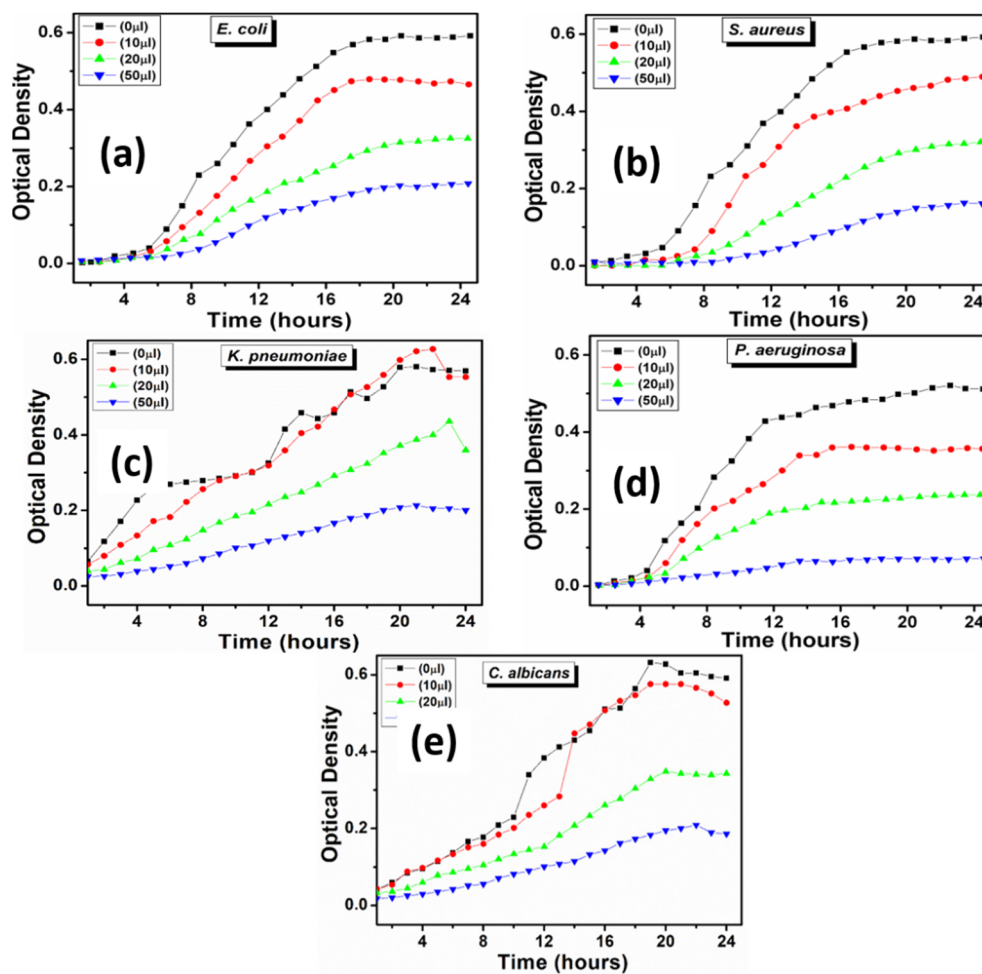


Figure 5. Growth curves of (a) *E. coli*, (b) *S. aureus*, and (c) *K. pneumoniae*, (d) *P. aeruginosa*, (e) *C. albicans* in the presence of silver nanoparticles.

We found that silver nanoparticles cause a growth delay in all the bacterial strains under all concentration used in the experiment. Figure 5 reflects the clear difference between antibacterial activities of the five microorganisms using silver nanoparticles (Figure 5a–d). We observed the silver nanoparticles exhibit the highest activity against *P. aeruginosa*, whereas it showed moderate activity against *E. coli*, *S. aureus*, *K. pneumoniae* and *C. albicans*. The small size of silver nanoparticles infers that it has a large surface area to come in contact with the bacterial cells, and as a result, it will have higher percentage of interaction [29]. Silver nanoparticles that are smaller than 10 nm interact with bacteria and generate electronic effects, which boost the reactivity of nanoparticles.

The comparative analysis on the antibacterial activity of silver nanoparticles against *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa* and *C. albicans* is shown in Figure 6. This result indicates that antibacterial activity toward *P. aeruginosa* is higher than that toward *E. coli*, *S. aureus*, *K. pneumoniae* and *C. albicans*. It is well-known that the structure and chemical composition of the cell wall are quite different among *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *C. albicans*. In chemical stress, such as the environment including antibiotics, the tolerance in *P. aeruginosa* is usually weaker than that in *E. coli*. This might be a possible reason why antibacterial activity toward *P. aeruginosa* is stronger than toward that of *E. coli*, *S. aureus*, *K. pneumoniae*, and *C. albicans* [30].

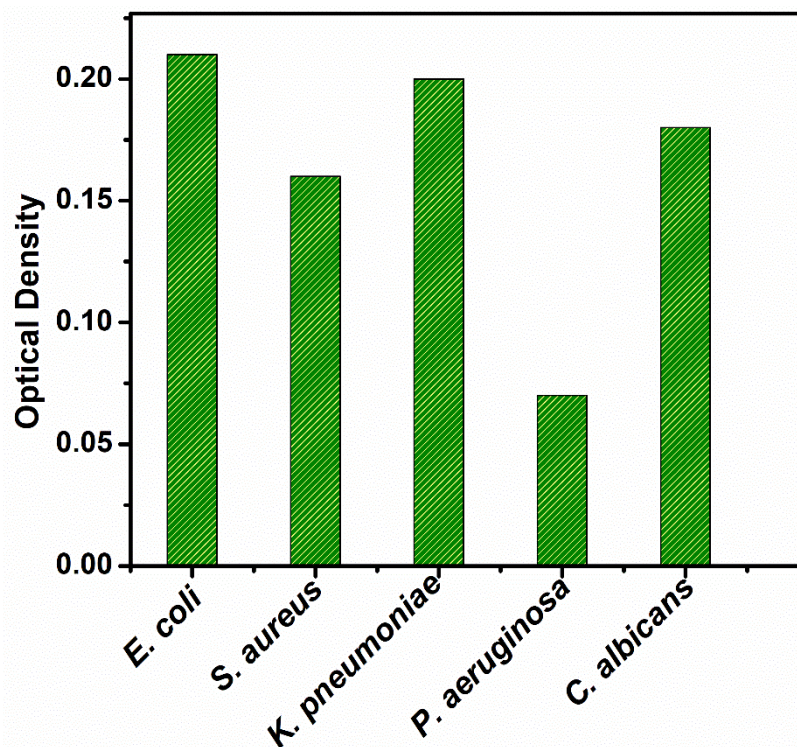


Figure 6. A comparative analysis on antibacterial activity of silver nanoparticles against *E. coli*, *S. aureus*, *K. pneumoniae*, *P. aeruginosa*, and *C. albicans*.

Antibacterial properties of silver nanoparticles against various pathogens have been reported by various researchers. Jun et al. [31] evaluated the effects of Ag nanoparticles on microorganisms against yeast, *Escherichia coli*, and *Staphylococcus aureus*. They observed that the low concentration of Ag nanoparticles was required for the inhibition of yeast and *E. coli*; however, *S. aureus* was found to show mild inhibition. In another work, Sheeana et al. [32] developed silver nanoparticle cluster coatings on the surfaces of copper. Their study was focused on the durability of the microbiocidal activity against pathogenic bacterial and fungal species, including *Escherichia coli* O157:H7 and *Candida auris*. The results showed that more than 90% of *E. coli* and *C. auris* were found to be non-viable. Other researchers [33] have prepared lichen-based Ag nanoparticles by using a green solid-state mechanochemical approach and evaluated the biological activity. In particular, they have compared the processing of lichen material of *Pseudevernia furfuracea* and *Lobaria pulmonaria* for extraction, as well as for synthesis of AgNPs, and tested the antibacterial and antioxidant activity of the extracts. Their results showed that due to the formation of AgNPs, the antibacterial activity was found to increase, while the antioxidant activity decreased. The influence of silver NPs as nano-food additives towards the human gut microbiome was investigated by Sheeana et al. [34].

Since most of the infection-causing multi-resistant bacterial strains have become a major warning to public health concerns, the high antibacterial activity of the silver

nanoparticles concerning both gram-positive and gram-negative bacteria means that their use as antibacterial agents is recommended in preventing the outbreaks caused by the above pathogenic strains.

4. Conclusions

In summary, spherical and monodispersed silver nanoparticles have been successfully prepared under microwave irradiation by using oxalic acid as a reducing agent and CTAB as a binding agent. XRD results show the highly crystalline and face-centered cubic structure of the silver nanoparticles. TEM and the selected area electron diffraction pattern confirmed the formation of spherical and highly dispersed silver nanoparticles (~6 nm) with an FCC structure. The UV-Vis absorption spectrum shows surface plasmon resonance at 420 nm, which is a characteristic of silver nanoparticles. The measured antibacterial activity of the synthesized silver nanoparticles clearly demonstrates the excellent activity of these nanoparticles against several pathogenic bacteria, showing the highest activity for *P. aeruginosa*. The method used in the present paper is relatively simple, fast, and environmentally friendly, and this can be scaled up for the bulk production of highly monodispersed silver nanoparticles, as well as other noble metals. This opens the opportunity for numerous applications of these nanoparticles to produce effective antibacterial agents for the management of emerging multidrug-resistant pathogenic bacteria.

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