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Assessment of the antimicrobial effect of silver nanoparticles synthesized from *Coriandrum sativum*

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Abstract

In this study the antimicrobial effect of silver nanoparticles (AgNPs) synthesized from *Coriandrum sativum (C. sativum)* leaf extract was evaluated by varying the incubation temperature from 25 °C to 40 °C, for 24 h of exposure. AgNPs were characterized using Ultraviolet-Visible (UV-vis), and Transmission Electron (TEM) spectroscopy, evidencing the presence of nanoparticles of spherical morphology with average sizes of 5.7, 13.4, and 6.0 nm for nanoparticle concentrations of 1, 10, and 100 mM, respectively. The efficacy of nanoparticles as microbicidal agents was evaluated with the halo inhibition method, impregnating nanoparticles in sensi-discs on agar planted with ATCC standard *Staphylococcus aureus* (*S. aureus*), and clinically isolated *Klebsiella pneumoniae* (*K. pneumoniae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*). AgNPs inhibited the growth of all three bacteria under all conditions. The clinically isolated bacteria obtained the smallest inhibitory diameters, with respect to the standard bacteria. Incubation temperature had a significant effect on all bacteria, with the greatest effect found with AgNPs-100mM at 25°C for *P. aeruginosa* and at 35°C for *S. aureus* and *K. pneumoniae*. These results showed the potential application against pathogenic microorganisms of AgNPs synthesized from *C. sativum* leaf in hospital environments within a temperature range from 25 to 40 °C.

Keywords

Silver nanoparticles; antimicrobial effect; *S. aureus*; *K. pneumoniae; P. aeruginosa*; *C. sativum*; hospital environments

Introduction

Silver nanoparticles (AgNPs) have emerged as novel nanomaterials for biological applications e.g., antibacterial and fungal agents.[1–3] AgNPs synthesized from *Coriander sativum* (*C. sativum*) are of great interest as an alternative ecofriendly synthesis method. Nanoparticle sizes <50 nm have been reported using *C. sativum*, which increases their interaction with the microorganisms of interest.[4–6] However, there must remain plenty of potential antimicrobial applications for *Coriander Sativum*-based AgNPs that remains unexplored.

Staphylococcus aureus (*S. aureus*), *Klebsiella pneumoniae* (*K. pneumoniae*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) are multidrug-resistant bacteria of notable clinical importance due to their rapid spread.[7–10] Senthilkumar *et al*. studied the inhibition of these bacteria after exposing them for 24 h at 37 °C to AgNPs synthesized with *C. sativum* stems.[11] Alsubki *et al*., evaluated the antimicrobial effect, ATCC standard bacteria and 16 h of exposure at 37 °C. Ashraf *et al*. investigated the antimicrobial potential of *C. sativum* leaf-mediated AgNPs against *S. aureus*.[6] Deshpande *et al*. used AgNPs against *S. aureus* and *K. pneumoniae* at 37 °C.[12]

S. aureus, *K. pneumoniae* and *P. aeruginosa* have the versatility to survive and reproduce over an ample temperature range (4 to 46 °C), which increases the probability of contamination of the human-host in hospital environments.[13–15] On the other hand, it has been reported that clinically isolated bacteria including *S. aureus* and *P. aeruginosa* are less susceptible to antibacterial agents than standard bacteria. Therefore, it is important to evaluate the antimicrobial effect of AgNPs under conditions that mimics the hospital environment, i.e., against isolated bacteria and temperatures from 25 °C to 40 °C.

In the present study, environmentally friendly AgNPs were synthesized using *C. sativum* leaf, with three different concentrations of 1, 10, and 100 mM. Antimicrobial properties of *C. sativum* leaf-based AgNPs were studied against hospital isolated pathogenic bacteria *K. pneumoniae* and *P. aeruginosa*, and ATCC standard *S. aureus*, during 24 h of exposure at 25, 35, and 40 °C.

Results and Discussion

Characterization of the Extract

The infrared spectrum of the *C. sativum* leaf extract (see Figure 1) shows a peak between 1568-1660 cm^{-1}, characteristic of the functional group C-C (Alkane). Intense absorption bands could also be noticed in regions of the infrared spectrum between 3221-3508 cm^{-1}, showing OH groups from water, carbohydrates, and amides. Absorption of 1367-1435 cm^{-1} is attributed to the vibrations of the C-O amide and peaks between 1028-1120 cm^{-1} correspond to the carboxylic acid group (COOH). In addition, a band between 2877-2956 cm^{-1} is associated with the functional group C-H (Alkane). [5,16,17]

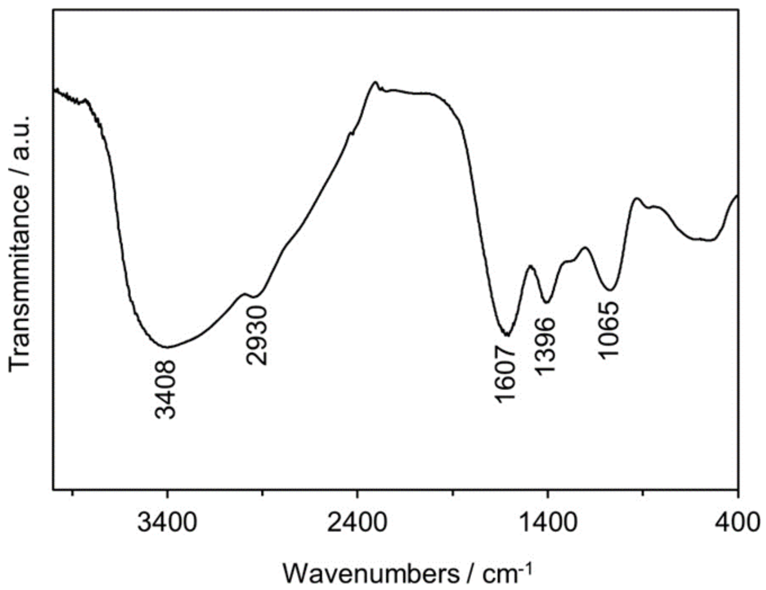

Figure 1: Fourier-transform infrared spectroscopy spectrum of coriander leaf extract

Characterization of Silver Nanoparticles:

The color change from colorless to brown when synthesizing the AgNPs demonstrates the reducing properties of coriander attributed to secondary metabolites such as flavonoids that may reduce silver ions to nanoparticles counterpart. [1,4,5]

UV-vis Characterization:

UV-vis spectra of silver nanoparticles suspended in NaOH with a pH ~ 10 are shown in Figure 2. AgNPs were labelled as AgNPs-1mM, AgNPs-10mM, and AgNPs-100mM for AgNPs obtained using $AgNO_3$ solutions at concentrations of 1, 10, and 100 mM, respectively. Maximum absorption peaks, determined by UV-vis spectroscopy, appeared at 421 nm, 423 nm and 430 nm for the AgNPs-1mM, AgNPs-10mM and AgNPs-100mM samples, respectively. These values confirmed the presence of AgNPs.[18]

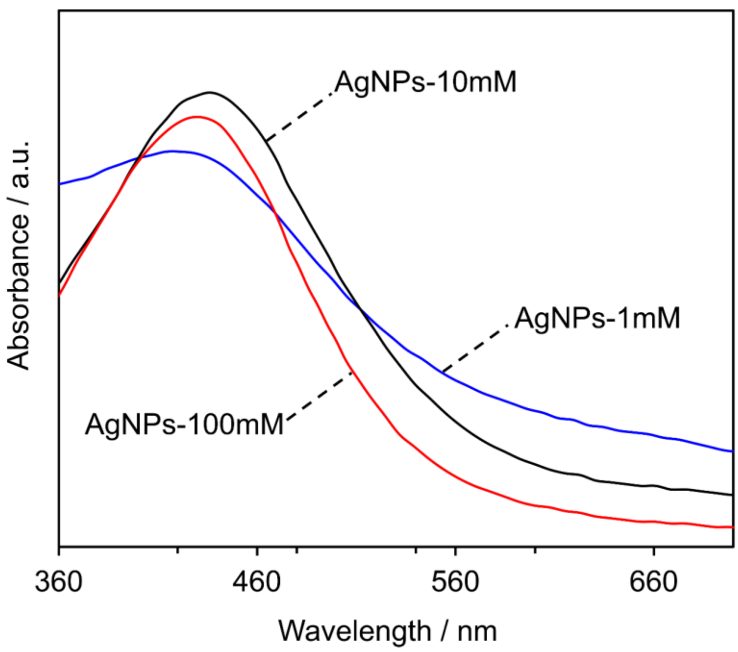

Figure 2: UV-vis spectra of *C. sativum* leaf-based AgNPs-1mM, AgNPs-10mM, and AgNPs-100mM.

TEM Characterization:

TEM analysis was performed on samples of reduced AgNPs with *C. sativum* to establish the presence, size, and morphology of AgNPs suspended in aqueous medium. Figures 3a, 3b, and 3c shows TEM images of AgNPs synthesized, which demonstrate a predominant spherical morphology for each concentration. The images show a good dispersion of AgNPs in the aqueous medium without agglomerations. The average nanoparticles diameter found was 5.7 nm, 13.4 nm and 6.0 nm for AgNPs-1mM, AgNPs-10mM, and AgNPs-100mM, respectively. The obtained nanoparticles sizes is between the typical sizes previously reported, i.e., 8 to 75 nm.[5] The nanoparticles obtained by this method of synthesis are relatively small, which makes them have a greater contact surface area, thus increasing their reactivity and their antimicrobial activity.

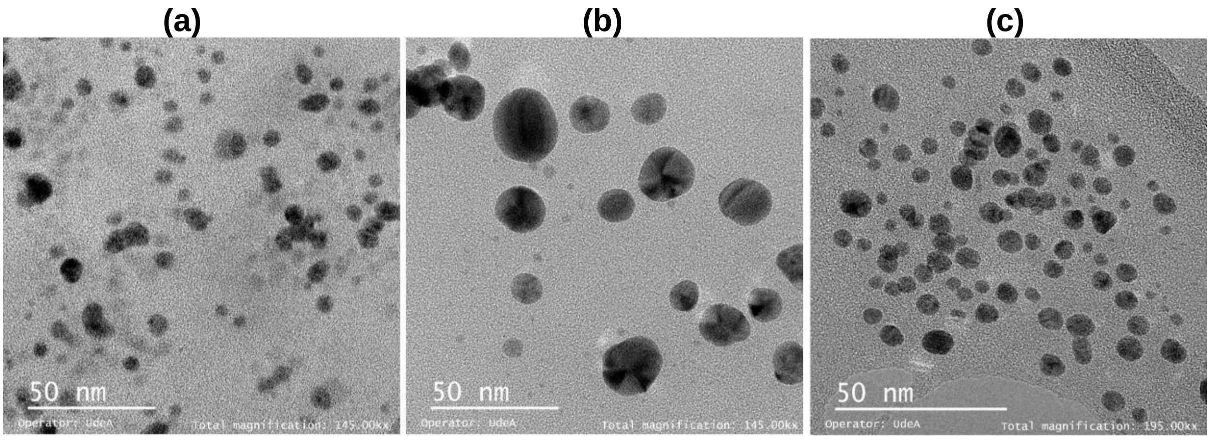

Figure 3: TEM characterization of (a) AgNPs-1mM, (b) AgNPs-10mM and (c) AgNPs-100mM with scale bar 50 nm.

Antimicrobial effect of AgNPs against bacteria

The antimicrobial effect of the AgNPs was determined against three multidrug-resistance bacteria according to the World Health Organization (WHO) with priority 1 (*S. aureus)* and priority 2 (*K. pneumoniae* and *P. aeruginosa*).[7] The average inhibition halo for each condition is shown in Table 1. The positive control of the standard antibiotic Ciprofloxacin was used, which had an inhibitory effect on the three bacteria.

Table 1: Main effects of inhibition halo formed by AgNPs against bacteria.

Temperature	Silver nitrate concentration (mM)	Average inhibition halo (mm)		
		S. aureus	***K. pneumoniae***	***P. aeruginosa***
25 °C	1	- [a]	6.3±0.2	8.0±0.1
	10	- [a]	6.7±0.4	8.3±0.4
	100	- [a]	6.8±0.4	9.0±0.1
35 °C	1	8.1±1	7.6±0.5	7.7±0.6
	10	10.1±1	8.2±1	8.4±0.6
	100	12.1±0.9	8.6±0.5	8.7±0.5
40 °C	1	7.2±0.4	7.2±0.4	6.5±0.1
	10	8.2±0.4	8.4±0.2	7.5±0.1
	100	9.5±0.7	8.5±0.1	8.0±0.1

[a]The bacteria did not grow in this condition.

Synthesized *C. sativum* leaf-based AgNPs induced growth inhibition in all three bacteria studied. The largest average diameter of inhibition was obtained with the ATCC standard bacteria *S. aureus*, which may be due to the higher susceptibility compared to clinically isolated bacteria *K. pneumoniae* and *P. aeruginosa*. Furthermore, gram-positive bacteria have an absence of the outer membrane characteristic of gram-negative bacteria, which makes them more resistant to antimicrobials. However, gram-positive bacteria have a thicker peptidoglycan layer that protects it from penetration by silver ions.[19,20]

The halo is indicative of bacterial growth inhibition on a Mueller Hinton agar plate inoculated with each bacterium. Incubation temperature and $AgNO_3$ concentration for nanoparticle synthesis seem to have a significant effect on the average diameters of the inhibition halos (ANOVA P-Value <0.05). However, the combination of both factors (temperature and concentration) only had a significant effect on *S. aureus*, which means that the impact of the concentration of AgNPs on the diameter of the inhibition zone depends on the temperature used. The maximum diameter indicated in *S. aureus* and *K. pneumoniae* was 12.1 and 8.6 mm, respectively, both obtained at 35 °C and AgNPs-100mM. In contrast, *P. aeruginosa* showed the largest halo of 9 mm at 25 °C and AgNPs-100mM.

The increase in the concentration of the synthesized *C. sativum* leaf-based AgNPs has a greater antimicrobial efficacy against the three bacteria studied. This result is attributed to the increased antimicrobial properties of silver at the nanoscale. Due to AgNPs have a high surface area, the chemical reactivity of the surface is increased, which enhances interaction with bacteria.[21] Although the mechanism of action of AgNPs in bacteria is not completely clear, it have been suggested that AgNPs can denature the outer membrane or peptidoglycan layer of bacteria.[22] This action induces the depletion of intracellular ATP due to the electrostatic attraction between the positive charge of the nanoparticles and the negative charge of the cell wall of the microorganisms.[22] The increased antimicrobial activity of the AgNPs obtained using a concentration of 100 mM is probably a result of a higher concentration of AgNPs and not of the change of the dimensions of the nanoparticles. It should be noted that the only component that is exerting inhibition against growth is AgNPs, since the controls that were performed (see Figure 4), do not inhibit the growth of these bacteria. Ciprofloxacin was also used as standard and positive control to show antimicrobial activity, where inhibition halos of 2.8 cm, 3.2 cm and 2.2 cm were obtained for *Staphylococcus Aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, respectively. Figures 5, 6 and 7 represent the inhibition halos formed by AgNPs-1mM, AgNPs-10mM, and AgNPs-100mM, at 35°C against *Staphylococcus Aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, respectively.

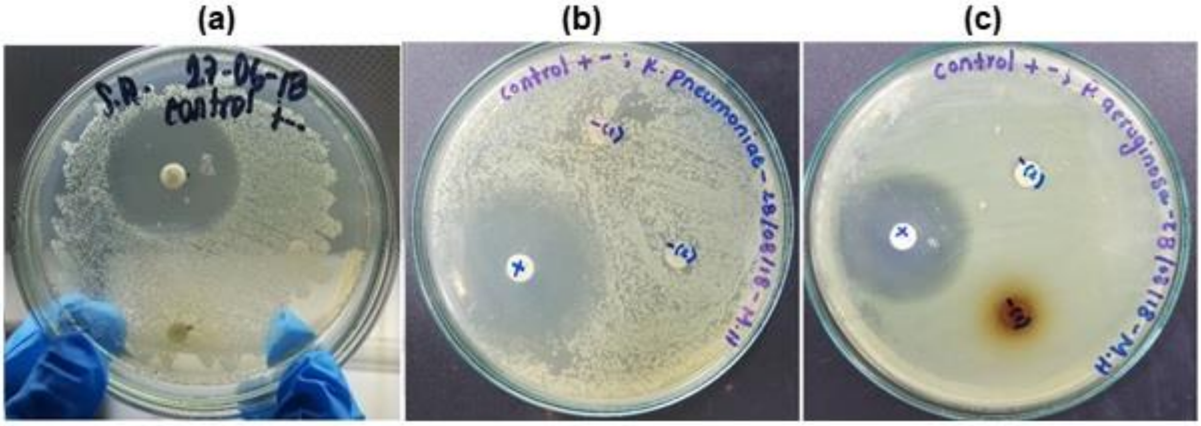

Figure 4: Controls: Ciprofloxacin (+), *Coriandrum sativum* (-1) *sodium hydroxide* (-2) against bacteria: (a) *Staphylococcus Aureus*, (b) *Klebsiella pneumoniae* and (c) *Pseudomonas aeruginosa.*

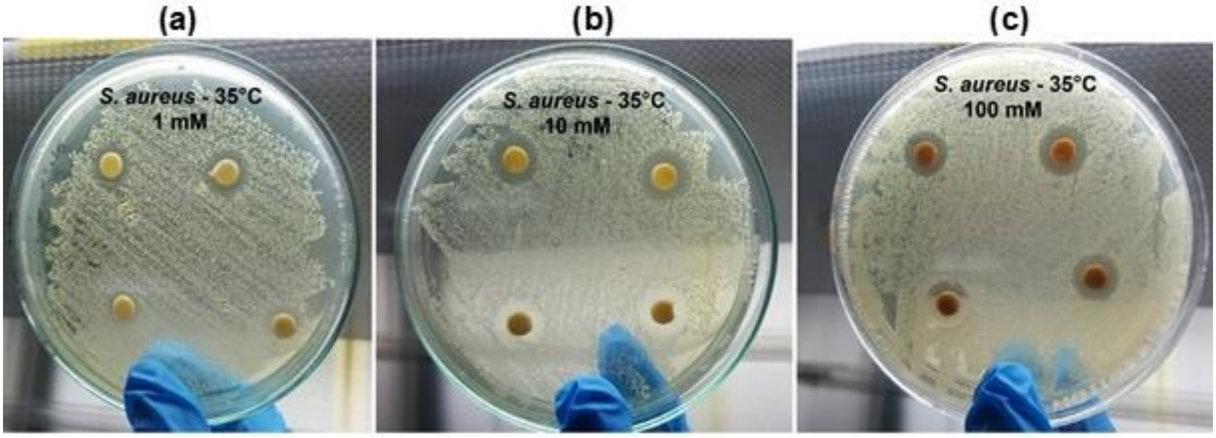

Figure 5: Inhibition halo formed by (a) AgNPs-1mM, (b) AgNPs-10mM, and (c) AgNPs-100mM, at 35°C against *S. aureus*.

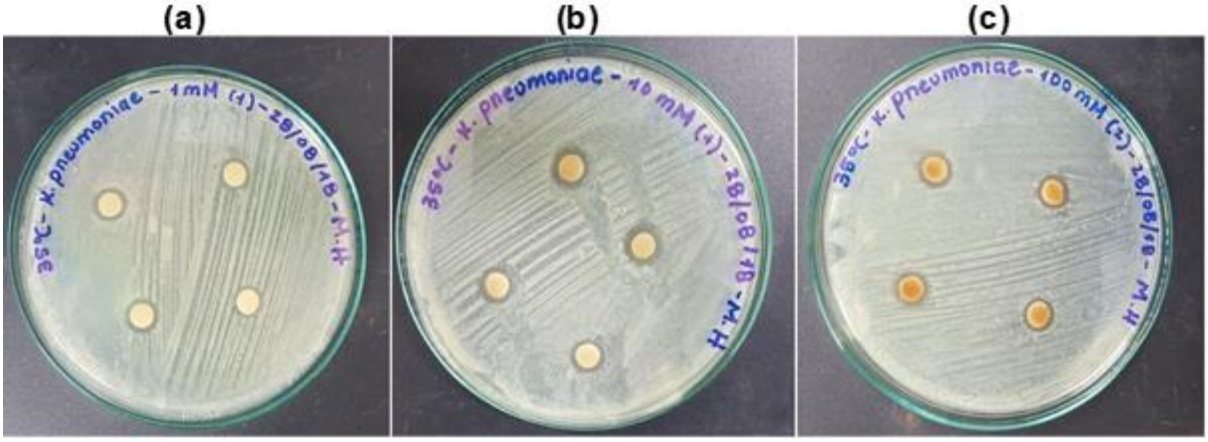

Figure 6: Inhibition halo formed by (a) AgNPs-1mM, (b) AgNPs-10mM, and (c) AgNPs-100mM, at 35°C against *K. pneumoniae*.

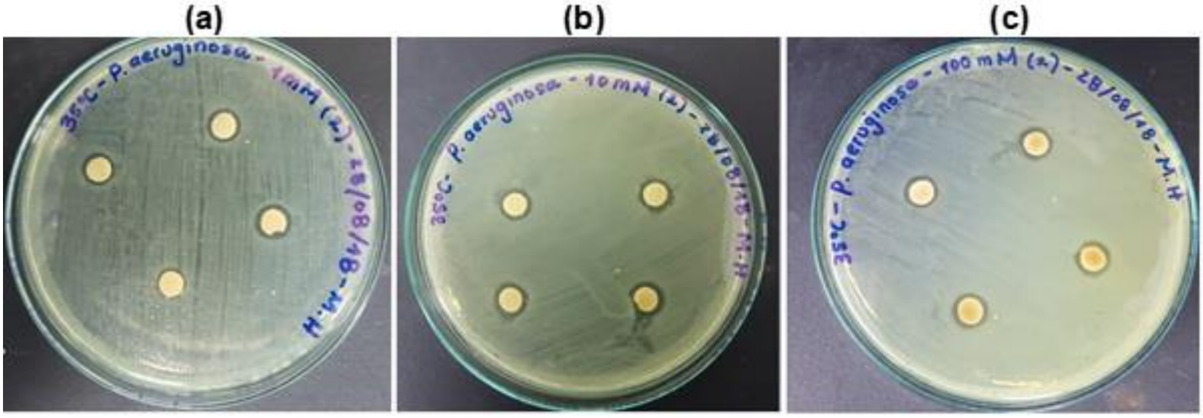

Figure 7: Inhibition halo formed by (a) AgNPs-1mM, (b) AgNPs-10mM, and (c) AgNPs-100mM, at 35°C against *P. aeruginosa*.

Conclusion

Synthesized *C. sativum leaf-based* AgNPs proved to be antimicrobial agents against bacteria of clinical interest and multidrug-resistant *S. aureus*, *K. pneumoniae* and *P. aeruginosa.* The increase in the concentration of $AgNO_3$ in the synthesis of AgNPs was directly correlated with the inhibition halo. Clinically isolated bacteria *K. pneumoniae* and *P. aeruginosa* were more resistant to AgNPs, with lower inhibition halos compared to standard *S. aureus* bacterium. Furthermore, temperature had a significant effect on the inhibition of all three bacteria, implying that *C. sativum leaf-based* AgNPs appear to be viable for use as an antimicrobial against clinically isolated pathogenic bacteria in hospital environments with a temperature range from 25 °C to 40 °C.

Experimental

Preparation of Coriander Leaf Extract

200 g of coriander leaves gathered from a local market, were used and prepared according to the procedure described in the literature.[4] In a typical synthesis, 100 g of leaves washed with distilled water were used and their size was reduced by crushing in a mortar and pestle. Then it was poured into a beaker with 250 mL of distilled water and heated on a plate with continuous stirring until boiling (~300 rpm and 10 minutes). 100 g of additional leaves were then added with continued stirring and heating. Subsequently, the leaves were removed, and the concentrated extract was heated until its volume was reduced by evaporation to 50 mL. The final extract was filtered, deposited in glass vials, and cooled to room temperature.

Synthesis of AgNPs

A solution of $AgNO_3$ purchased from Merck Millipore (Emsure, ACS reagent) was used as a precursor. A stock solution of 100 mM $AgNO_3$ was prepared, and dilutions of 10, and 1 mM were made.[23] 1.5 mL of coriander extract was combined with 25 mL of $AgNO_3$ solution. Subsequently, the temperature of the solution was increased to 55 °C and nanoparticles were collected by centrifugation at 7500 rpm for 20 min. The AgNPs were suspended in distilled water adding 0.1 M NaOH (purchased from Merck Millipore) drops until reaching a pH of 10. The suspensions were brought to ultrasound for 30 minutes, allowed to settle for a period of one week, to remove any precipitate that may had formed

Characterization

To carry out the characterization of the coriander leaf extract, an IRAffinity model Fourier transform infrared spectroscope (FTIR) was used, with a resolution of 4 cm^{-1} and a wavelength range between 4000 and 400 cm^{-1}. The characterization of the AgNPs was carried out through the ultraviolet visible spectroscope (UV-Vis) model Evolution 60S to determine the wavelength between 400-450 nm using samples diluted at 1% v/v. TEM analysis was performed using the high resolution (0.1 nm) FEI Tecnai G2 F20 S-TWIN HR(S)TEM equipment.

Assessment of the Antimicrobial Effect of AgNPs

To evaluate the antimicrobial activity of AgNPs, gram-negative bacteria strains *K. pneumoniae* and *P. aeruginosa* were isolated from local hospital samples and the gram-positive bacteria *S. aureus* was purchased to Thermo Scientific (ATCC 25923). To determine the effectiveness of AgNPs as a microbicidal agent, the disk diffusion or inhibition halo method was used.[24] Each inoculum of bacteria was suspended in saline and turbidity was adjusted to McFarland's 0.5 standards (~1.5x108 CFU/ml).[25] Petri dishes were prepared with 15 mL of Mueller-Hinton Agar and the entire plate was inoculated homogeneously with a sterile cotton swab. Circular filter paper discs of 6 mm of diameter were soaked with 10 µl of AgNPs dispersion of 1 mM, 10 mM and 100 mM were put on the freshly seeded agar and incubated at 25 °C, 35 °C, and 40 °C for 24 hours. After the incubation period, the diameter of the halo around the filter paper was determined. Negative controls were NaOH and coriander extract and positive control Ciprofloxacin Sensi-disc (Becton, Dickinson, and Company).

Statistical Analysis

A multifactorial analysis of variance ANOVA was carried out to determine the statistical significance of each factor (concentration of silver nitrate solution and incubation temperature) and the interactions between them on the growth of the inhibition halo.

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