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Research Article

ANTIBACTERIAL ACTIVITY OF GREEN SYNTHESIZED SILVER NANOPARTICLES

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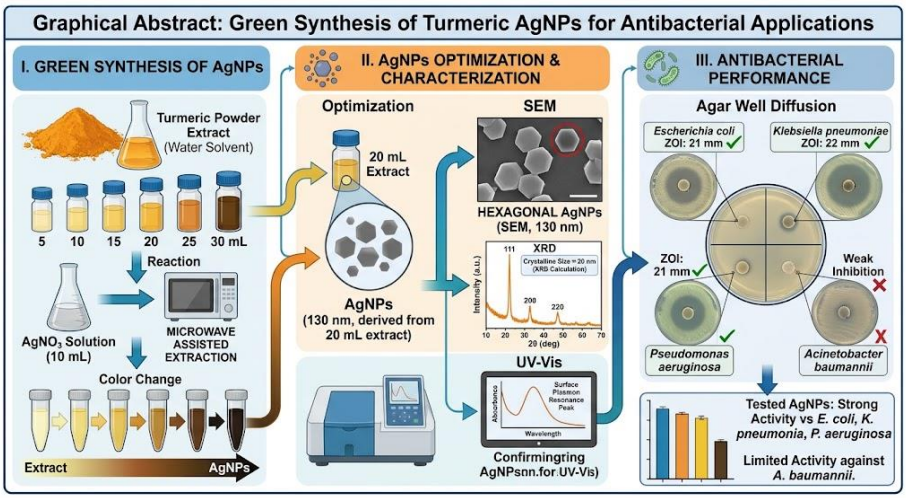
ABSTRACT

Nanoparticles have gained acknowledgment owing to their antibacterial activity. Whereas their green synthesis has added to their importance. In the present study, green synthesis of silver nanoparticles was done using turmeric powder extract employing microwave assisted extraction method using water as a solvent. Nanoparticles synthesis was optimized using different concentrations of plant extracts 5, 10, 15, 20, 25, and 30 mL. The particles were characterized using UV-Visible spectroscopy, SEM and XRD and antibacterial activity using agar well diffusion method. Results showed that color change of silver nanoparticles was noticed using visual and UV-Visible spectrophotometric analysis. SEM analysis revealed hexagonal shaped silver nanoparticles. X-Ray diffraction analysis of turmeric silver nanoparticles was done to calculate the average crystalline size which was 20 nm. Overall, particles with smallest size as per SEM analysis (130 nm) were formed by AgNPs formed by 20 mL plant extract combined with 10 mL of silver nitrate solution. These NPs were then used to test antibacterial activity. Antibacterial activity of pure plant extract and silver nanoparticles were checked against four different Gram-negative bacterial species i.e., *Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa* and *Acetinobacter baumannii.* Tested AgNPs were more active against *E. coli, K. pneumonia,* and *P. aeruginosa* with zone of inhibition of 21, 22, and 21 mm whereas they were not very active against *A. baumannii.*

KEY WORDS: *Curcuma longa,* Scanning electron microscopy (SEM) analysis, X-Ray diffraction analysis

GRAPHICAL ABSTRACT:



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1: INTRODUCTION

New strategies are required for combating increased cases of drug resistance amongst bacteria and fungi. Nanotechnology applications in various medicine fields have been widely discovered particularly in field of drug delivery. In the 1-100 nm range, nanoparticles show strong antibacterial activity (Zhang *et al*., 2008). The National Nanotechnology Initiative (NNI), U.S. defined nanoparticles as microscopic particles with at least one of the three dimensions less than 100 nm (Kim *et al*., 2011).

Silver (Ag) has natural antimicrobial properties and low toxicity. Silver nanoparticles are widely used in medical sciences. They have been proved as an active antibacterial agent and used as biocide to kill or inhibit the growth of different bacterial, fungal and viral species. Green synthesis of metal nanoparticles is valuable above physical and chemical methods as it is sustainable and ecological process as it is not essential to consume high temperature, energy, toxic chemicals and pressure. Plants are free from dangerous chemicals and natural covering agents so they can offer better platform for the synthesis of silver nanoparticles. Now a days, silver nitrate is the most important silver salt used in nanotechnology to synthesize silver nanoparticles (Sharma *et al*., 2013).

Turmeric or *Curcuma longa* is a member of Zingiberaceae family and a perennial herb extensively cultivated in Asia typically in China and India. The plant rhizome portion produces a yellow powder that is used medicinally. Turmeric powder that gives curry powder a characteristic color yield from dried *C. longa.* Its rhizome has been traditionally used as antimicrobial agent as well as an insect repellant (Rudrappa and Bais, 2008). Curcuminoids i.e., flavonoids are the most active constituents of turmeric that is a combination of diferuloylmethane (curcumin), monodesmethoxy curcumin and bisdexmethoxy curcumin. In turmeric, curcumin is the most active constituent and makes up almost 90% of the curcuminoid content in turmeric. Other components include proteins, resins and sugars (Heath *et al.,* 2004).

In the present research work, potential of *Curcuma longa* (turmeric) powdered plant extract was checked to synthesize silver nanoparticles by using $AgNO_3$, to characterize them using different techniques and to analyze their antibacterial activity against four Gram negative bacterial strains i.e., *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Acetinobacter baumannii.*

2: MATERIAL AND METHODS

Present study was carried out to synthesize and characterize silver nanoparticles by green synthesis method using powdered plant material of *Curcuma longa* L. Commercially available pure powdered plant material was purchased from local market and stored in airtight jars for further use. Powdered plant material was extracted with microwave by using water as a solvent.

2.1: Synthesis of silver nanoparticles

Aqueous solution (4 mM) $AgNO_3$ was prepared, and 10 mL of $AgNO_3$ solution was mixed with different concentrations of plant extract (i.e., 5, 10, 15, 20, 25 and 30 mL) respectively. Then solutions were placed at room temperature for 24 hours. After 24 hours, solutions were analyzed by UV-Visible spectrophotometry in the range of 300-700 nm and by color change. The images of all solutions were taken at each step. Then the solutions were centrifuged for 15 minutes at 10,000 rpm. The pellet was stored at 4°C in ethanol after disposal of supernatant.

2.2: Characterization of Ag-NPs:

Silver nanoparticles solutions were then subjected to Scanning electron microscopy (SEM), Powder X-Ray diffraction analysis and antibacterial activity (Ghaffar *et al*., 2022).

2.3: Scanning electron microscopy

Average size and apparent morphology of silver nanoparticles were verified by scanning electron microscopy. SEM analysis was done using JSM-6480 scanning electron microscope. By dropping a very small amount of sample on a carbon coated copper grid thin films of the samples were prepared. Extra solution was removed by using blotting paper. Then the films were allowed to dry on SEM grid and nanoparticles images were taken (Ghaffar *et al*., 2022).

2.4: Powder X-Ray Diffraction analysis:

X-ray diffraction analysis of powdered silver nanoparticles was done by using X'pert Pro MPD PAN analytical X-ray diffractometer Holland limited. CuKα was used as a characteristic radiation with λ= 1.54 with 2θ configuration. Measurements were made in 20° to 80° in 2θ range. The data was analyzed using specific XRD X'pert software. The crystalline domain size of nanoparticles was calculated from the width of XRD peaks using Debye-Scherrer's formula.

$$D = 0.94\lambda/ß\ Cos\theta$$

Where D is the average crystallite domain size perpendicular to the reflecting planes, λis the X-ray wavelength, ß is the full width at half maximum (FWHM) and θ is the diffraction angle.

Analysis of antibacterial activity of silver nanoparticles

Silver nanoparticles antibacterial activity was verified by using agar well diffusion process. Different concentrations of silver nanoparticles were used as experimental. Aqueous plant extract and silver nitrate solution were used as control. Glassware was sterilized properly before use. After autoclaving nutrient agar media was poured in the petri plates under sterile conditions and then allowed to solidify and left in incubator to check contamination. Then freshly prepared overnight cultures of five Gram negative bacterial strains *Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumonia, Aceinobacter baumannii*were inoculated on petriplates containing media. Two wells were made in each plate using cork borer and labeled as A, B, C and D. 15-20 µL of the silver nanoparticles and pure plant extracts (namely aqueous, methanol, ethanol, acetone and chloroform extracts) were added in wells. Then the plates were kept at incubation for 24 to 36 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed as a clear region around the well. The maximum zone of inhibition was measured in all samples against different bacterial strains.

2.5: Statistical Analysis:

Means were compared using one way ANOVA with Tuckey's test with 5% level of significance.

3: RESULTS

3.1: Synthesis of silver nanoparticles:

Different concentration solutions of turmeric extract affected the formation of silver nanoparticles. The experiment was set up for 2, 4 and 24 hours that allowed the silver nitrate to react with the different concentrations of plant extract for the formation of silver nanoparticles. The reduction of silver ions to silver nanoparticles was observed as visible color change from orange yellow to light brown after 2 hours. After 24 hours dark brown color change was observed in all the concentration solutions of silver nanoparticles. Color change was also monitored with the help of UV-Visible spectrophotometric

analysis. Wavelength range was 300 to 700nm for this study. Spectrophotometric analysis noted that for each concentration of plant extract λ_{max} shifted from 300 nm to in the range between 426 to 476nm after 24 hours. There was also a significant change recorded in lambda maximum after 2 and 4 hours of reaction as shown in Table 1.

Table 1: UV-visible analysis of green synthesized AgNPs

Sr. No	Extract Quantity mL	$\lambda_{(max)}$ Wave length (nm) with maximum absorption			
		At Start	After 2 Hours	After 4 hours	After 24 hours
TNP1	5	300±0.696	437±1.690	436±1.821	426±0.730
TNP2	10	300±1.259	543±2.013	438±2.142	462±1.054
TNP3	15	300±1.351	462±2.070	450±2.056	470±1.016
TNP4	20	300±1.097	461±2.267	466±2.347	476±1.175
TNP5	25	300±1.280	483±2.251	472±2.364	469±1.096
TNP6	30	300±1.196	536±1.769	536±2.786	473±1.161

3.2: Scanning electron microscopy analysis:

Scanning electron microscopy was done to analyze the surface morphology and shape of silver nanoparticles. Average size of silver nanoparticles was also determined. Hexagonal shape silver nanoparticles were observed overall. While the average size of synthesized AgNPs was observed to be 256, 310, 300, 130, 155, and 185 nm when 5, 10, 15, 20, 25, and 30 mL of plant extract was used respectively. The figures (a, b) show the images of SEM analysis of *C. longa* based silver nanoparticles with 20 and 30 mL of plant extract.

3.3: XRD analysis

For further confirmation of *C. longa* silver nanostructure X-ray powder diffraction analysis was done. Characteristic silver peaks were determined in X-ray diffractogram pattern. By using the Debye' Scherrer's crystalline nature of the silver nanoparticles was also confirmed.

$$D=0.9\lambda/FWHM.\ Cos\ \theta$$

Where λ is the characteristic X-ray wavelength with the value of 1.54Å, FWHM is the full width at half maximum of the highest peak and θ is the Bragg's angle. The XRD pattern revealed that hexagonal shape silver nanoparticles were present in the sample. The average calculated crystalline size was 20 nm.

3.4: Antibacterial assay

To analyze the antibacterial activity, silver nanoparticles (synthesized from 20 mL of plant extract) were tested against five different Gram negative pathogenic species i.e., *Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa* and *Acetinobacter baumannii* using agar well diffusion method. Figure 3 shows that there was a significant effect of AgNps on the growth of all the bacteria. In all bacterial strains, antibacterial activity of AgNps was significantly higher than simple water extract of *C. longa.* Figure also shows that inhibitions zones of 21, 22, and 21 mm were recorded for AgNPs against *E. coli, K. pneumonia, and P. aeruginosa respectively.* Whereas against*, A. baumannii* AgNPs were not effective.

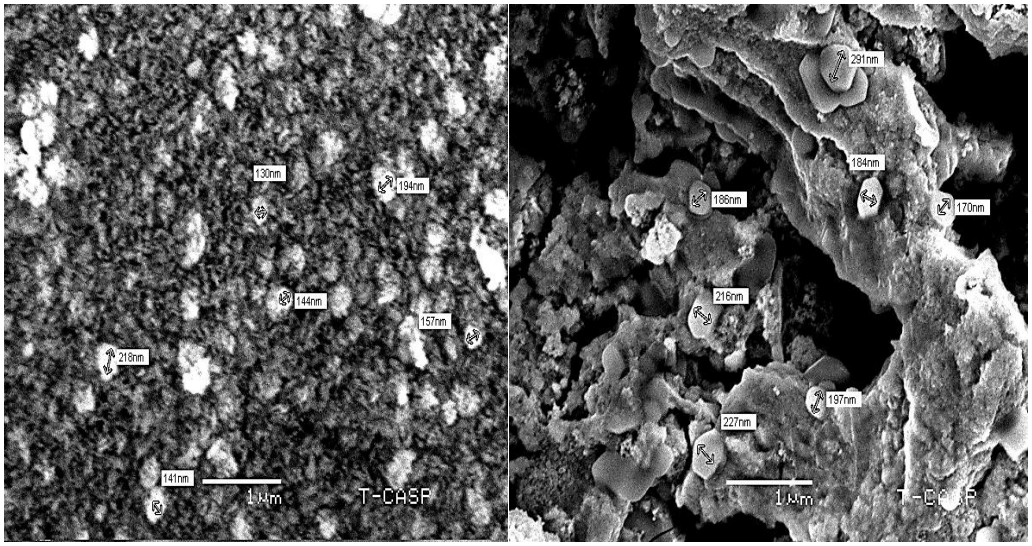

Figure 1: SEM analysis of AgNPs synthesized fro water extract of *C. longa* using 10 mL of silver nitrate and a) 20 mL of plant extract b) 30 mL of Plant extract X-Ray diffraction analysis

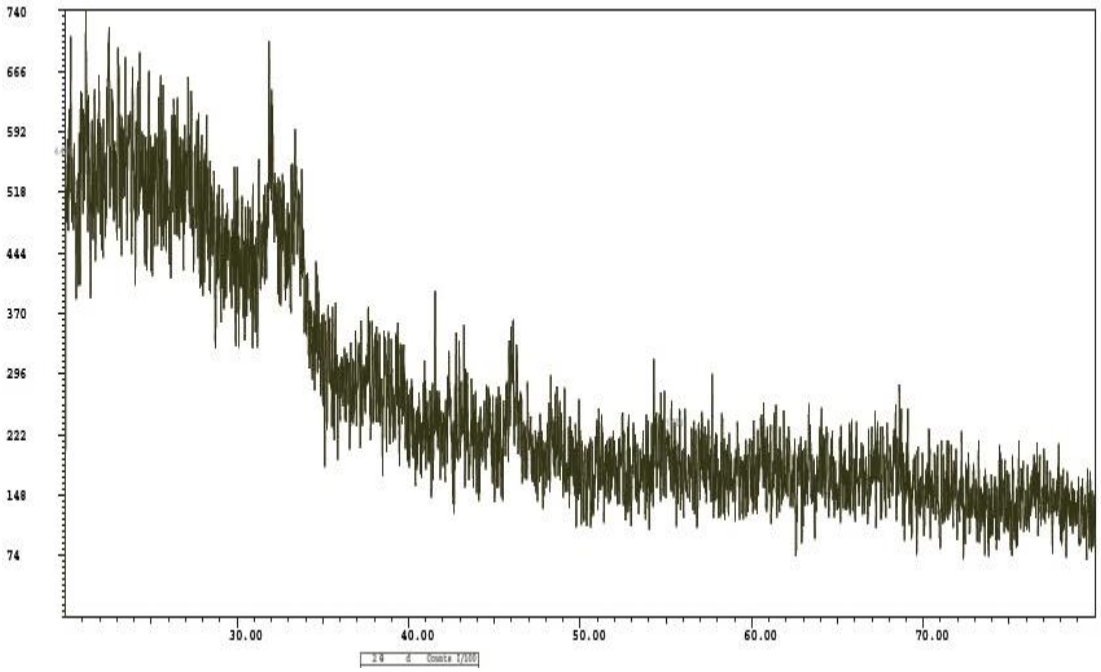

Figure 2: X-ray diffractogram of silver nanoparticles formed by 20 mL of water extract of *C. longa*

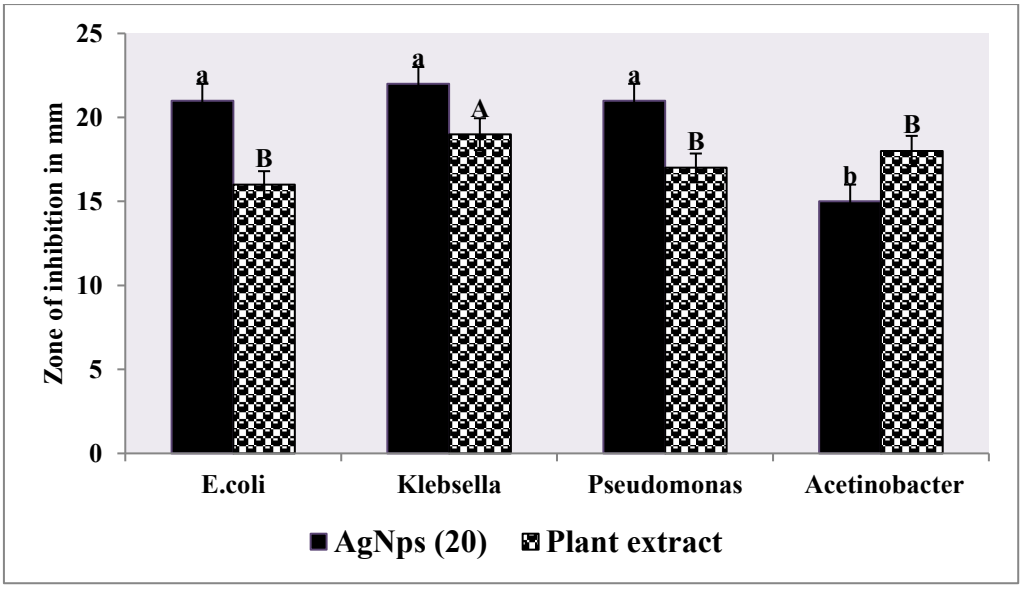

Figure 3: Antibacterial activity of AgNPs synthesized from water extract of *Curcuma longa* against different bacterial strains

4: DISCUSSION

Green synthesis of nanoparticles is a cost effective and environmentally friendly method. It ensures the synthesis of NPs without involving any complicated physical method but biochemical processes i.e., biochemical reduction of metal ions. In the present study when plant extracts were mixed with silver nitrate solution, due to surface plasmon resonance, the color change was observed in all the concentrations of reacting solutions. Electrons oscillate continuously in the silver nanoparticles. The particle size and shape affect these specific oscillations, and it also affects the nanoparticles' interaction with light. Therefore, these particles have different colors and sizes (Shameli *et al*., 2012). Kuber *et al.* (2006) reported the spectrum of UV-Visible analysis of silver ions present in aqueous medium with *Aspergillus fumigatus* showed a peak at 420 nm corresponding to the silver nanoparticles surface plasmon absorbance. Das *et al*., 2013 studied the Ag-NPs formation by calculating the SPR bands of the *Moringaoleifera* and Ag/ *Moringa oleifera* emulsions in the range of 300–800 nm wavelength.

Furthermore, number of extracts significantly affected the amount of plant metabolites that influenced the formation of NPs in turn. Earlier research has also shown that amount of plant metabolites is directly affecting the size, shape and other characteristics of synthesized NPs (Javad *et al*., 2019; Naseer *et al*., 2024). Parsad and Swamy in 2012 analyzed the *Szyzigium cumini* silver nanoparticles to analyze their morphology and dimensions using scanning electron microscopy. SEM has been recognized as an authentic assay to analyze the shape and approximate size analysi for NPs (Patil and Chougale, 2021).

X-Ray diffraction analysis gives the crystalline size of the NPs and is mostly used for identifying the NPs after their synthesis (Shitu *et al*., 2023). In the present stuy, XRD analysis of synthesized NPs showed a crystalline size of 20 nm. Earier, Das *et al*., 2013 determined the average crystalline size 40 to 50 nm of *Moringa oleifera* silver nanoparticles

using X-ray diffraction analysis technique. Kumar in 2010 evaluated the average crystalline size 10 nm of *Parthenium hysterophorus* silver nanoparticles with the help of XRD analysis.

There is a difference between sizes of NPs analyzed by SEM (130 nm) and XRD (20 nm) in the present study, when 20 mL of plant extract was mixed with 10 mL of 4 M silver nitrate solution. XRD shows the inner crystal size of NPs using Xray beams whereas SEM shows overall particle size with outer margins. Therefore, it gives higher readings than XRD (Hirschle *et al*., 2016).

Inhibition of the bacterial growth occurred due to the change in the bacterial structure. Silver nanoparticles destroy the cell membrane of bacterial cells and enter the cell. Silver ions react with the respiratory enzymes of the bacteria thus activating the reactive oxygen species that can cause destruction of bacterial cell due to the damage of its DNA (Prabhu and Polouse, 2012; Moges, W. and Misskire,).

Rao and Svithramma in 2011 checked the antibacterial efficacy of silver nanoparticles of *Svensonia hyberadensis,* silver against *Klebsiella pneumonia, Escherichia coli* and *Pseudomonas aeruginosa*. Maximum zone of inhibition was observed in *Pseudomonas aeruginosa* i.e., 15 mm followed by *Escherichia coli* 10 mm and *Klebsiella pneumoniae* 8 mm. Karthik *et al*., 2014 also evaluated the antibacterial activity of *Hibiscuss cannabinus* and *Punica grantum* ethanol, acetone and chloroform extracts against *E. coli, Klebsiella pneumonia* and *Pseudomonas aeruginosa* strains. In both plants ethanol extract exhibited the highest antibacterial activity against *Escherichia coli* with the 12 mm zone of inhibition. Chloroform extracts of both plants showed the maximum antibacterial action against *Klebsiella pneumonia* with the inhibition zone of 12 mm and acetone extract of both plants showed the equal antibacterial activity with the zone of inhibition of 9 mm against all the bacterial strains.

In the present study, *A. baumannii* showed an enhanced resistance against NPs formed that may be attributed to its defence mechanism depending upon capsular polysaccharide shielding, lower outer membrane permeability, heavy metal efflux system, and enhanced oxidative stress response (Scoffone *et al*., 2025). But overall, NPs synthesized from the water extract of *C. longa* showed significant antibacterial activity.

5: CONCLUSION

Rapid green synthesis of *C. longa* silver nanoparticles was observed as an eco-friendly method. Plant extract acts as a reducing agent for silver nitrate. The silver nanoparticles were characterized by using different techniques i.e., Uv-Visible analysis, Scanning electron microscopy and X-ray diffraction analysis. The average size of the silver nanoparticles was 130 nm determined through SEM analysis while the crystalline average size of silver nanoparticles was 20 nm calculated via X-ray diffraction analysis. The antibacterial activity of silver nanoparticles and pure plant extract was evaluated against five Gram negative bacteria i.e., *Klebsiella pneumonia, Escherichia coli, Pseudomonas aeruginosa and Acetinobacter baumannii*using agar well diffusion method. Green synthesis is an eco-friendly method, and it has advantage of producing large scale production of silver nanoparticles. These synthesized silver nanoparticles have potential in different biomedicinally related fields due to their antibacterial activity.

Declarations

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AI USAGE: Gemini was used with proper prompt to make graphical abstract

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