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ECO-FRIENDLY SYNTHESIS OF SILVER NANOPARTICLES USING *CALOTROPIS GIGANTEA* LEAF EXTRACT: STRUCTURAL CHARACTERIZATION AND EVALUATION OF ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES

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ABSTRACT
Nanoparticles are unique small-scale particles compared to their large bulk counterparts. *Calotropis gigantea* is widely used in traditional medicine. Plant extract contains micronutrients, which acts as a reducing factor when synthesized with metal nanoparticles. The study provides eco-friendly synthesis of silver nanoparticles using *Calotropis gigantea* leaf extract and their biological potential. The investigation involves various characterization techniques: resultant AgNPs were confirmed by UV-Vis spectroscopy at 390 nm and XRD analysis with crystalline nature and equivalent particle size of 30 nm. The FT-IR technique confirms a metal-ligand interaction of Ag^+ ions at a peak around 518 cm^{-1}. The structure and detailed morphology of AgNPs were confirmed using SEM and TEM techniques. Additionally, AgNPs exhibited substantial antibacterial activity against E. coli and S. aureus using the agar well diffusion method; the zone of inhibition was observed at 50, 100, and 150 µg/mL, respectively. The antioxidative property of AgNPs were tested using the DPPH radical scavenging assay at concentrations ranging from 100 to 500 µg/mL. The study underscores that the comprehensive characterization techniques that provide the synthesis of AgNPs from *Calotropis gigantea* leaf extract and their potential antibacterial and antioxidant properties pave the way for further development in the field.

Keywords: Synthesis, *Calotropis gigantea*, silver nanoparticles (AgNPs), characterization, antibacterial activity, and antioxidant activity.

1. INTRODUCTION
The synthesis of metal nanoparticles has been widely used in the field of nanoscience and technology. [1] There are multiple methods to synthesize metal nanoparticles with their desired characterization. By using a large number of physical and chemical methods of synthesis, metal nanoparticles involve toxic substances, which reclassify hazardous chemicals and high energy requirements, leading to environmental contamination and human health issues, which may cause neurological damage, respiratory illness, and even cancer exposure to carcinogens. [2] To overcome it, the development of synthesizing metal nanoparticles by eco-friendly methods has become a significant approach of research as it bypasses the harsh conditions and toxicity and is more advantageous as a harmless, hazard free, and sustainable approach. [3]

The biological method of synthesizing metal nanoparticles is an “bottom-up” approach using microorganisms (bacteria, fungi, yeast, algae, viruses) and plant extracts. The plant often considers most efficient using leaves, flowers, stem, or root extracts, which produce eco-friendly polyphenols that perform as a reducing agent with Ag^+ ions. [4] The *Calotropis gigantea* comes under the family Apocynaceae, which is typically known as crown flower, and has a whiteish-purple colour. It commonly occurs on wasteland. It has been used as a folk medicine in India for many years, and has been reported to have a variety of uses as effective in treating skin, bacterial killing, circulatory, respiratory, neurological, asthma, and even cancer. The Phyto active compounds present in the plant, such as Tannins and glycosides, show the potential abilities to fight against the toxic pathogen. [5,6]

Ag^+ ions play a crucial role in the field of biomedical science due to the presence of the phytochemical properties. Silver nanoparticles are known as capable inhibitor for the antibacterial effect, anti-inflammatory, dental implantation, and medicinal activities. Due to their potential ability the are been used for treatment of various diseases. [7] The aim of synthesizing the AgNPs by using the *Calotropis gigantea* leaf extract is to validate the influence of the concentration of Ag^+ metal ions and the concentration of leaf extract. The plant compounds present in the leaf extract which are dependable for reduction of $AgNO_3$, were confirmed were confirmed using the colour change indication. Characterization techniques confirm the successful formation of silver nanoparticles, including UV-Visible Spectroscopy, X-Ray Diffraction (XRD), and Fourier Transform Infrared Spectroscopy (FT-IR). Their structural and detailed morphological appearance by using the techniques Scanning Electron Microscopy (SEM) and Transmission Electron Microscopy (TEM) to authenticate the potential abilities of silver nanoparticles synthesized by using *Calotropis gigantea* leaf extract. [8-10]

Furthermore, the formed AgNPs synthesized by using *Calotropis gigantea* leaf extract comprises the bio active metabolites including glycoside, steroids, proteins, flavonoids, alkaloids, and tannins with a capable antibacterial inhibitor against human pathogen E. coli and S. aureus using agar well diffusion methods as inhibiting property with minimum concentration, and antioxidative prospect by using DPPH radical scavenging assay was determined. [11,12] The study provides a sustainable, cost-friendly approach to synthesizing silver nanoparticles (AgNPs), illuminating their potential biological significance.

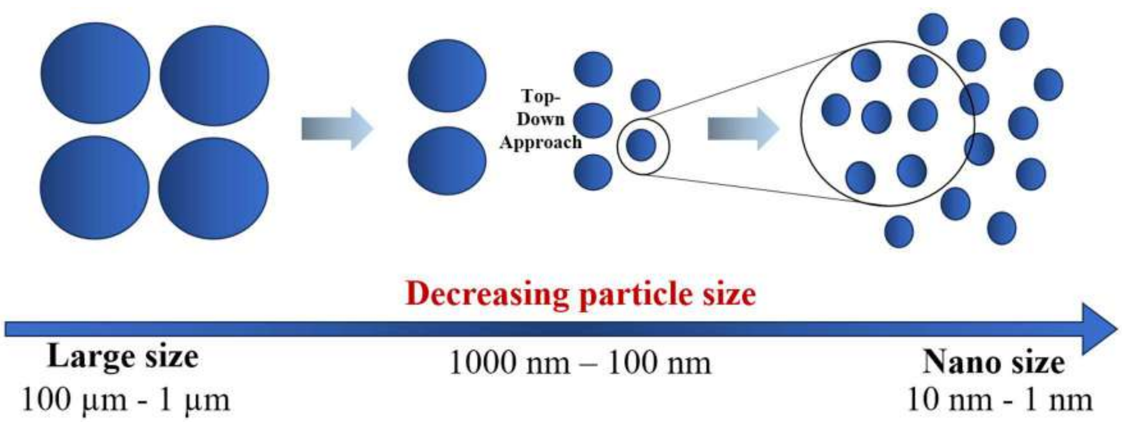

Fig 1.1: Bulk particles reduction to nano size particles.

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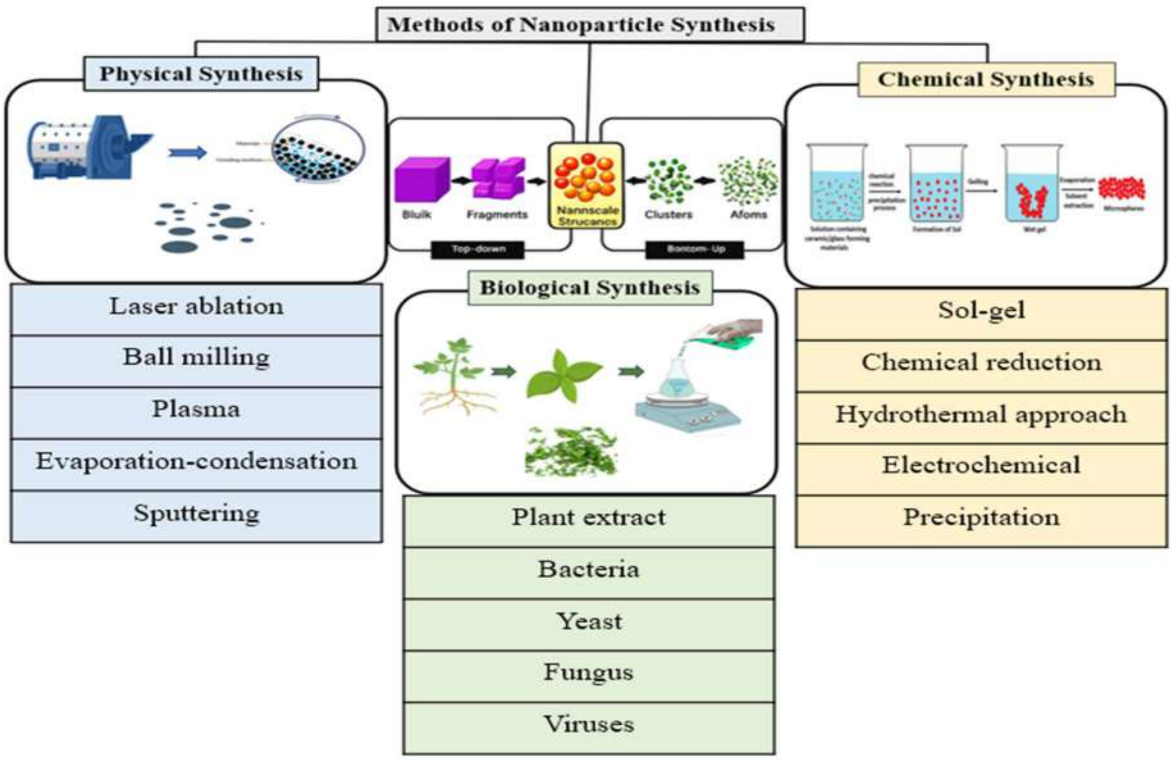

Fig 1.2: Conventional methods to synthesis nanoparticles.

2. METHODOLOGY

2.1 Chemicals and reagents

The chemical, including Silver Nitrate ($AgNO_3$), was used to synthesize silver nanoparticles. Develop a comprehensive protocol based on all the suitable conditions and focusing on both preventive and curative strategies for overcoming challenges posed by various factors (temperature, time of incubation, and contamination).

2.2 Preparation procedure of *Calotropis gigantea* leaf extract

The green and healthy leaves of the plant *Calotropis gigantea* were collected from the local region of Nagpur, Maharashtra, India. The leaves were washed multiple times under running tap water. After complete washing, the leaves were allowed to dry under shade conditions. After 15-20 days, the leaves were completely dried. The dried leaves were collected and crushed into small pieces, and the resulting material was further processed using an electric mixture grinder to obtain a uniform fine powder. The freshly prepared powder of the plant *Calotropis gigantea* leaf was stored in an air-tight condition for further use. [13]

To prepare the *Calotropis gigantea* leaf extract, the freshly prepared *Calotropis gigantea* leaf powder was weighed exactly 10 gm. 100 mL of distilled water was poured into a glass conical flask, and the leaf powder was added and stirred vigorously. By using an Electrical Magnetic Stirrer, the mixture solution was heated at 80°C for 30-35 minutes under continuous stirring conditions. After heating, the mixture solution was allowed to cool down at room temperature and was filtered using Whatman 42 filter paper. For better and clearer results, the solution was centrifuged at 1500 rpm for 15 minutes. The resultant *Calotropis gigantea* leaf extract was stocked in the refrigerator at 4°C for synthesis of silver nanoparticles as a precursor solution. [14]

Table 2.2: Summary of plant *Calotropis gigantea* leaf extraction

Plant Used (Common name)	Part of plant	Plant metabolites	Incubation	Temp (°C)	Formation of plant material	Storage
Calotropis gigantea (crown flower)	Leaves	Tannins, flavonoids, steroids, Proteins, and alkaloids	15-20 days	Room temperature	Powder form	Air-tight condition

2.3 Synthesis of silver nanoparticles (AgNPs)

Silver nanoparticles synthesis process as follows: A 9 mM stock solution of Silver Nitrate was prepared (10 mL plant extract in 100 mL $AgNO_3$ solution) in vigorous motion. The reaction solution was incubated in dark condition at room temperature for 24 hours. After 24 hours, the visible change in color was observed from pale yellow to brownish-black, indicating the formation of AgNPs successfully. Further, the solution was centrifuged at 7,000 rpm for 10-15 minutes. The acquired palette was repeatedly washed with distilled water and 70 % ethanol. The resultant precipitate was carefully collected and allowed to dry using an electrical Hot Air Oven at 80°C overnight. The silver nanoparticles were collected and scraped off. The AgNPs were stored in an air-tight condition. [3,15]

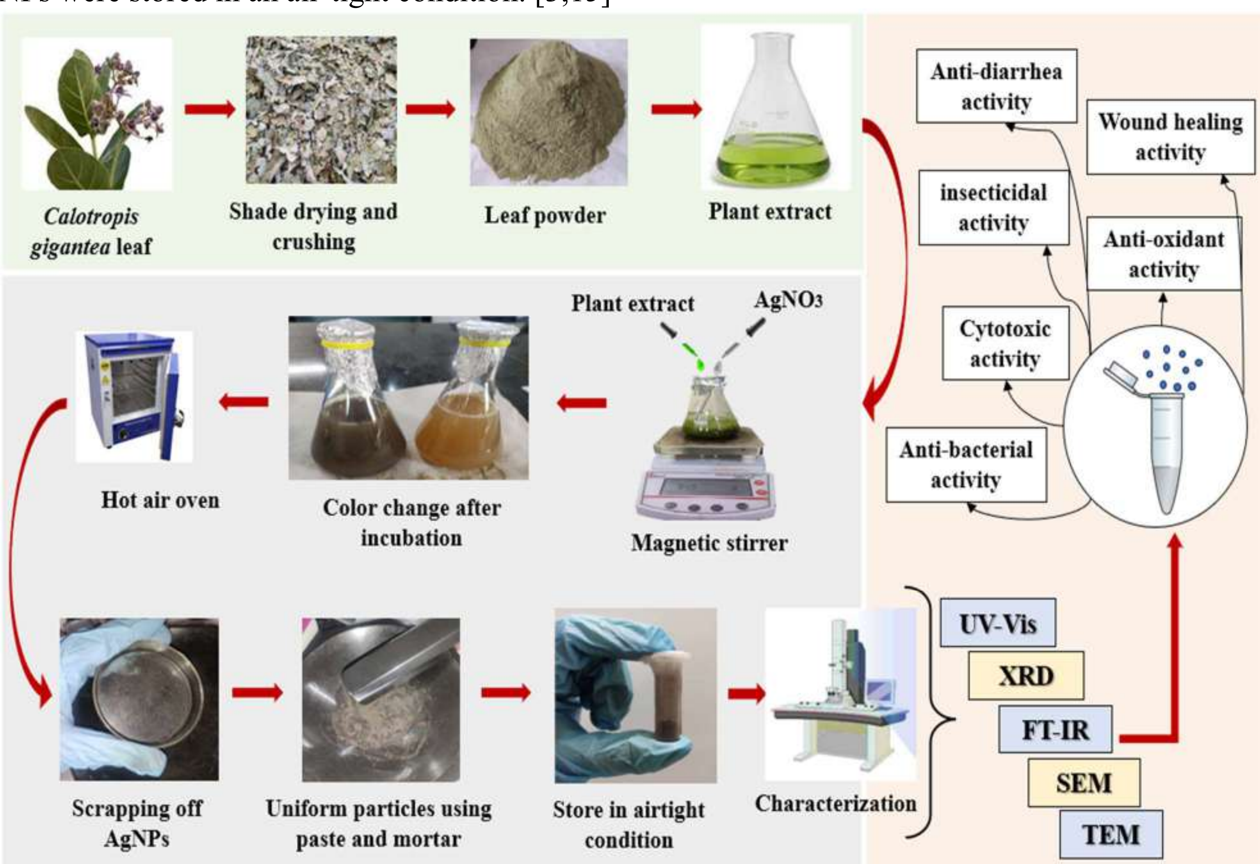

Fig 2.3: Green synthesis of silver nanoparticles

2.4 Characterization of silver nanoparticles (AgNPs)

The formation of silver nanoparticles (AgNPs)was confirmed, the sample was characterized by discoloration from pale yellow to brownish-black that occurred at the time of incubation. The green synthesized AgNPs by *Calotropis gigantea* leaf extract were measured by UV-Vis spectroscopy at 300-500 nm wavelength, and X-Ray Diffraction (XRD) was measured at 2-Theta degree. The Fourier Transform Infra-Ray spectroscopy (FT-IR) was observed from 4000-500 cm^{-1} Wavenumber. Scanning Electron Microscopy (SEM) was used to analyse the surface morphology including, shape and size of the particles at 10 to 1µm. Transmission Electron Microscopy (TEM) was used to analyse detailed morphology and structure of particles at 20 to 5 nm of silver nanoparticles. [15-19]

2.5 Antibacterial activity of AgNPs + *Calotropis gigantea* leaf extract

The examination of AgNPs potential synthesized by *Calotropis gigantea* leaf extract against the human pathogen *Escherichia coli* and *Staphylococcus aureus,* by using the Agar well diffusion method. Muller-Hinton Agar (MHA) was used to grow the cultures and were incubated in a bacteriological incubator for 24 hours. The agar plate preparation follows: 200 mL of MH agar was prepared in distilled water and heated in an electric microwave. The petri plates were washed and sterilized at 121°C for 15 minutes using a vertical autoclave. At room temperature, the agar was poured into the plates and allowed to rest for 40-45 minutes. Further, the petri plates were stored at 4°C in a refrigerator for further use. [21] Concentrations were prepared in DMSO universal solvent and subjected to serial dilutions for 50 µg/mL, 100 µg/mL, and 150 µg/mL. The antibacterial potential was compared to Ampicillin (Standard), and DMSO (negative control), highlighting potential human health concerns with AgNPs synthesized by *Calotropis gigantea* leaf extract. On freshly prepared agar plates, 20 µl broth medium of E. coli and S. aureus was poured on separate petri plates and spread uniformly using a spreader. 6.0 mm diameter agar wells were punctured by using a sterile borer. The prepared concentrations of silver nanoparticles, ampicillin, and negative control were added to the respective wells and incubated at 37°C for 24 hours. The zone of inhibition (ZOI) was observed and measured using a vernier caliper. The experiments were repeated multiple times independently, and the data were calculated as mean and standard deviation (SD). [10,20]

2.6 Antioxidant activity of AgNPs + *Calotropis gigantea* leaf extract

The antioxidant effect of silver nanoparticles was tested by using 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The *in-vitro* radical scavenging activity of AgNPs was compared with Ascorbic acid. 0.1 mM DPPH solution was prepared in 100 mL of methanol in dark conditions. [14] Multiple concentrations including, 100, 200, 300, 400, and 500 µg/mL of AgNPs (Sample) and Ascorbic acid (Standard) were prepared in Methanol. For each concentration, the sample or standard were stirred with freshly prepared DPPH solution vigorously using vortex shaker. In dark condition, the mixture solutions were incubated at room temperature for 30-35 minutes. The visible colour change was observed in the reaction mixture solution. The absorbance was calculated using UV-Visible Spectroscopy at 517 nm. Methanol was taken as blank. [22] The percentage of inhibition for antioxidant activity of AgNPs synthesized by *Calotropis gigantea* leaf extract was calculated by using the following formula,

(%) Percentage of inhibition = [(control – sample) / control] x 100

Whereas

- Control = Absorbance of DPPH solution
- Sample = Absorbance of AgNPs / Ascorbic acid

3. RESULT AND DISCUSSUION

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3.1 Successful synthesis of silver nanoparticles (AgNPs)

Synthesis of AgNPs by *Calotropis gigantea* leaf extract has provided a nontoxic, cost affiant and eco-friendly approach. Aqueous extract of *Calotropis gigantea* leaves used as a capping agent with $AgNO_3$ stock solution showed the colour reaction from pale yellow to brownish-black mixture solution after 24 hours of incubation. [3] The change in colour indicates the formation of AgNPs. The control stock solution of $AgNO_3$ showed no colour change after incubation as compared to $AgNO_3$ with *Calotropis gigantea* leaf extract. [23] Various factors play a key role in verifying the characteristics of AgNPs synthesis, including temperature: green synthesis is conducted at room temperature, with increasing temperature, the reaction mixture rate also increases, formation of Ag^+ ions to nanoparticles cause to smaller particles size. Incubation time: AgNPs with respect to the time of incubation may undergo aggregation and affect the properties of synthesized particles. pH: the ideal pH rate of the mixture solution ranges from 7 to 9 as alkaline to nature. Concentration of extract: the volume of compounds presents in the plant extract also affect the balance rate of the mixture solution, which may result in aggregation of molecules and inappropriate size and shape formation of the silver nanoparticles. Therefore, maintaining proper parameters could obtain unique properties of AgNPs synthesized by *Calotropis gigantea* leaf extract. [24, 25]

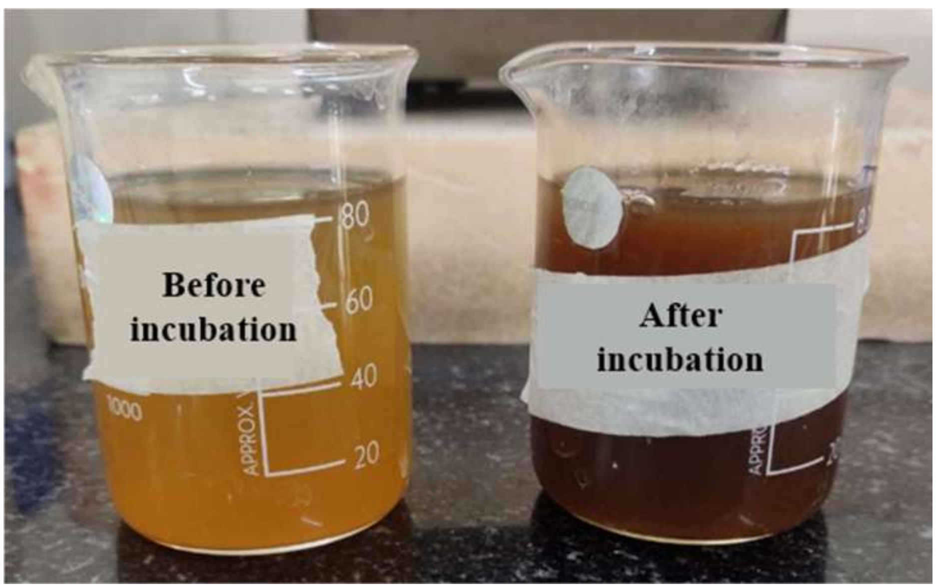


Fig 3.1: The colour change after 24 hours of incubation from pale yellow to brownish black after the addition of $AgNO_3$

3.2 Characteristics of AgNPs by UV-Visible Spectroscopy

The UV-Visible spectra validate the formation of AgNPs synthesized by *Calotropis gigantea* leaf extract, evidenced by the solution transitioning from pale yellow to brownish-black upon the addition of $AgNO_3$ to *Calotropis gigantea* leaf extract. The colour shift indicated successful synthesis, whereas analysis using particle size determination from UV-vis absorbance spectra, at wavelength ranging from 300 to 700 nm, revealing the highest peak observed at 390 nm absorbance, corresponds to the evidence by the emerging the surface plasmon resonance (SPF) bands. The study confirms the formation of AgNPs by using UV-Visible spectra, following the reduction of Ag^+ ions with the plant extract. [3, 26]

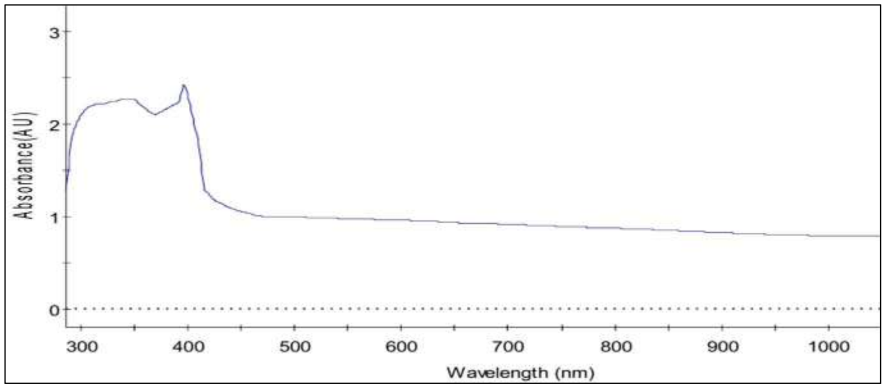


Fig 3.2: UV-vis spectra of AgNPs

3.3 X-Ray Diffraction of AgNPs

The nature of silver nanoparticles synthesized by plant extract was determined by the XRD analysis technique. The Bragg reflections was examined by the XRD pattern at 2-theta (Degree) values. The peaks observed at approximately 27.98° (2 theta) plane - (110), 32.38° (2 theta) plane - (111), 44.41°(2theta) plane - (200), 64.65° (2theta) plane - (220), and 76.98° (2theta) plane - (311) sets of lattice planes. In the study, the dominant AgNPs crystalline plane, the 32.38° (2 theta) plane - (110), is the highest peak, strongly suggesting it may arise from copper nanoparticles phases from the plant extract used for synthesis. The peak at 44.41° (2 theta) plane - (200) confirms the Face-Centered Cubic of the formed particles. The AgNPs proves the formation of pure crystalline particles with an average size of 30 nm. [27,28]

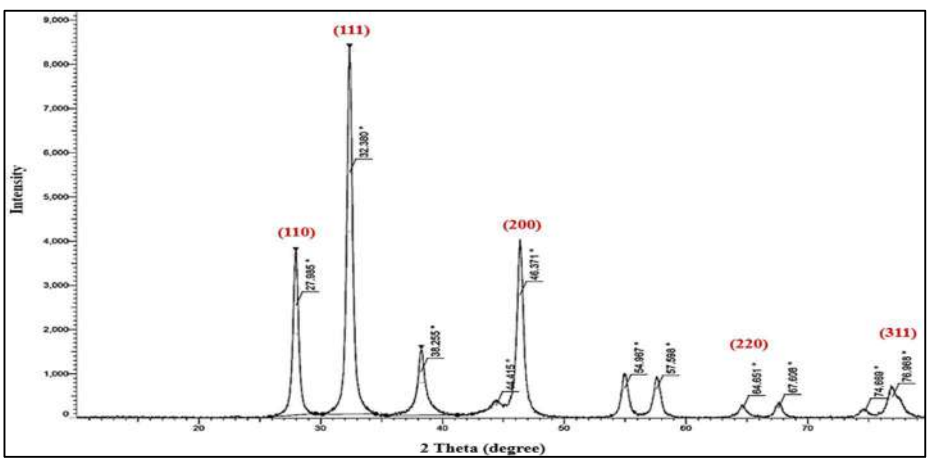
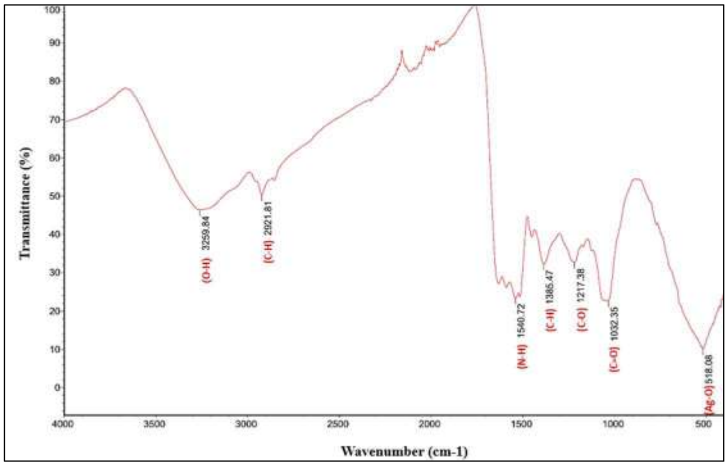


Fig 3.3: XRD spectra of AgNPs

3.4 Fourier Transform Infrared spectroscopy of AgNPs

The FT-IR spectra of AgNPs synthesized by *Calotropis gigantea* leaf extract was used to analyse the formation of functional groups by the AgNPs on a wavelength ranging from 4000-500 cm^{-1}, at the absorption peaks (3259, 2921, 1540, 1385, 1217, 1032, and 518 cm^{-1}) respectively. [2,29] The spectral profile of AgNPs indicated the existence of 3259.84 cm^{-1} (O–H stretch) Alcohols/phenols groups, 2921.81 cm^{-1} (C–H stretch) Alkanes groups, 1540.72 cm^{-1} (N–H stretch) Proteins/Amino groups, 1385.47 cm^{-1} (C–N stretch) Phenol compounds, 1217.38 cm^{-1} (C–O stretch) Secondary Metabolites, 1032.35 cm^{-1} (C=O stretch) Glycoside, and 518.08 cm^{-1} (Ag–O stretch) Metal-Oxygen vibration, confirming the formation of AgNPs. [29,30,38]



Absorption (cm⁻¹)	Functional Group	Type of Vibration	Intensity
3259.84	O–H	Stretching	Broad
2921.81	C–H	Stretching	Moderate
1540.72	N–H	Bending	Strong
1385.47	C–H	Bending	Moderate
1217.38	C–O	Stretching	Moderate
1032.35	C=O	Stretching	Weak
518.08	Metal–O (Ag–O vibration)	Stretching	Strong

Fig 3.4: FT-IR spectra of AgNPs

3.5 SEM analysis: surface morphology of AgNPs

Scanning Electron Microscopy of silver nanoparticles by *Calotropis gigantea* leaf extract exhibited surface morphology, structure, size, and stability of the particles. The images were obtained with 100% form of *Calotropis gigantea* leaf extract with silver ions. [30] The AgNPs were analysed at 10 and 1 µm. The study observed the surface morphological dimensions and the irregular quasi-spherical formed particles with an average dimetric size of 20 nm, confirming the formation of AgNPs. [31]

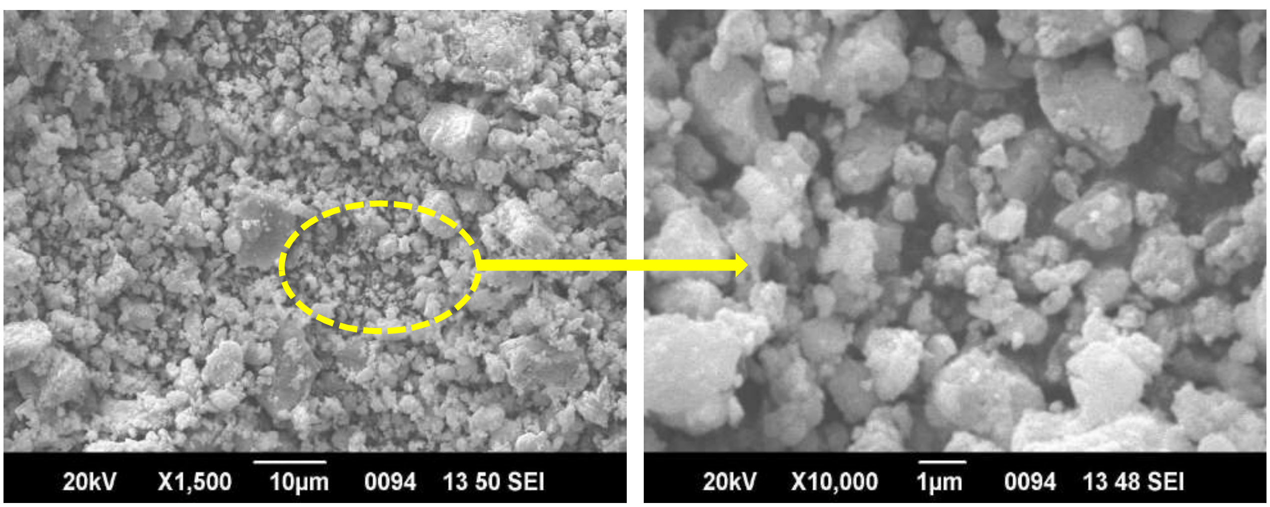


Fig 3.5: SEM image of AgNPs

3.6 TEM analysis: detailed structure of AgNPs

The Transmission Electron Microscopy analysis expose the structure of AgNPs synthesized by *Calotropis gigantea* leaf extract. The technique used to obtain the actual nanoscale size and crystallinity of AgNPs. The high-resolution image undergoes the ratio between the distance if objective lens and specimen with the image plane. [27] The sample preparation is extremely important in order to obtain the highest-quality images possible. The shape and size of the AgNPs at 20 -5 nm proved the spherical shape and the approximate size ranged from 15-20 nm. [28,32]

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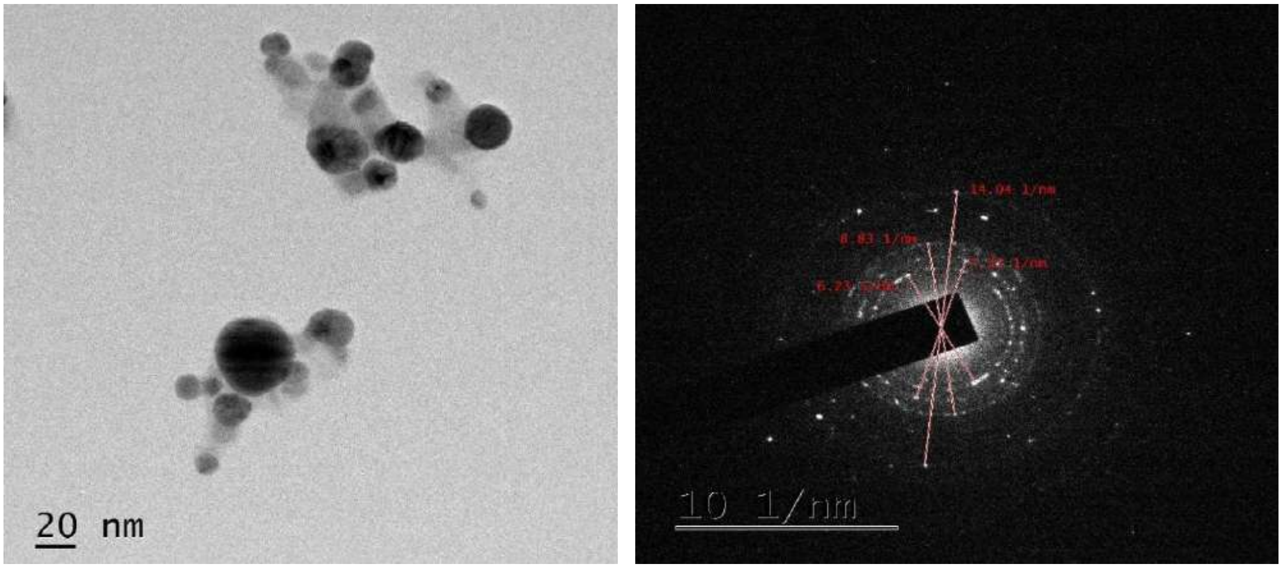
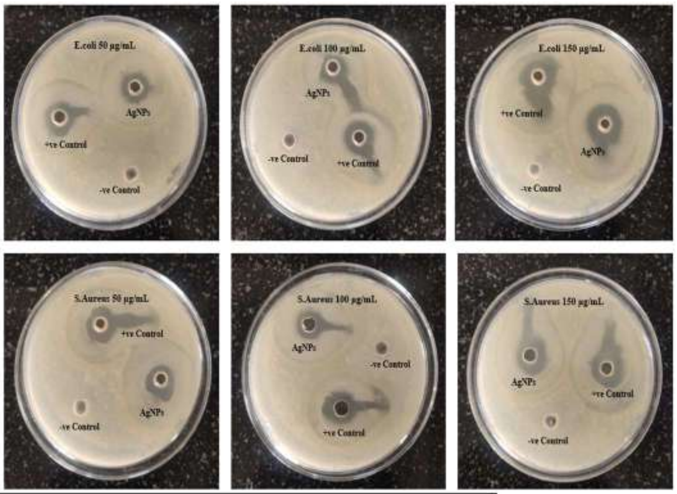


Fig 3.6: TEM image of AgNPs

3.7 Antibacterial potential of AgNPs + *Calotropis gigantea* leaf extract

The antibacterial potential emphasizes human relevance; silver nanoparticles synthesized by *Calotropis gigantea* leaf extract were aimed against human pathogenic bacteria, Escherichia coli and Staphylococcus aureus. [33,34] The concentration for sample and standard was taken (50, 100, and 150 µg/mL) for both the bacterial cultures. The antibacterial properties were compared with the antibiotic Ampicillin (standard) and DMSO (negative control). The visible zone of inhibition diameter was observed ranging from 10 to 25 mm. For E. coli, the inhibition zone was observed at 50 µg/mL with diametric size of 10 mm (sample) and 15 mm (standard), 100 µg/mL at 15 mm (sample) and 20 mm (standard), and 150 µg/mL at 20 mm (sample) and 25 mm (standard). Whereas, for S. aureus, the inhibition zone was observed at 50 µg/mL with diametric size of 10 mm (sample) and 10 mm (standard), 100 µg/mL at 15 mm (sample) and 20 mm (standard), and 150 µg/mL at 25 mm (sample) and 30 mm (standard). No zone of inhibition was observed for DMSO (negative control). [35,36] The most significant effect was observed at 150 µg/mL against both bacteria, showing the strongest antibacterial potential of AgNPs synthesized by *Calotropis gigantea* leaf extract. [37] The antibacterial potential of AgNPs undergoes standard deviation (SD) for E. coli, AgNPs (sample) 15 ± 5 mm, and ampicillin (standard) 20 ± 5 mm. For S. aureus, AgNPs (sample) 16.67 ± 7.64 mm and ampicillin (standard) 21.67 ± 7.64 mm were observed. The antibacterial potential of AgNPs highlighting a strong and promising effect against the pathogen as human health and environmental concern. [38,39]



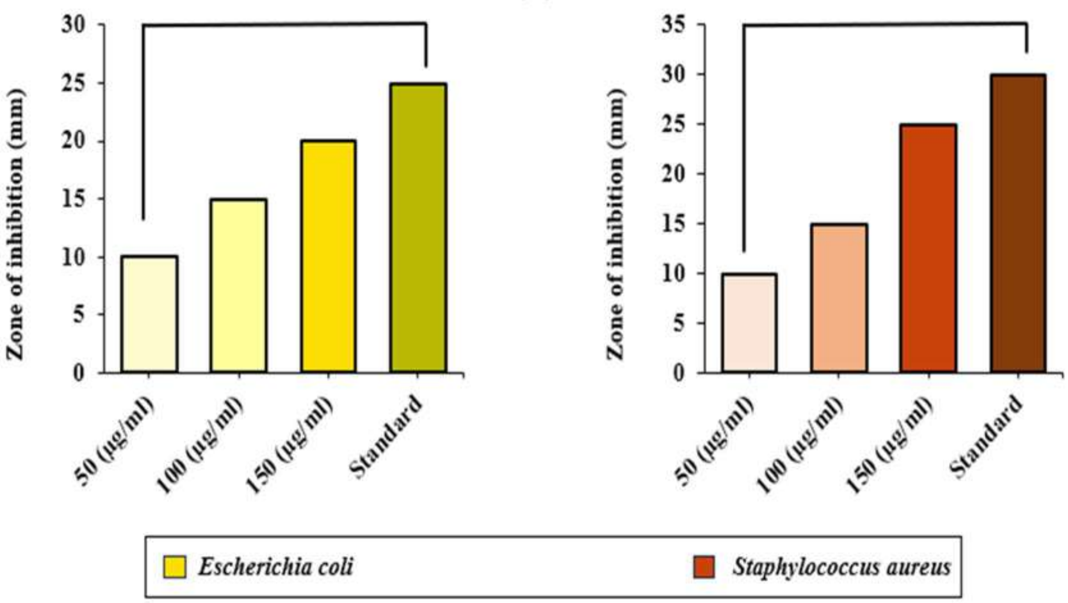
(B)

Table 3.7: Antibacterial activity of Silver Nanoparticles by the agar well diffusion method

Bacterial strains	**Zone of inhibition (mm)**	
	Treatment	**Concentration (µg/ml)**
E. coli	AgNPs	15 ± 5
	Control	20 ± 5
S. aureus	AgNPs	16.67 ± 7.64
	Control	21.67 ± 7.64

*Data is mean ± SD of three replicates.

(A)

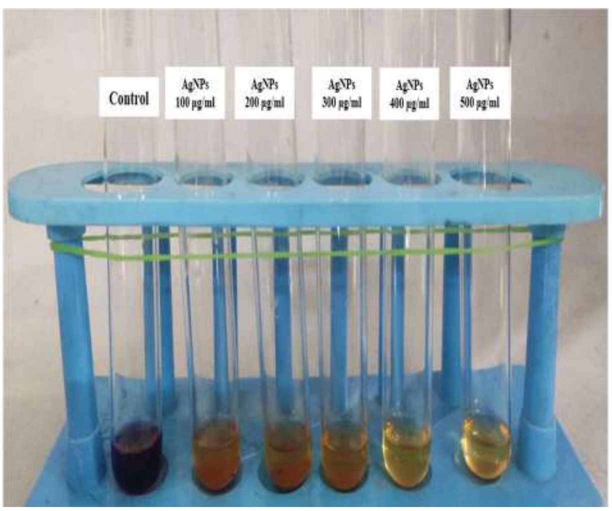


(C)

Fig 3.7: Antibacterial activity of AgNPs synthesized by *Calotropis gigantea* leaf extract (A) Zone of inhibition observed at concentration 50 µg/ml, 100 µg/ml, and 150 µg/ml by using agar well diffusion method, (B) Standard deviation was calculates for gram-positive and gram-negative bacteria, and (C) Comparative analysis of sample with standard using different concentrations.

3.8 Antioxidant property of AgNPs + *Calotropis gigantea* leaf extract

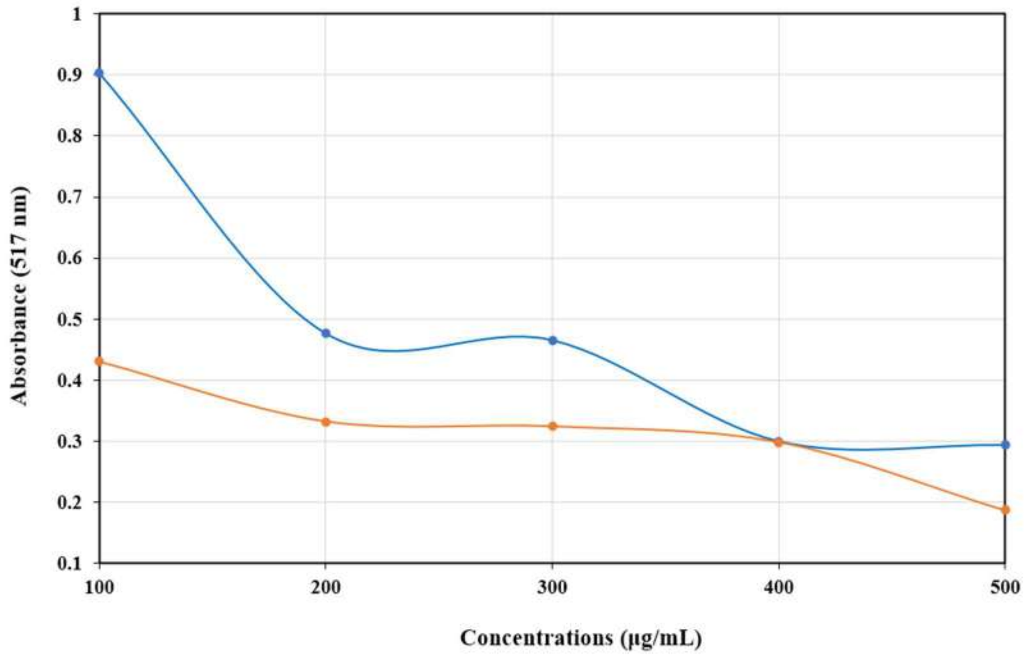
The antioxidative property of silver nanoparticles synthesized by *Calotropis gigantea* leaf extract was tested, the concentrations were taken as (100, 200, 300, 400, 500 µg/mL). The DPPH converted unpaired electrons into paired electrons with AgNPs as the sample. [15,40] The percentage of inhibition was compared to that of ascorbic acid (standard). The absorbance was calculated by using the UV-Visible spectroscopy at wavelength 517 nm. The percentage of inhibition was observed at 100 µg/mL as 20.58 % for (sample) and 62.18 % (standard), 200 µg/mL as 58.13 % for (sample) and 70.80 % (standard), 300 µg/mL as 59.10 % for (sample) and 71.50 % (standard), 400 µg/mL as 73.70 % for (sample) and 73.79 % (standard), and 500 µg/mL as 74.23 % for (sample) and 83.64 % (standard). The lowest antioxidative effect was observed at 100 µg/mL with inhibition percentage (20.58%) and the highest antioxidative effect was observed at 500 µg/mL with inhibition percentage (74.23%) compared with ascorbic acid. The result shows an effective antioxidant property of AgNPs synthesized by *Calotropis gigantea* leaf extract with more the 50 % inhibition potential. [41-45]



(A)

Concentrations (µg/mL)	Inhibition percentage (%)	
	AgNPs (sample)	Ascorbic acid (standard)
100	20.58	62.18
200	58.13	70.80
300	59.10	71.50
400	73.70	73.79
500	74.23	83.64

(B)



(C)

Fig 3.8: Antioxidant activity of AgNPs synthesized by *Calotropis gigantea* leaf extract (A) Change in colour observed compared to control using DPPH assay, (B) Percentage of inhibition was more than 50%, and (C) Comparative analysis using different concentration of sample with standard.

4. CONCLUSION

The eco-friendly synthesis of silver nanoparticles by *Calotropis gigantea* leaf extract as capable attribute. The synthesized nanoparticles are quite uniform in their size and shape, and the reaction was confirmed by the visible color change from pale yellow to brownish-black after 24 hours of incubation. The characterization techniques involving, UV-Visible spectroscopy, XRD, FT-IR, SEM, and TEM validate the formation of nanoparticles, purity, chemical bonding, structural, and morphological demonstration of AgNPs. Additionally, the AgNPs synthesized by *Calotropis gigantea* leaf extract showed the potential antibacterial inhibition property against the bacteria Escherichia coli and Staphylococcus aureus. A strong antioxidant potential ability was also observed in AgNPs compared to ascorbic acid. The study concludes that AgNPs synthesized from *Calotropis gigantea* leaf extract represent a promising, biologically significant approach for healthcare aspects against human pathogens.

Declaration

The authors declare that they have no known competing financial interests.

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