

GREEN SYNTHESIZED SILVER NANOPARTICLES (AGNPs) USING AQUEOUS EXTRACT OF *CALOTROPIS PROCERA* AND ITS ANTIMICROBIAL ACTIVITY ON CLINICAL BACTERIA ISOLATES

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ABSTRACT

The green synthesis of nanoparticles, utilizing aqueous plant extract as a capping and stabilizing agent, has attracted significant attention in various domains, particularly in pharmaceuticals and drug delivery. In this investigation, silver nitrate (AgNO₃) salts were employed as precursors to fabricate silver nanoparticles using *Calotropis procera* (leaves/flower) extract, and the resulting nanoparticles were characterized. Fourier Transform Infrared (FTIR) spectroscopy revealed three primary functional groups at peaks of 2851.4 cm⁻¹, 1543.1 cm⁻¹, and 1323.2 cm⁻¹, responsible for capping and stabilizing the synthesized *C.p*-AgNPs. Scanning Electron Microscopy (SEM) demonstrated that the synthesized *C.p*-AgNPs exhibited spherical shapes with an average particle size ranging from 20 nm to 30 nm. Energy-dispersive X-ray (EDX) analysis of the synthesized *C.p*-AgNPs indicated the presence of pure silver (Ag) at 54.32% in the region of 2.7 to 3.1 keV. Furthermore, the antimicrobial activity of *C.p*-AgNPs was examined, with the best inhibition observed at 0.5 mg/mL on Gram-negative bacteria *S. aureus* (12.0 mm) and *Streptococcus* spp (13.0 mm), and on Gram-positive bacteria *E. coli* (16.0 mm) and *Salmonella* spp (14.0 mm). The antimicrobial efficacy was dose-dependent, suggesting the potential for eradicating resistant human pathogenic bacteria. The antibacterial potential of *C.p*-AgNPs could be enhanced by increasing their concentration, depending on the specific application. Based on the study's findings, *C.p*-AgNPs derived from *Calotropis procera* can be employed for various biomedical purposes, such as textile coating by incorporating *C.p*-AgNPs in fibers and food storage by nanocapsulation of food items to extend their shelf life.

Keywords: silver nanoparticles, *Calotropis procera*, *Salmonella* spp, *E. coli*, *S. aureus*, *Streptococcus* spp.

1.0 INTRODUCTION

Nanoparticles have attracted significant attention across various domains due to their substantial impact on drug delivery and targeting, reducing toxicity, enhancing efficacy, and creating new avenues for pharmaceutical and drug delivery enterprises (Lutfi *et al.*, 2011; Blanco *et al.*, 2015; Jakerst *et al.*, 2017). Capping agents play a vital role in functionalizing and stabilizing synthesized nanoparticles. These biologically acceptable reducing, stabilizing, or capping agents are carefully chosen to ensure compatibility with living systems (Ocoy *et al.*, 2018; Javed *et al.*, 2020). They shield nanoparticles from agglomeration and enhance reduction kinetics by forming complex structures with metallic ions in precursor salts (Campisi *et al.*, 2016; Sharma *et al.*, 2021).

Silver nanoparticles (AgNPs) were synthesized using *Artemisia*

capillaris extract, and their antimicrobial activity was tested on methicillin-resistant *Staphylococcus aureus* (MRSA) (Jang *et al.*, 2015). AgNPs were also synthesized using aloe vera extract and tested against *Staphylococcus epidermidis* and *Pseudomonas aeruginosa* (Tippayawat *et al.*, 2016). Additionally, AgNPs from *Coffea arabica* seed extract were tested on *Lactobacillus acidophilus* (Dhand *et al.*, 2016), AgNPs from *Rosmarinus officinalis* leaf extracts exhibited activity against *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Ghaedi *et al.*, 2015), and AgNPs from *Calotropis gigantea* flower extract showed efficacy against *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans* (Kemala *et al.*, 2022). Furthermore, silver (Ag) and zinc (Zn) NPs extracted from *Calotropis procera* fruits or leaves demonstrated activity against *Vibrio cholerae* and *Escherichia coli* (Salem *et al.*, 2015). AgNPs from *Helicteres isora* fruit extract exhibited activity against extensively drug-resistant (XDR) *Pseudomonas aeruginosa* isolates (Mapara *et al.*, 2015). AgNPs from *Caesalpinia sappan* extract demonstrated efficacy against *Streptococcus faecalis*, *Escherichia coli*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and *Candida albicans* (Jun *et al.*, 2015).

Antimicrobial resistance is a persistent natural occurrence, with factors such as excessive and illicit antimicrobial use, inappropriate usage, sub-therapeutic dosing, and noncompliance with prescribed treatment courses accelerating the emergence of drug resistance (CDC, 2019).

Plants have been long recognized for their therapeutic attributes, with aboriginal cultures worldwide employing traditional herbal medicine for centuries to address various ailments (Li *et al.*, 2017). The recent surge in demand for herbal medicines is attributed to their efficacy in treating diseases with minimal side effects (Priyanka and Brahmeshwar, 2017). Concerns have arisen regarding the use of modern medicines, such as chemotherapy, hormone-blocking therapy, monoclonal antibodies, and their equivalents, for diseases like cancer, given their adverse effects, therapeutic complexities, and cost implications. Ethnopharmacological remedies present a viable alternative to address these issues (Tanmoy *et al.*, 2017). Ethnopharmacology serves as an approach to identify potential medicinal compounds by assessing plants with therapeutic potential (Tita *et al.*, 2020). Throughout history, plants and their secondary metabolites have consistently served as an exemplary source of medicine, renowned for their antibacterial and anticancer effects (Maya *et al.*, 2016). *Calotropis procera* (Plate 1), commonly referred to as the Apple of

Sodom or Sodom Apple, belongs to the Apocynaceae family and the Asclepiadaceae subfamily (milkweed family) (Al-Rowaily *et al.*, 2020). It is a perennial shrub or small tree native to North Africa, tropical Africa, and parts of Asia, reaching a height of up to 2.5 m. It holds significance in traditional medicinal practices across North Africa, the Middle East, South Asia, and Southeast Asia (Al Sulaibi *et al.*, 2020). Additionally, it has been employed for fiber, fuel, fodder, and timber purposes since ancient times (Batool *et al.*, 2020). Numerous studies explore its antimicrobial, anti-inflammatory, analgesic, antidiabetic, antihypertensive, and anticancer properties. Its therapeutic potential stems from bioactive compounds like alkaloids, glycosides, flavonoids, tannins, glucosides, saponins, and terpenoids (Rahman *et al.*, 2016; Kalu *et al.*, 2022).



Plate 1. *Calotropis Procera* plant showing leaves, flowers, and fruit/pods.

Several studies have explored the antimicrobial properties of biosynthesized silver nanoparticles (AgNPs). However, only a limited number of these studies have utilized *Calotropis procera* as the stabilizing and capping agent. Notably, Salem *et al.* (2015) synthesized AgNPs using *Calotropis procera* and observed antimicrobial activity specifically against *V. cholerae* and *E. coli*. Meanwhile, Kemala *et al.* (2022) employed *Calotropis gigantea* flower extract, demonstrating activity against *E. coli*, *S. aureus*, and *Candida albicans*.

There is limited existing literature in Nigeria on the biosynthesis of silver nanoparticles using *C. procera* aqueous extract and evaluating its antimicrobial efficacy against clinically resistant strains of *Salmonella* spp., *Escherichia coli*, *Staphylococcus aureus*, and *Streptococcus* spp.

This study aimed to synthesize and characterize silver nanoparticles using the aqueous extracts of *Calotropis procera* (a combination of leaves and flowers). Subsequently, the antimicrobial activity (Minimum Inhibitory Concentration, MIC) of the nanoparticles was evaluated against clinically resistant isolates of two gram-negative bacteria (*Salmonella* spp. and *Escherichia coli*) and two gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus* spp).

2.0 MATERIALS AND METHODS

2.1 Collection and Identification of Plant Extract and Bacterial Isolates

Calotropis procera leaves and flowers were obtained from Chikun LGA, Kaduna, Nigeria, and authenticated by a taxonomist at the Department of Biological Science Herbarium, Ahmadu Bello University with voucher number V/N-ABU900086. Clinical isolates of *Staphylococcus Aureus* (sourced from Urine), *Streptococcus* spp (sourced from Sputum), *Salmonella* Spp (sourced from Stool), *Escherichia Coli* (sourced from Urine), and *Staphylococcus Aureus* (sourced from High Vaginal Swab) were collected at Oxford Hospital Chemical Pathology, Hematology, and Microbiology diagnostic laboratory in Kaduna State, Nigeria.

2.2 Preparation of Aqueous Silver Nitrate (AgNO₃)

A 1 mM AgNO₃ (Silver trioxonitrate (V)) solution was prepared by weighing 0.0169 g of AgNO₃ using an analytical balance and dissolving it in distilled water to reach the 100 mL mark of a 100 mL volumetric flask.

2.3 Preparation of Aqueous *Calotropis procera* Extract and AgNPs Synthesis

Following the method of Chhangte *et al.* (2021) with slight modifications, *Calotropis procera* leaves (8.6 g) and flowers (7.4 g) were sliced into small pieces, pounded, and mixed with 100 mL of distilled water. The resulting extract was filtered, and 30 mL of the filtrate was added to 70 mL of 1 mM AgNO₃ solution in a 100 mL conical flask. The mixture was heated to 70 °C, stirred mechanically, and the pH adjusted to 9 using 0.1M NaOH (sodium hydroxide). The color change from light green to dark brown confirmed the synthesis of AgNPs. The synthesized *C.p*-AgNPs were obtained through centrifugation (KA-1000 model), eliminating the supernatant, and then oven-dried at 40 °C.

2.4 Characterization of Green Synthesized Nanoparticles

The synthesized silver nanoparticles (AgNPs) were characterized using Agilent Technology UV-Vis spectroscopy (Agilent Cary 5000 UV-Vis-NIR), Agilent Technology Attenuated Total Reflectance Fourier Transform Infrared Spectroscopy (ATR-FTIR) model (Agilent Cary 630 FTIR), Scanning Electron Microscopy (SEM), and Energy Dispersive X-ray (EDX) SEM-EDX, NLNG model at the Multiuser Laboratory of Chemistry Department, Faculty of Physical Science, Ahmadu Bello University.

UV-Vis spectroscopy observed the synthesis of AgNPs, with successive dilutions of the initially prepared nanoparticles solution. For FTIR analysis, the Agilent ATR-FTIR instrument was used to collect the infrared spectrum reflecting the absorption of infrared light by the functional groups within *Calotropis procera* extracts and the synthesized silver nanoparticles. SEM was employed to capture images depicting the morphology and surface characteristics of the nanoparticles, and EDX analysis identified and quantified elements on the nanoparticle surface.

2.5 Antimicrobial Screening

The antimicrobial screening was carried out at Oxford Hospital Chemical Pathology, Hematology, and Microbiology diagnostic laboratory in Kaduna State, Nigeria.

Streptococcus spp (High Vaginal Swab) *Salmonella* spp (Stool), *Escherichia Coli* (Urine), and *Staphylococcus Aureus* (High Vaginal

Swab) were isolated, characterized, and identified using biochemical method. High profile positive/negative 10 tipped multiple susceptibility antibiotic discs containing Amoxicillin, Perfloracin, Erythromycin, Septrin, Streptomycin, Ciprofloxacin, Rocephin, Zinnacef, Ampiclox, Gentamycin, Sparfloxacin, Chloramphenicol were used for screening of resistant bacteria, while the prepared sample mixtures antimicrobial disk were used for minimum inhibitory concentration determination against the screened resistant bacteria using Kirby-Bauer disk diffusion test, as outlined by Olgica *et al.*, (2018), utilizing Mueller-Hinton Agar (MHA).

- Sgu for 5 mg of *C. procera* extract dissolved in 1 mL distilled water and was applied on 5mm diameter autoclaved filter paper to prepared the antimicrobial disk)
- Tg1 for 0.1 mg AgNPs dissolved in 1 mL distilled water and was applied on 5mm diameter autoclaved filter paper to prepared the antimicrobial disk)
- Tc2 for 0.25 mg AgNPs dissolved in 1 mL distilled water and was applied on 5mm diameter autoclaved filter paper to prepared the antimicrobial disk)
- Sg3 for 0.5 mg AgNPs dissolved in 1 mL distilled water and was applied on 5mm diameter autoclaved filter paper to prepared the antimicrobial disk)

To ensure sterility, area sterilization involved the use of a disinfectant and an open burner. A sterile loop was then employed to select a well-isolated bacterial colony from a pure culture plate, and the colony was subsequently transferred to the broth medium under aseptic conditions, as described by Sudhir *et al.*, (2015). Inoculation of the entire surface of a Mueller-Hinton agar plate included streaking in three directions (north-south, east-west, and diagonally) to achieve uniform distribution. Following inoculation, the plate was allowed to briefly dry, facilitating bacterial adhesion to the agar surface, as suggested by Sudhir *et al.*, (2015). The application of prepared antimicrobial disks onto the agar surface was performed using sterile forceps with gentle pressure for proper contact. Plates were then inverted and incubated for 24 hours at 37 °C. Post-incubation, the plates were examined, and the diameter of the zone of inhibition around each disk was measured and recorded, indicating susceptibility or resistance, as detailed by Sudhir *et al.*, (2015).

For confirmed clinical isolates of *Staphylococcus Aureus* from High vaginal swab (HVS), *Escherichia Coli* from stool, *Streptococcus* Spp from sputum, and *Salmonella* Spp from stool, antimicrobial susceptibility tests were conducted using the prepared samples:

3.0 RESULTS AND DISCUSSION

3.1 Synthesis of AgNPs

When *C. procera* leaf/flower extract was mixed with AgNO₃ solution, heated at 60 °C for 10 minutes, and alkalized, a visible colour change from light green to dark brown or reddish-brown occurred. This colour change is attributed to the Surface Plasmon Resonance phenomenon in silver nanoparticles, resulting from the excitation of free electrons in nanoparticles, as explained by Kemala *et al.*, (2022). Phytometabolites have been reported to act

as electron donors, mediating the reduction of silver ions and initiating surface plasmon resonance (SPR), as noted by Al-otibi *et al.*, (2021) and Kemala *et al.*, (2022). SPR is a complex process defined as the excitation and coherent oscillation of electrons in an incoming electromagnetic field, as described by Pryshchepa *et al.*, (2020) and Kemala *et al.*, (2022). No further color change was observed after 24 hours, indicating the completion of the reduction process, consistent with literature reports by Kero *et al.*, (2017), which indicated the synthesis of silver nanoparticles at 24 hours using *Lippia citriodora* leaf extract.

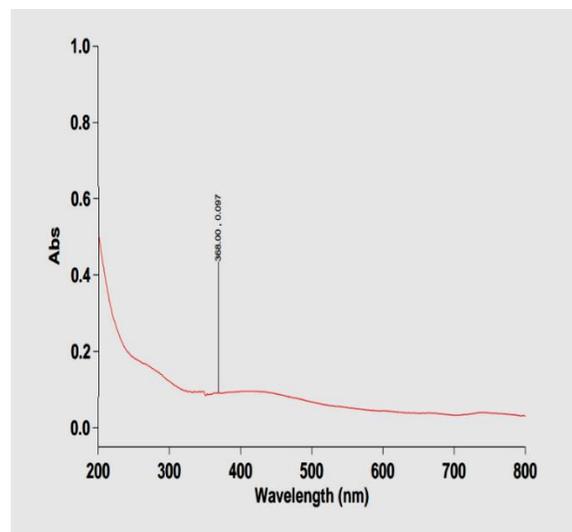


Figure 1: UV spectrum of *C.p*-AgNPs

The UV-VIS spectrum, depicted in figure 1, reveals absorbance peaks at a λ_{max} of 368 nm, which is consistent with the characteristic absorption pattern of AgNPs. This observation aligns with the findings of Hao and Qinghua (2017), who conducted a study on the green synthesis of silver nanoparticles and their antimicrobial activities. In their research, they reported a distinct surface plasmon adsorption peak at approximately λ_{max} 400 nm, indicative of silver nanoparticles.

Similarly, Nadia *et al.* (2014), in their investigation on the antimicrobial activity of latex silver nanoparticles using *Calotropis procera*, identified a surface plasmon resonance band with a peak centered around λ_{max} 290 nm.

Moreover, Chhangte *et al.* (2021), in their review of recent literature on the green synthesis of silver nanoparticles using plant extracts and their antimicrobial activities, highlighted that the absence of peaks in the 335-560 nm region in UV-Vis spectra is sometimes employed as an indication of the absence of aggregation in nanoparticles.

3.2 ATR-FTIR spectra of *C. procera* extract and the synthesized *C.p*-AgNPs

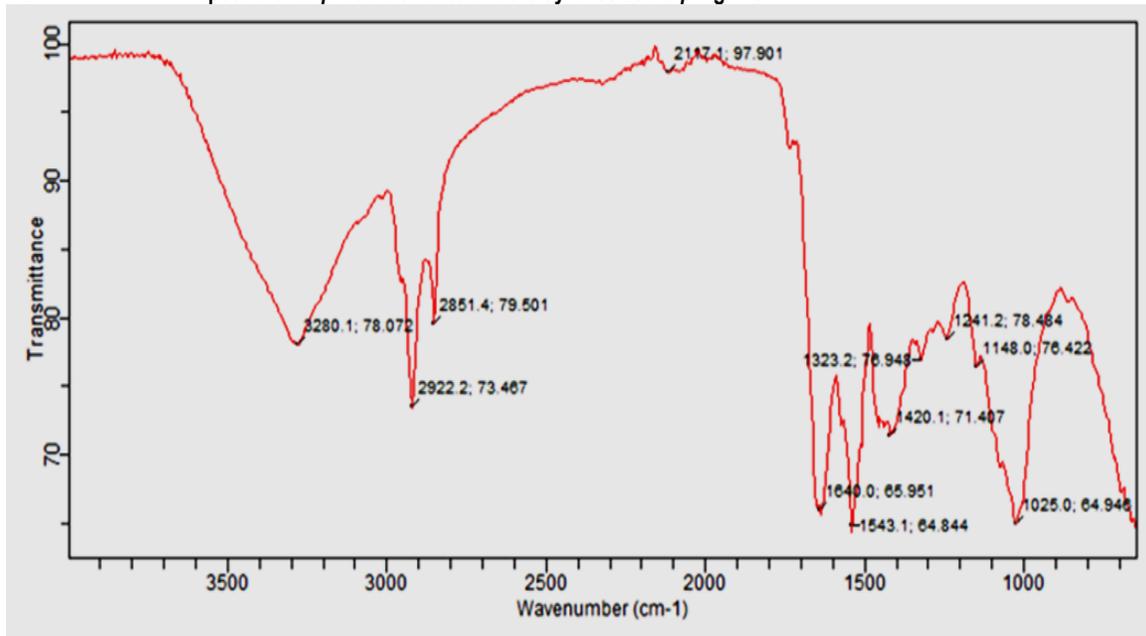


Figure 2a: ATR-FTIR of aqueous *C. procera* extract

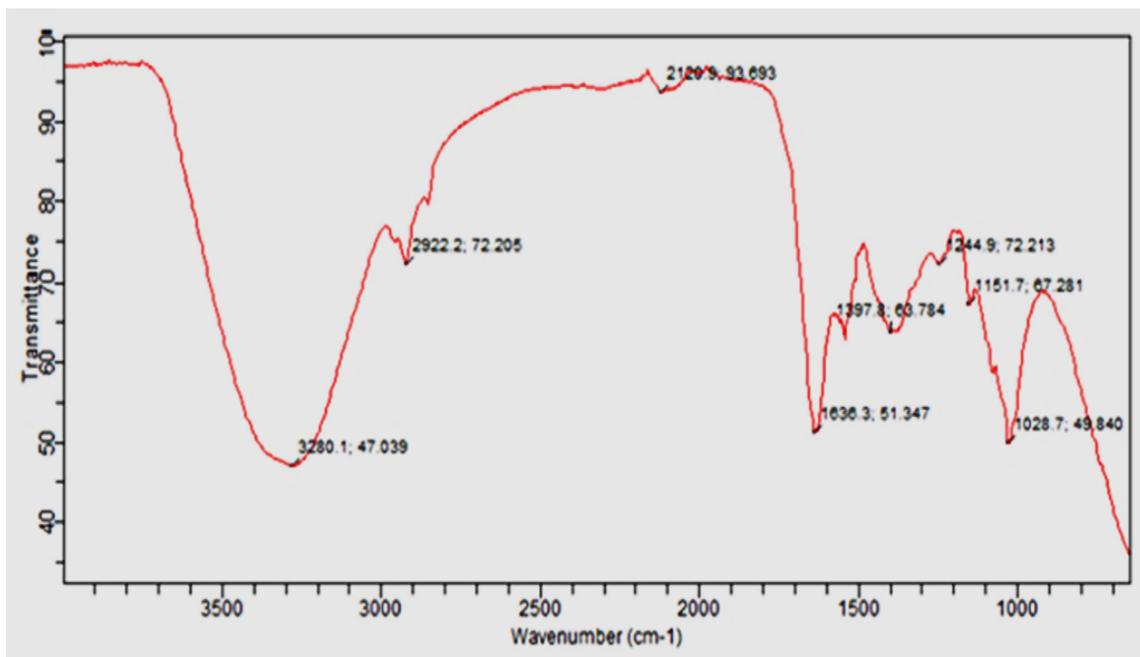


Figure 2b: ATR-FTIR of *C. procera* -AgNPs

The ATR-FTIR spectroscopy results for both *C. procera* extract and the synthesized *C.p*-AgNPs are illustrated in figures 2a and 2b. The key characteristic peaks observed at 3280.1 cm⁻¹, 2922.2 cm⁻¹, and 2851.4 cm⁻¹ were attributed to Amine N-H stretching, alcohol and carboxylic acid O-H stretching, and C-H stretching of terminal alkyne, vinyl, alkane, and aldehydes, as reported by Félix *et al.* (2021). Félix *et al.* (2021) also noted multiple bands between 3600

and 3200 cm⁻¹, indicative of O-H stretching from phenol and N-H stretching from amide (acetaminophen), along with C-H stretching at 3000 – 2840 cm⁻¹. Furthermore, peaks at 2117.1 cm⁻¹, 1640.0 cm⁻¹, 1543.1 cm⁻¹, 1420.1 cm⁻¹, and 1323.2 cm⁻¹ were assigned to C=C, C=N, -C=C-, C≡C, and C≡N stretching vibrations of alkyne, alkene, benzene, and alkyne, with support from Kihokoi (2019), Monowar *et al.* (2021), and Félix *et al.* (2021). The literature also

includes references to peaks at 2110 cm^{-1} and 1523 cm^{-1} corresponding to $\text{C}=\text{C}$ and $\text{C}=\text{N}$ stretching vibrations (Monowar *et al.*, 2021), and a peak at 1329 cm^{-1} attributed to $-\text{C}=\text{C}-$ stretching for the benzene ring (Kihitokoi, 2019). Félix *et al.* (2021) reported $\text{C}=\text{C}$ double bond stretching at 1690 – 1635 cm^{-1} . Peaks at 1241.2 cm^{-1} , 1148.0 cm^{-1} , and 1025.0 cm^{-1} were assigned to C-O stretching and $-\text{CH}_2-$ bending vibrations, aligning with the findings of Ibrahim *et al.* (2016), who reported C-O stretching at 1200 cm^{-1} and $-\text{CH}_2-$ bending vibrations at 1100 – 1000 cm^{-1} . Distinctive peaks at 2851.4 cm^{-1} , 1543.1 cm^{-1} , and 1323.2 cm^{-1} observed in *C. procera* silver nanoparticles (*C.p*-AgNPs) disappeared, possibly due to conjugation resulting from synthesis temperature and/or sodium hydroxide addition. Two peaks at 3280.1 cm^{-1} and 2922.2 cm^{-1} remained unchanged, while the remaining six peaks exhibited variations in wave numbers. These changes in functional groups, coupled with others showing reduced or increased wave numbers, contribute to the reduction, capping, and stabilization of synthesized *C.p*-AgNPs, in accordance with Javed *et al.* (2020). This observation aligns with Shakeela *et al.* (2019), who reported similarities between plant extracts and AgNPs, suggesting that residual plant material serves as capping agents for AgNPs, preventing agglomeration and stabilizing the medium. The shifts within the peaks (3000 cm^{-1} to 1600 cm^{-1}) and some unchanged peaks indicate the potential binding of proteins from the plant extract with Ag, potentially contributing to the capping of metal NPs and preventing agglomeration, as discussed by Shakeela *et al.* (2019).

3.3 Scanning Electron Microscope (SEM) Micrographs and Energy Dispersive X-ray (EDX) of Synthesized *C.p*-AgNPs

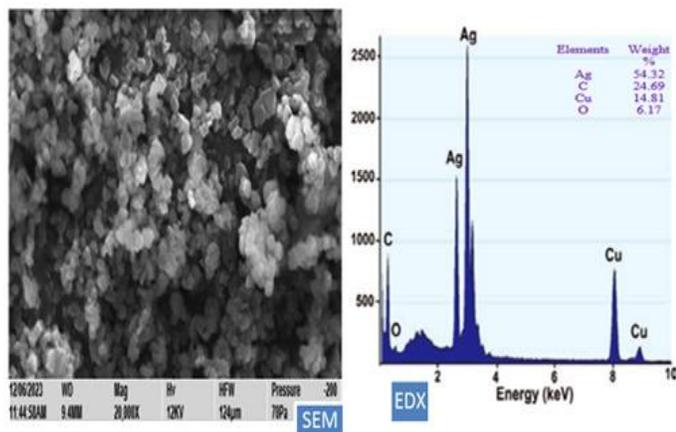


Figure 3: SEM-EDX of *C.p*-procera synthesized silver nanoparticles *C.p*-AgNPs

The SEM micrographs of synthesized *C.p*-AgNPs, as presented in figure 3, reveal predominantly spherical nanoparticles with an average particle size of 20 – 30 nm. The non-uniform distribution and agglomeration of nanoparticles may be attributed to the precursor concentration, biomolecules in *C. procera* leaf extracts, and the presence of secondary metabolites in leaves and flower extracts. Similar findings were reported by Chhangte *et al.* (2021) in their study on the green synthesis of silver nanoparticles using plant extracts, indicating spherical particle sizes ranging from 10 – 20 nm. Alkammash (2017) also observed predominantly spherical particles with sizes ranging from 8 – 20 nm, with other non-

spherical shapes present.

The analysis presented in figure 4, through Energy Dispersive X-ray (EDX) revealed the presence of pure Silver (Ag) at a range of 2.7 to 3.1 keV, constituting 54.32% of the material. This robust signal within the metallic silver range serves as confirmation of the formation of silver nanoparticles utilizing *C. Procera*. Additional peaks were identified for Carbon (C) at 24.69%, Copper (Cu) at 14.81%, and Oxygen (O) at 6.17%. Notably, a significant percentage of Copper atoms were observed on the surface of *C.p*-AgNPs nanoparticles, potentially stemming from impurities in the precursor or agrochemicals used on the *C. Procera* plant sourced from a farm. These findings align with results reported by Dada *et al.* (2017), who observed a similar elemental composition in AgNPs synthesized using the leaf extract of *Calotropis procera*.

3.4 Antimicrobial Assay

The antimicrobial assay of *C. procera* extracts and *C.p*-AgNPs is detailed in table 1 and figure 4. The susceptibility test revealed that *C. procera* extracts at 5 mg/mL exhibited no inhibition against the four tested bacterial strains. This lack of inhibition is attributed to the relatively low concentration of the extract used, as aqueous plant extracts typically require higher concentrations to demonstrate positive effects on bacterial growth, especially for resistant strains (Gideon, 2023).

Table 1: MIC Zone of inhibition of *C.p*-procera synthesized silver nanoparticles *C.p*-AgNPs

Bacteria Isolates	Diameter of inhibition zone (mm)				
	<i>C.p</i> -procera extract	<i>C.p</i> -procera Silver Nanoparticles, AgNP	0.1 mg/mL	0.25 mg/mL	0.5 mg/mL
<i>Salmonella</i> spp	0.0	0.0	9.0	14	14
<i>E. coli</i>	0.0	5.0	12.0	16.0	16.0
<i>S. aureus</i>	0.0	0.0	7.0	12.0	12.0
<i>Streptococcus</i> spp	0.0	5.0	10.0	13.0	13.0

Furthermore, *C.p*-AgNPs at 0.1 mg/mL displayed negative inhibition against *Salmonella* spp. and *S. aureus*. This result is attributed to the inherent nature of these bacterial strains, which necessitate a higher concentration of synthesized silver nanoparticles for inhibition. Comparable findings were reported by Mathew *et al.* (2023) and Siriporn *et al.* (2016), where lower concentrations of plant extracts and biologically synthesized silver nanoparticles were insufficient to inhibit the growth of various gram-positive and gram-negative bacteria at concentrations below 1 mg/mL



Figure 4: Antimicrobial susceptibility test of *C.procera* synthesized silver nanoparticles *C.p-AgNPs*

C.p-AgNPs at concentrations of 0.25 and 0.5 mg/mL exhibited effective inhibition, resulting in zones of 12 mm and 16 mm against *E. coli*, 7 mm and 12 mm against *S. aureus*, 10 mm and 13 mm against *Streptococcus*, and 9 mm and 14 mm against *Salmonella* spp, respectively. The observed inhibition can be attributed to the elevated concentration of *C.p-AgNPs*. Furthermore, escalating concentrations of silver nanoparticles enhance the probability of interaction between the nanoparticles and bacteria, thereby facilitating their penetration into bacterial cells (Cai *et al.*, 2015).

Silver nanoparticles release silver ions (Ag^+) into the surrounding medium, exhibiting antimicrobial properties. Elevated concentrations of silver nanoparticles result in a more substantial release of silver ions, thereby amplifying inhibitory effects on bacterial growth (Gong *et al.*, 2017). With increased concentrations, the likelihood of physical interactions between silver nanoparticles and bacteria rises. These nanoparticles can affix to the bacterial cell membrane, disrupting its structure and functionality, or permeate the cell wall, causing intracellular damage (Li *et al.*, 2010). Inducing oxidative stress in bacteria through reactive oxygen species (ROS) generation, silver nanoparticles exhibit a heightened ROS production at higher concentrations, leading to increased oxidative stress within bacterial cells and a larger zone of inhibition (Zhang *et al.*, 2013). Elevated concentrations of silver nanoparticles can potentiate the effectiveness of co-administered antimicrobial agents, such as antibiotics or disinfectants, through synergistic interactions, resulting in a more pronounced inhibitory effect on bacterial growth (Yuan *et al.*, 2018). Consistent findings were reported by Siriporn *et al.* (2016) and Kero *et al.* (2017), where an augmentation in biosynthesized silver nanoparticles corresponded to larger zones of inhibition against *S. aureus*, *E. coli*, *S. typhi*, *Bacillus subtilis*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.

4.0 Conclusion

The study demonstrates the bio-reduction of aqueous silver ions using the leaf/flower extract of the *Calotropis procera* plant. Characterization of the resulting product was carried out through UV-Vis, ATR-FTIR, SEM, and EDX analyses. The metal ions were reduced, leading to the formation of silver nanoparticles with an average particle size of 20 – 30 nm, as indicated by SEM analysis. The UV-Vis analysis of AgNPs revealed a surface plasmon resonance (SPR) characteristic peak at 368 nm. EDX confirmed the presence of pure silver (Ag) at a region of 2.7 to 3.1 keV. ATR-FTIR showed the disappearance of three distinctive peaks at

2851.4 cm^{-1} , 1543.1 cm^{-1} , and 1323.2 cm^{-1} , with others exhibiting reduced and increased wavenumbers. These changes are responsible for the reduction, capping, and stabilization of the synthesized *C.p-AgNPs*. The leaves/flowers of *Calotropis procera* emerge as a promising source for green synthesis of silver nanoparticles.

Furthermore, the antimicrobial activity of *C.p-AgNPs* was investigated, revealing dose-dependent inhibition. The *C.p-AgNPs* exhibited the best inhibition at 0.5 mg/mL against gram-negative bacteria *S. aureus* (12.0 mm) and *streptococcus* spp (13.0 mm), as well as gram-positive bacteria *E. coli* (16.0 mm) and *salmonella* spp (14.0 mm). The efficacy of the antimicrobial activity suggests the potential to eradicate resistant human pathogenic bacteria, and adjusting the concentration of *C.p-AgNPs* could further enhance their antibacterial potential.

Based on these findings, *C.p-AgNPs* derived from *Calotropis procera* can be utilized for various biomedical purposes. Additionally, incorporating *C.p-AgNPs* in fiber for textile coating and nanocapsulation of food items to increase shelf life are suggested applications.

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None.

Conflict of interests

The authors declare no competing conflict of interest.

Thank you for your adequate outstanding review of my manuscript. All corrections highlighted were effectively addressed, also in regards to the preparation of the disk, it is also addressed which can be seen in page 6. The antimicrobial activity was completely carried out in the oxford hospital laboratory, it was isolated and characterized by the laboratory technologist before we did the resistance test and our prepared disk Minimum inhibitory concentration using disk diffusion method was carried out on the resistant bacteria.

As for MBC minimum bactericidal concentration was not determine based on the scope of our work.

Also the topic has been annul to emphasize the major aim of the work as directed by my supervisor.

I hope you will understand and consider all the corrections made noteworthy.

Yours faithfully

Mamman A. James

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