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Calotropis procera Mediated Synthesis of Silver Nanoparticles and its Antibacterial Effect on Selected Pathogens

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Authors' contributions

This work was carried out in collaboration between both authors. Author AOH designed the study, performed the analysis, and wrote the first draft of the manuscript. Author ADA reviewed and edited the manuscript and supervised. Both authors read and approved the final manuscript.

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ABSTRACT

This study investigates the green synthesis and antibacterial potential of silver nanoparticles (AgNPs) derived from *Calotropis procera* leaf extract. The AgNPs was characterized using Ultraviolet-Visible (UV-VIS) Spectroscopy, Fourier Transform Infrared Spectroscopy (FTIR), Scanning and Transmission Electron Microscopy (SEM and TEM), Energy Dispersive X-ray (EDX) and X-ray Diffraction (XRD). The spectrum from UV-VIS had a peak centered at 445 nm confirming the formation of AgNPs, SEM-TEM micrographs showed them as aggregated, spherical in shape and about 15.30 – 17.66 nm in size, while EDX revealed Ag with highest proportion (75.56 %). The FTIR analysis revealed the presence of functional groups such as alcohols, ethers, esters, alkenes,

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carboxylic acid and amines. The XRD confirmed the crystalline nature of the synthesized CP-AgNPs. The synthesized AgNPs showed strong antibacterial activity against *Escherichia coli* (18.1±0.2 mm), *Enterobacter sichuanensis* (16.8±0.5 mm), *Pseudomonas aeroginosa* (14.4±0.3 mm), *Staphylococcus aureus* (22.2±0.5 mm) and *Streptococcus agalactiae* (21.5±0.1 mm). This study demonstrates an efficient, eco-friendly method for synthesizing AgNPs with strong antibacterial potential, which could be valuable for developing alternative antimicrobial agents.

Keywords: Antibacterial; Calotropis procera; pathogens; silver nanoparticles.

1. INTRODUCTION

"Calotropis procera is a soft-wooded, evergreen plant belongs to family Apocynaceae and subfamily Asclepiadaceae. It is primarily found in dry and semi-arid environments. This plant has many uses; it can be used for medicine, fuel, fodder, wood and fibre production, phytoremediation, and nanoparticle synthesis" [1]. "It is acknowledged as an ornamental species as well. Numerous studies have reported finding metabolites in different portions of the plant, including flavonoids, tannins, terpenoids, saponins, alkaloids, steroids, and cardiac glycosides" [2,3]. "Research has demonstrated that *C. procera* extracts have potent antipyretic, analgesic, depressive, and neuromuscular blocking properties" (Garabadu et al., 2019). According to Singhal et al. [4], *C. procera* is also used as a remedy for conditions like rheumatism, asthma, leprosy, toothaches, skin diseases, and elephantiasis by number of indigenous tribes over the world. Bark and leaf extracts of *C. procera* showed significant antibacterial activity against *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, and *Klebsiella pneumoniae* [5]. "*C. procera* leaf extracts also significantly lower blood glucose, suggesting that they may have antihyperglycemic effects" [6].

"Green synthesized nanoparticles from *C. procera* are more efficient and environment friendly than those derived from chemicals. Since nanoparticles derived from *C. procera* can create natural capping agents without any hazardous chemicals, and thus they can be used as an excellent platform for the synthesis of nanoparticles. When compared to other biological techniques, the rate of metal nanoparticle synthesis using plant extract is stable, significantly faster, and incredibly mono-dispersive" [4,7]. "Additionally, the cost-effectiveness of nanoparticle synthesis is increased by the use of plant extracts, which lowers the cost of microorganism isolation and growth" [8]. "Metallic nanoparticles based on the oxides of magnesium, copper, zinc, cadmium, titanium, silver, and gold have been studied because of their antibacterial activity and preservation capabilities" [9]. "Among metallic nanoparticles, silver nanoparticles (AgNPs) have drawn particular interest because of their potential use in biological applications such as waste treatment, food production, agriculture, biomolecular detection and diagnostics, medicine administration, and therapies" [10]. Similar to other therapeutic herbs, *C. procera* (L.) has qualities that are purgative, anti-inflammatory, antibacterial, anticoagulant, and parasiticidal. Therefore, the objective of this research is to synthesize and characterize *C. procera* AgNPs (CP-AgNPs) and to assess its antibacterial effect on selected pathogens.

2. MATERIALS AND METHODS

2.1 Collection of Leaf Samples

Fresh *Calotropis procera* leaves were collected from the Botanical garden of Moshood Abiola Polytechnic, Abeokuta, Ogun State. A sample of the plant was authenticated by the Botanists of Enviromental Biology Unit, Science Laboratory Department, School of Science & Technology, Moshood Abiola Polytechnic, Abeokuta, Ogun State.

2.2 Preparation of Aqueous Extract of *C. procera* Leaves

This was carried out according to the modified method of Essien et al. [11]. The leaves were washed with tap water and rinsed with distilled water to remove dust and particle pollutants. They were then size reduced and sun-dried, ground into fine powder and stored in an airtight container for future use. The powder (4.0 g) was mixed with 100 mL of distilled water, sealed, and left for 48 h at room temperature. Afterwards, the mixture was filtered to get the *C. procera* aqueous leaf extract. The resultant extract was then kept at 4 °C till further use.

2.3 Synthesis of Silver Nanoparticles

The synthesis was performed by the method of Dada et al. [12] and Essien et al. [13]. The *C. procera* aqueous leaf extract (1 mL) was added to 9 mL of 10 mM solution of $AgNO_3$. The reaction was incubated at 25 ^oC at 60 rpm for 48 h in the dark condition. A colour change from colourless to brown was observed which is due to the reduction of silver ions (Ag^+) to silver nanoparticles (Ag^o)**.** The solution was centrifuged at 15,000 rpm for 15 min and particles formed washed in deionized water to remove any residual organic compounds present in the AgNPs. The wet particles were dried at 60 °C in an oven (NYC-101 oven, FCD-3000 serials, Medical and Scientific, UK) for 12 h, stirred in absolute ethanol to reduce aggregation and then dried at 60 °C for 30 min in an oven to expel the solvent.

2.4 Characterization of Biosynthesized Silver Nanoparticles

2.4.1 UV–visible absorption spectrophotometer

UV–Vis spectral details were recorded in a UV–visible absorption spectrophotometer (Camspec M 106) in the wavelength between 200 nm and 700 nm to monitor the formation of AgNPs in the reaction medium between *C. procera* extract and $AgNO_3$. The UV–visible spectrum of the CP-AgNPs was also obtained at a similar wavelength range.

2.4.2 Fourier Transformed infrared (FTIR) spectroscopy

The type of bonds present in the CP-AgNPs was assessed using FTIR spectroscopy (Nicolet iS10 FT-IR Spectrometer) in the wave number range of 350-4400 cm^{-1}.

2.4.3 Scanning electron microscope – Energy dispersive X-ray (SEM-EDX)

A morphological evaluation to determine the microstructure, particle distribution and elemental composition of the CP-AgNPs was performed in a scanning electron microscope (SEM) having an energy dispersive X-ray analysis (EDX) unit (JOEL JSM-7600F). The sample was analyzed using an accelerating voltage of 15 kV [11]. Samples for SEM analysis were prepared by coating them in gold using a Balzers' Spluttering device.

2.4.4 Transmission electron microscopy (TEM)

The particle size and structure of the CP-AgNPs was assessed by TEM (JOEL JEM-F200) operating at 200 kV accelerating voltage. The average particle size and distribution was determined from the TEM micrograph using software Image J.

2.4.5 X-ray Diffraction (XRD)

XRD was carried out using Rigaku D/Max-lllC X-ray diffractometer developed by the Rigaku Int. Corp. Tokyo, Japan. Powdered samples were pelletized and sieved to 0.074 mm, these were taken in an aluminum alloy grid (35mm x 50mm) on a flat glass plate and covered with a paper. The samples were compacted by gently pressing them with the hand. Each sample was run through the diffractometer which is based on passing X-ray beam through a sample. This was set to produce diffractions at scanning rate of 2 ^{0}/min in the 2 to 50^0 at room temperature with a CuKa radiation set at 40 kV and 20 mA. The X-ray on passing through the samples gives peaks that is typical of each type of diffracted along a group of planes.

2.5 Antibacterial Activity of CP-AgNPs

Antibacterial activity of the aqueous extract and synthesized CP- AgNPs were determined by the agar well diffusion method. This was assayed against spoilage bacteria (*Pseudomonas aeruginosa*, *Enterobacter sichuanensis*, *Escherichia coli*) isolated from previous study. The method of Mohamed et al. [13] was used. Two days culture of the spoilage bacteria were spread on Mueller–Hinton agar. The agar plates (Mueller–Hinton agar) seeded with the test organisms was punched with a sterile cork- borer (6 mm diameter) to make open wells. The aqueous extract of *C. procera*, CP-AgNPs and Ciprofloxacin were added into the open wells and left for 10 minutes to allow diffusion on agar plates. All plates were inoculated at 37°C for 24 h and zone of inhibition (in mm) measured. The antimicrobial property of aqueous extract and AgNPs was determined by measuring the zone of inhibition around the discs after incubation. This was done in triplicates and recorded as mean ± standard error of mean.

3. RESULTS AND DISCUSSION

The CP-AgNPs was successfully synthesized from silver nitrate ($AgNO_3$) and *C. procera* aqueous leaf extract as the synthesized AgNPs became dark brown in an aqueous solution, suggesting the reduction of Ag^+ into Ag° with the $AgNO_3$ serving as a precursor and extracts as a reducer or capping agent. The UV-Vis spectroscopic study confirmed the synthesis of CP-AgNPs as distinct surface plasmon resonance band with a peak centered at around 445 nm was obtained (Fig. 1). This is in agreement with a similar study by Dada et al. [12] and Nagime et al. [14] whose UV-Vis spectroscopy findings revealed the synthesis of CP-AgNPs with a peak centered on 420 nm and peak of ~400 nm respectively. The finding is in contrast to the study by Nawab et al. [15] peak was observed at 271 nm. The FTIR spectra peaks were found to occur in the range between 579.00 and 4257.00 cm^{-1} (Fig. 2). The FTIR spectrum shows several significant peaks indicative of various functional groups. A broad peak at 1020 cm^{-1} indicates C-O stretching in alcohols, ethers, or esters, and the peak at 1159 cm^{-1} is also related to C-O stretching in these functional groups. The 1251 cm^{-1} peak is attributed to C-O stretching in ethers or esters, while the 1455 cm^{-1} peak corresponds to C-H bending in alkanes or possible C=C stretching in alkenes. The peak at 1650 cm^{-1} suggests C=C stretching in alkenes or aromatic compounds, while the strong peak at 1716 cm^{-1} is characteristic of C=O stretching in carbonyl compounds such as ketones, aldehydes, or esters. The 2600 cm^{-1} peak indicates broad O-H stretching, which is commonly found in carboxylic acids. Additionally, the peak at 2925 cm^{-1} reflects C-H stretching in alkanes. The peaks at 3207 cm^{-1} and 3492 cm^{-1} suggest the presence of O-H stretching in alcohols or phenols and N-H stretching in amines. The FTIR analysis reveals the presence of functional groups such as alkyl halides, aromatic compounds, alcohols, ethers, esters, alkenes, carbonyl compounds, carboxylic acids, and amines. These findings are in concordance with similar studies by Akshaya et al. [16] and Mohamed et al. [13]. Awad et al. [17] also recorded similar FTIR analysis results during the synthesis of AgNPs by *Aspergillus niger* strain. These various peaks indicated the presence of biomolecules which are responsible for the reduction and stabilization of AgNPs.

The SEM and TEM image for aqueous extract of *C. procera* AgNPs is presented in Figs. 3 and 4. The SEM images had width 50 µm while the view field value is 13 mm. The SEM image TEM micrographs of the synthesized *C. procera* AgNPs showed them as aggregated, spherical in shape, well dispersed with a diameter of about 15.30 – 17.66 nm and an average particle size of 16.79 nm in size. This is similar to the work of Marimuthu et al. [18] and Oraibi et al. [19]. This affirms that it may function as a reducing and capping agent in the synthesis of AgNPs. The EDX analysis gives qualitative as well as quantitative status of the constituents of a nanoparticle. The elements identified in the CP-AgNPs (EDX) with their weight and atomic size is shown in Fig. 5 with silver (Ag) having the highest proportion (75.56%). The strongest peak observed (3KeV) was from Ag. Generally, metallic silver nanocrystals showed typical optical absorption peak approximately at 3 keV due to their Surface Plasmon Resonance [12]**.** One of the advantages of green synthesis is the presence of other phytochemical elements from the EDX spectrum which may possible arise from the *C. procera* extra serving as the capping agents. This is supported by the findings of other researchers [20,21,22,23]. The XRD result confirmed the crystalline nature of the particles [13]. It is a powerful technique used to study the crystal structure of materials. Peak intensity is relative to quantity of arranged semi-crystalline structures and/or variations in electron density between crystalline and amorphous lamellae [24] while the sharp peaks correlated to crystalline region. A number of Bragg reflections at 2θ values of 38.10°, 44.3°, 77.5°, are shown corresponding to 111, 200 and 311 planes respectively (Fig. 6). The peak positions are expected for AgNPs and serve as a means of identifying silver nanoparticles in XRD analysis. The strong and sharp peaks revealed the crystalline nature of AgNPs. The peaks assigned in the AgNP sample agree with a similar study by Nawab et al. [15].

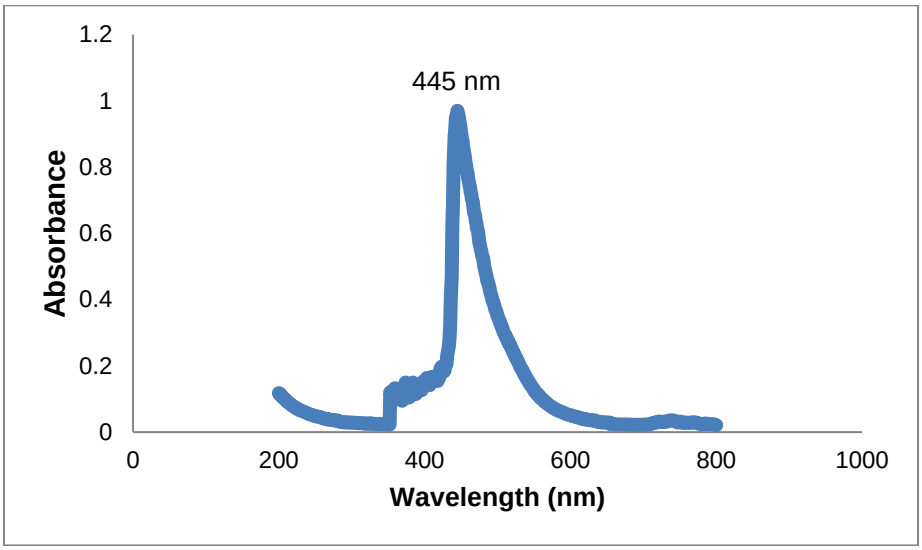

Fig. 1. The UV-Vis spectra of the synthesized CP-AgNPs

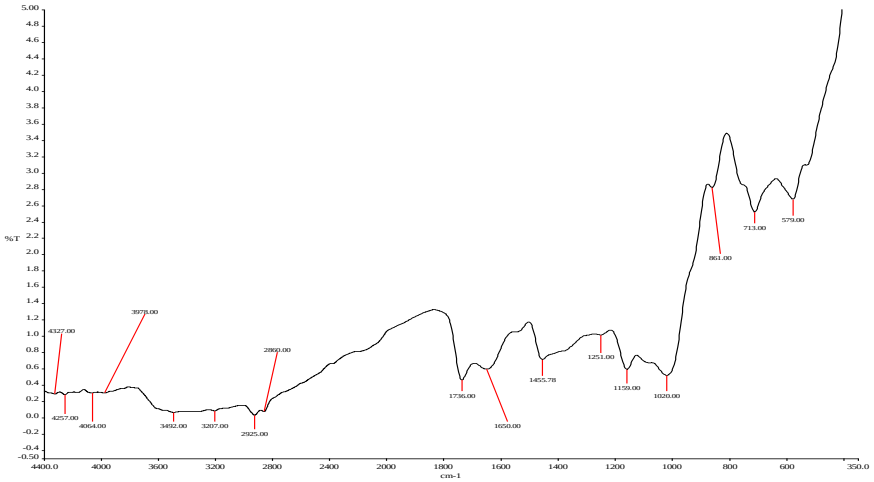

Fig. 2. FTIR Analysis of synthesized CP-AgNPs

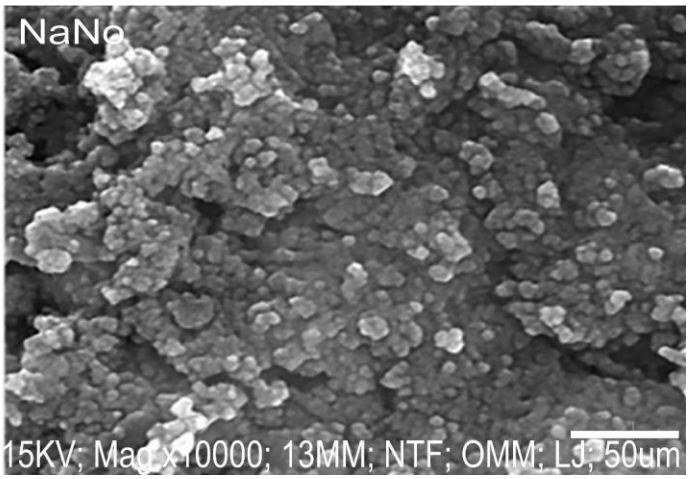

Fig. 3. SEM micrograph of synthesized CP-AgNPs at magnification of 10000X

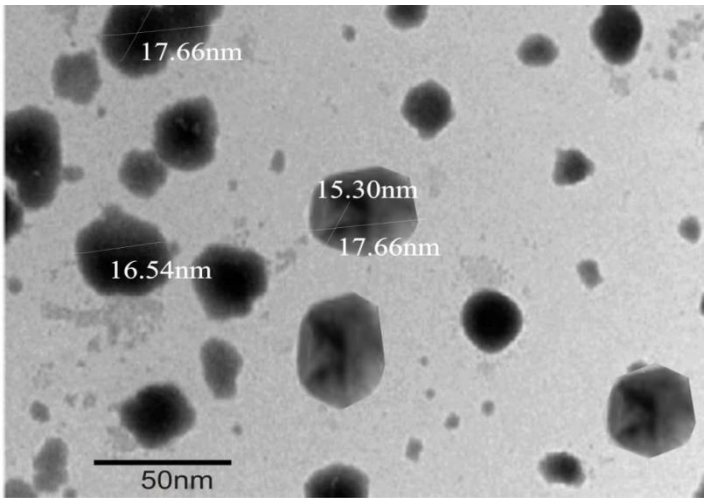

Fig. 4. TEM micrograph of synthesized CP-AgNPs

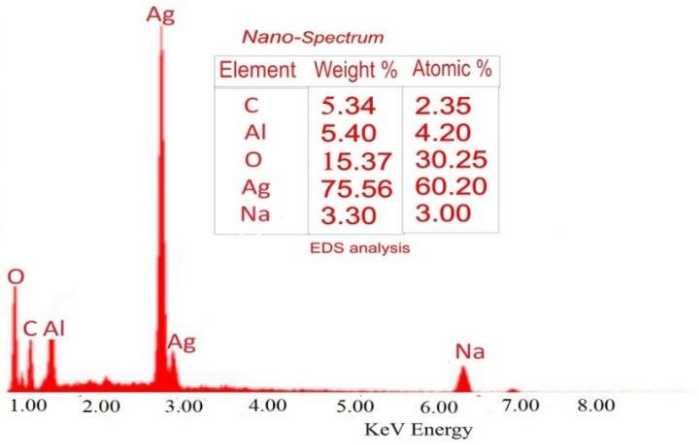

Fig. 5. EDX spectrum of synthesized CP-AgNPs

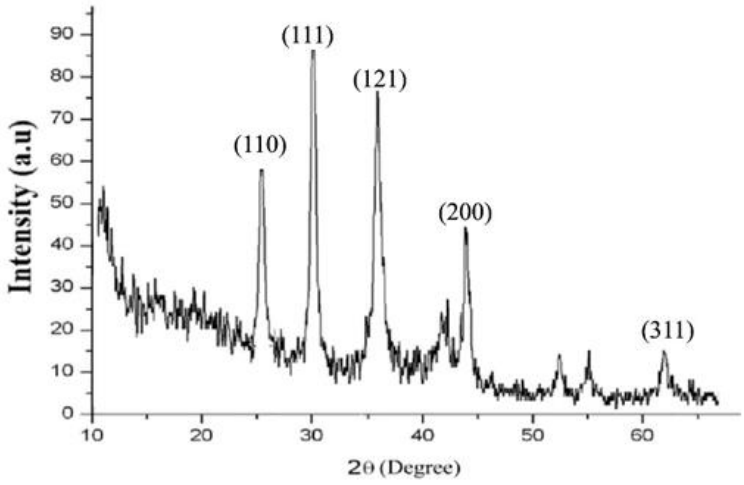

Fig. 6. XRD of synthesized CP-AgNPs

Table 1. Antibacterial activity of CP-AgNPs on tested bacteria

Test Organisms	Zone of Inhibition, Mean ± SE (mm)			
	***C. procera* extract (300 µL)**	***C. procera* AGNPs (10 µL)**	***C. procera* AGNPs (20 µL)**	**Ciprofloxacin (20 µL)**
Pseudomonas aeruginosa JF513146.1	10.5±0.1	11.7±0.2	14.4±0.3	18.1±0.4
Enterobacter sichuanensis CP027986.1	11.1±0.3	12.4±0.3	16.8±0.5	19.3±0.4
Escherichia coli KF917161.1	12.8±0.5	13.9±0.1	18.1±0.2	22.1±0.2
Staphylococcus aureus CP045560.1	9.3±0.3	15.2±0.2	22.2±0.5	25.3±0.4
Streptococcus agalactiae CP050455.1	10.5±0.5	14.8±0.3	21.5±0.1	23.1±0.3

Values are represented as means of triplicate readings ± Standard error of mean

The CP-AgNPs exhibited good antibacterial activity against Gram-negative bacteria and Gram-positive bacteria (Table 1). A zone of inhibition was observed in the area surrounding the test sample containing the well. The CP-AgNPs exhibited higher effect than the use of crude extract. The Gram-negative bacteria strain (*E. coli*, *E. sichuanensis* and *P. aeruginosa*) revealed a zone of 18.1±0.2 mm, 16.8±0.5 mm and 14.4±0.3 mm whereas Gram-positive bacteria (*Staphylococcus aureus* and *Streptococcus agalactiae*) showed a zone of 22.2±0.5 mm and 21.5± 0.1 mm. The strong antimicrobial activity depends on the large surface area of the nanoparticles which gave more surface area for interaction with the organisms and is also possible that the AgNPs not only interact with the surface of membrane, but also can penetrate inside the bacteria. Additionally, the high susceptibility of the synthesized AgNPs against these organisms indicated that it could be a valuable candidate for the treatment of infectious diseases [25]. Mohamed et al. [13] also reported that latex of *C. procera* has successfully synthesized Ag-NPs which exhibited good antibacterial activity against Gram-negative bacteria such as *E. coli, Serratia* sp*. and P. aeruginosa.*

4. CONCLUSION

AgNPs synthesized from aqueous extract of *C. procera* leaves are efficient and environment friendly, and thus, can be used as an effective stabilizing reducing agent for the synthesis of plant derived AgNPs. The SEM, TEM result showed the formed CP-AgNPs as highly stable spherical shaped particles, showing strong antibacterial activity against *E. coli*, *E. sichuanensis* and *P. aeroginosa*. The findings of this study proved that the green AgNPs can serve as a new technology for sustainable control of pathogens without any side effects.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

COMPETING INTERESTS

The authors declare no conflicts of interest.

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