Development and Analysis of Biogenic Nanoparticles Derived from Plant Extracts: A Sustainable Method to Improve Antimicrobial Efficacy

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Abstract

Silver nanoparticles have attracted significant interest owing to their remarkable antibacterial characteristics, particularly when synthesized using environmentally friendly approaches. This study introduces a sustainable approach for synthesizing AgNPs using Calotropis procera extract from leaves as a natural reducing component to convert a silver salt solution into nanoparticles which are stabilized using polyvinylpyrrolidone (PVP). The characterization of PVP-coated AgNPs was conducted using UV-Visible spectroscopy, FTIR, XRD and HR-TEM techniques. The UV-Visible spectra displayed a prominent peak at 468 nm, indicating the successful and stable formation of the nanoparticles. The FTIR analysis validated the successful preparation and stabilization of silver nanoparticles with PVP and the X-ray diffraction (XRD) pattern indicated a monoclinic crystalline structure.

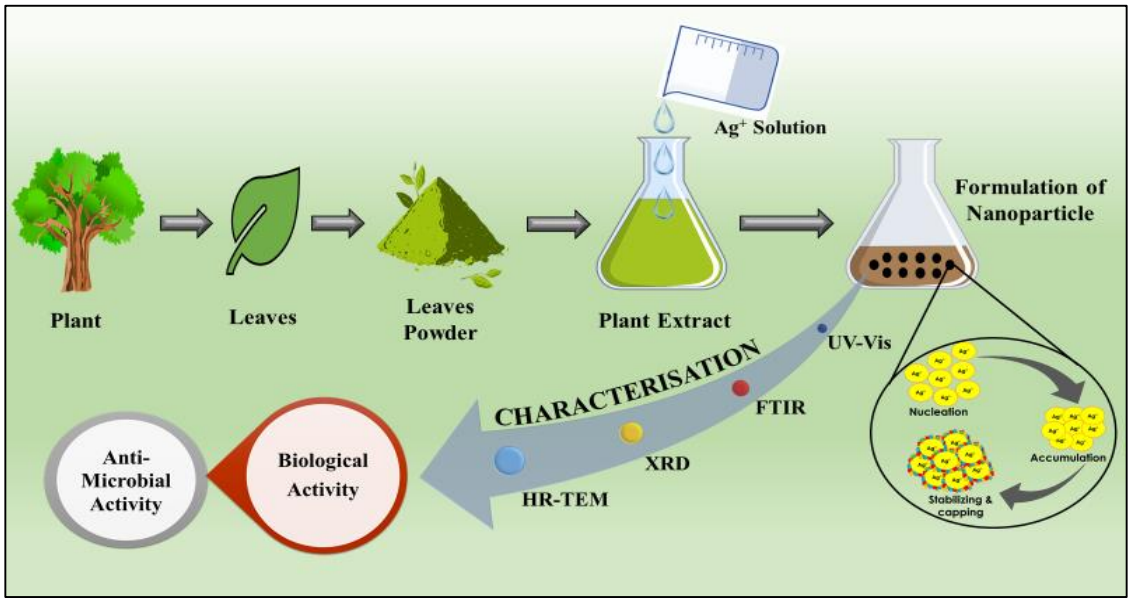
HR-TEM demonstrates that the nanoparticles are spherical in shape and are evenly distributed. The synthesized AgNPs showed an average diameter of 14.36 nm and have been functionalized with Polyvinylpyrrolidone (PVP) to increase their biological activity. An antibacterial efficiency of PVP-capped AgNPs has been evaluated against several bacterial strains including S. aureus, P. aeruginosa, GPB and E. coli, demonstrating significant activity against all analyzed pathogens.

Keywords: Green Synthesis, *Calotropis procera*, Silver Nanoparticles, Polyvinylpyrrolidone (PVP), Antimicrobial Activity.

Introduction

Green synthesis removes the dependence on hazardous chemicals, leading to a process that is not only economically viable but also environmentally friendly, energy-efficient and time-efficient. This approach employs a variety of eco-friendly materials such as plant extracts, microbes, algae, yeast and enzymes as sources for synthesis[9,10,14,37]. As a biotechnological method, green chemistry seeks to prevent, manage and remediate pollution at the molecular level, addressing issues related to global warming and the greenhouse effect. Environmental contaminants' detrimental effects have been connected to a variety of health issues including cardiovascular and respiratory ailments, immune system abnormalities, infertility and skin conditions such as dermatitis.

The production of nanoparticles from plant sources exemplifies a green chemistry approach that integrates nanotechnology with botanical resources, offering a viable and sustainable alternative to traditional synthetic methods[5,35].



Graphical Abstract

Research in nanotechnology prominently includes the creation of various nanoparticles such as silver, gold, iron and copper. Among these, silver nanoparticles are particularly notable for their unique properties including high catalytic efficiency, excellent electrochemical conductivity and significant antimicrobial effectiveness[12.33]. These attributes make silver nanoparticles especially suitable for applications in diverse fields including biomedicine[29], agriculture[13], photochemistry[34] and food science[21]. Silver is recognized for its efficacy against various microorganisms and its primary use in biotechnology today is largely due to its strong antibacterial properties.

Calotropis procera (Ait.), a member of the *Asclepiadaceous* family, is widely cultivated in India and in tropical and subtropical regions of the African and Asia continent. This plant is particularly noted for its substantial latex production, a milky substance with various potential uses[24.27]. In traditional folk medicine, *Calotropis procera* is utilized to treat a range of health conditions including pneumonia, sickness malaria, bronchial asthma joint pain, rashes, vomiting, diarrhoea, elephantiasis, skin ailments and dysentery[22.30]. It also demonstrates a variety of advantageous characteristics including anticoagulant, antipyretic, analgesic, anti-inflammatory, anticancer, antioxidant and antimicrobial properties, along with efficacy against *Mycobacterium tuberculosis*[1,7,8,23.25].

A diverse selection of plant leaf extracts including those from *Lantana camara*[3], *Arbutus unedo*[18], *Ocimum sanctum*[32], *Enicostemma axillare*[26], *Coriandrum sativum*[17], *Artemisia absinthium*[4], *Capparis zeylanica*[28], *Azadirachta indica*[2], *Padina tetrastromatica*[15], *Psidium guajava*[31], *Chenopodium album*[11], *Pulicaria glutinosa*[16] and *Stevia rebaudiana*[36], have been thoroughly studied by researchers for the environmentally friendly synthesis of silver nanoparticles.

In this work, *C. procera* leaf extract was utilized to produce silver nanoparticles in a speedy, easy and environmentally friendly manner. The generated nanoparticles underwent analysis via UV-Vis spectroscopy, FT-IR, XRD and HR-TEM techniques and their antibacterial properties were assessed using the agar well diffusion method.

Material and Methods

Materials: The leaves of *C. procera* utilized in the experiment were procured from a local market in Gujarat. All compounds purchased were of scientific grade and utilized without additional purification. Silver nitrate of analytical reagent grade ($AgNO_3$) was sourced from S.D Fine, while polyvinylpyrrolidone (PVP, molecular weight 40,000) was also acquired from S.D Fine Chem. Throughout the investigation, double-distilled water was employed for solution preparation. The UV spectrum was recorded using a Shimadzu-1800 UV-Vis spectrophotometer. The analysis using FTIR was performed within the range of 4000–400 cm^{-1} utilizing a Shimadzu FTIR spectrometer alongside a KBr disc. X-ray diffraction (XRD) investigation was conducted with a Rigaku D/max 40 KV spectrometer. The structural morphology was analyzed utilizing high-resolution transmission electron microscopy (HR-TEM) with a JEOL JEM 2100 plus model.

Preparation of Plant extract: Double distilled water was employed to cleanse 10 grams of fresh *Calotropis procera* leaves to eliminate any undesirable substances. Subsequently, the leaves went through a drying process by being pressed between sheets of filter paper. Once dried, the clean *Calotropis procera* leaves were ground using a grinder. The Soxhlet extraction method was utilized, combining 10 grams of the leaf powder, contained in thimbles, with 350 milliliters of double-distilled water to produce the leaf extract. The extraction continued until the solvent dripping from the siphon tube of the thimble became colourless, transitioning to a reddish-brown. The crude extract was subsequently cooled and filtered through Whatmann No. 1 filter paper and preserved in an airtight container at -4°C for future analysis.

Synthesis of AgNPs using *Calotropis procera* leaf extract: A freshly prepared extract of *Calotropis procera* leaves (30 ml) was mixed with a 0.03 mM $AgNO_3$ solution (90 ml) in an Erlenmeyer flask at room temperature for a duration of 2 hours, with continuous stirring.

As illustrated in figure 1, after this period, the solution underwent a significant colour change from light brown to dark brown, indicating a reduction of Ag^+ to Ag^0.

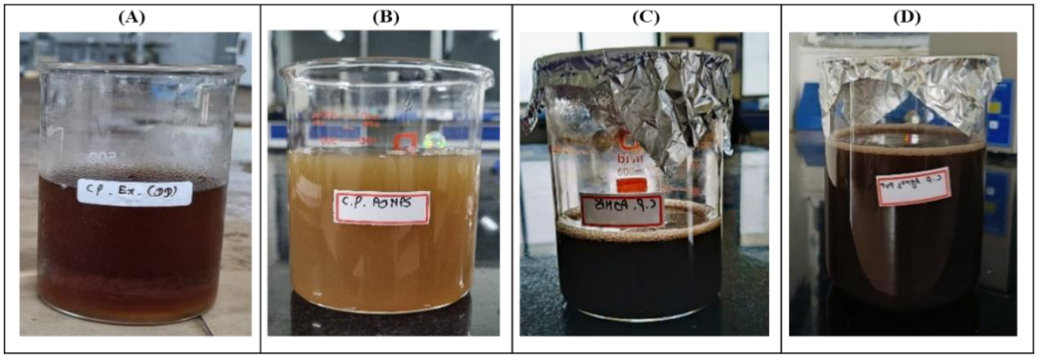


Figure 1: (A) Plant extract (B) Plant extract + AgNPs (C) AgNPs Colour change (D) Plant extract + AgNPs + PVP (Polyvinylpyrrolidone)

Synthesis of PVP encapsulated AgNPs: 0.50 grams of polyvinyl pyrrolidone (PVP) was immersed in 250 millilitres of double-distilled water and mixed for one hour at ambient temperature. A solution of silver nanoparticles (AgNPs) measuring 120 milliliters was gradually combined with the aqueous PVP. Following this period of mixing, there was a distinct colour shift, with the hue changing from dark brown to light brown. After 10 minutes at room temperature about 25 °C, the mixture was centrifuged for 30 minutes.

Characterization: The spectral properties of nanoparticles of silver (AgNPs) synthesized and coated with polyvinylpyrrolidone (PVP) were analyzed in the wavelength range between 200 and 800 nm using a Shimadzu UV-1800 UV-Vis spectrometer. Fourier-transform infrared (FTIR) spectroscopy was conducted at a resolution of 5 cm^{-1} across the range of 4000 to 400 cm^{-1} to verify the presence of functional biomolecules associated with both synthetic AgNPs and PVP-functionalized AgNPs. A Rigaku D/max 40 KV, a spectrum analyzer was employed for XRD (X-ray diffraction) analysis to assess the purity of the samples. The structural morphology of the synthesized AgNPs and PVP-functionalized AgNPs was examined through HR-TEM.

Antimicrobial activity: The agar well diffusion technique was employed to assess the biological properties of PVP, AgNPs and PVP-functionalized AgNPs, focusing on their effects against both Gram-negative and Gram-positive bacterial strains. A range of bacterial species, such as *E. coli*, *S. aureus*, *Pseudomonas aeruginosa* and Gram-positive bacteria (GPB), were utilized to assess the antibacterial efficacy of silver nanoparticles. For this assessment, a Petri dish was evenly coated with nutrient agar medium and a growth inhibition experiment was carried out. The antibacterial efficacy of the AgNPs and PVP-AgNPs was then evaluated by placing 100 µL of each in the disc's center[20]. The culture media was then incubated in an aerobic environment at 37 °C for a duration of 24 hours.

Results and Discussion

UV-Visible spectroscopy: The synthesis of PVP-AgNPs was confirmed through analysis conducted with UV-vis spectroscopy. An initial evaluation of the AgNPs indicated a distinct color change from light brown to dark brown, this being additionally supported by UV-Vis spectroscopy. The observed color change signifies the formation of AgNPs which occurs due to a reduction of silver ions (Ag^{+}) to metallic nanoparticles (Ag^{0}). As illustrated in figure 2, the peaks corresponding to AgNPs and PVP-AgNPs were identified at wavelengths of 468 nm respectively. Research suggests that the surface plasmon resonance (SPR) characteristics of most metallic nanoparticles are influenced by their size and morphology[19].

FTIR studies: The FTIR spectra of the biosynthesized PVP-AgNPs revealed significant peaks (fig. 3) at 3460, 3395, 3027, 2927, 2855, 1748, 1646, 1585, 1550, 1514, 1394, 1296 and 972 cm^{-1}. The peak at approximately 3460 cm^{-1} is attributed to the O-H stretching characteristic of alcohols. The band observed at 3395 cm^{-1} is associated with the N-H stretching of aliphatic primary amines. The peak at 2927 cm^{-1} signifies a robust and extensive O-H stretching bond, typical of carboxylic acids. The peak at 1748 cm^{-1} corresponds to the C=O stretching of six-membered lactones present in esters and δ-lactones. A significant band at 1646 cm^{-1} indicates the C=C stretching absorption characteristic of monosubstituted alkenes. Additional notable bands in the spectrum are identified at 1585, 1550 and 1514 cm^{-1} associated with N-H stretching of amine groups and significant N-O stretching bands from nitro compounds. The C-O stretching of aromatic esters is evidenced by a prominent band at 1296 cm^{-1}.

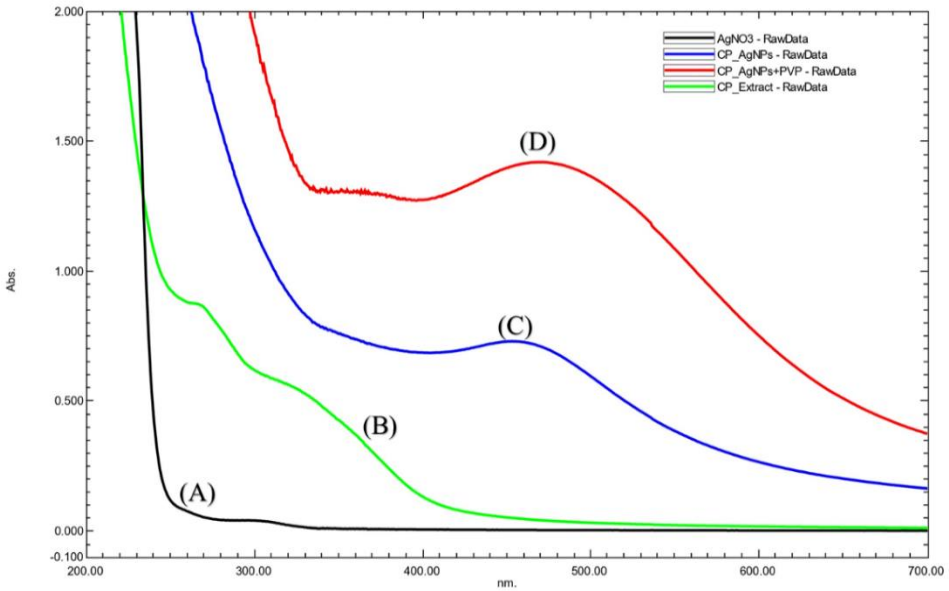


Figure 2: UV-Vis absorbance spectra of (A) Silver salt $AgNO_3$ (B) Plant extract (C) Ag-NPs (D) PVP-AgNPs

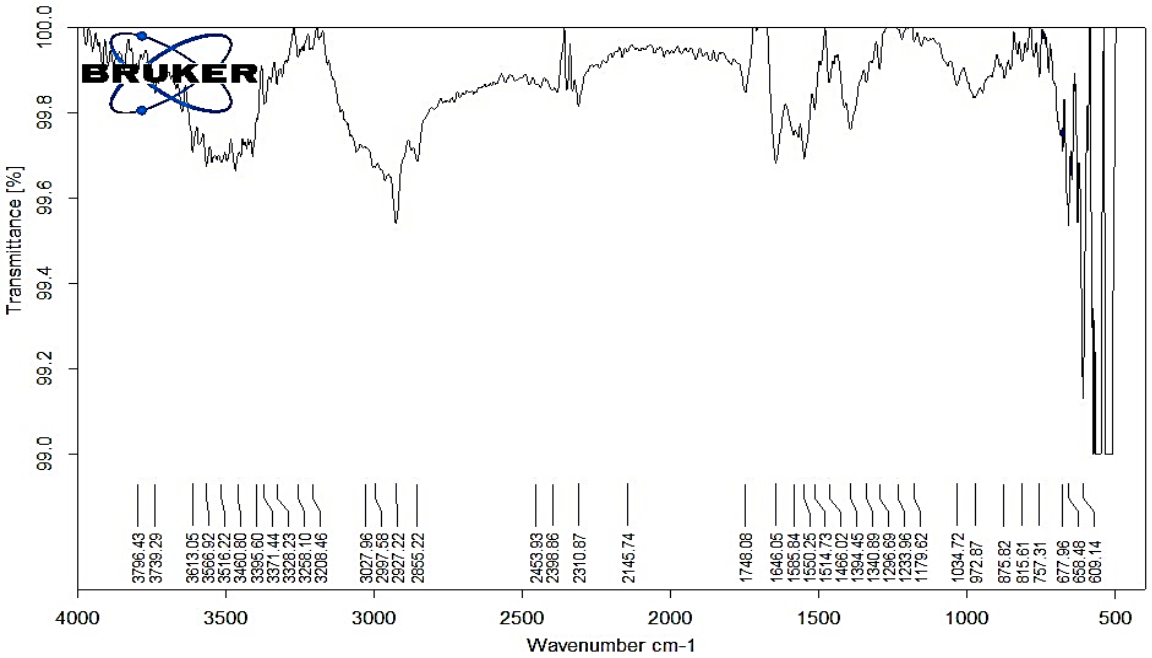


Figure 3: FTIR spectrum of PVP-AgNPs

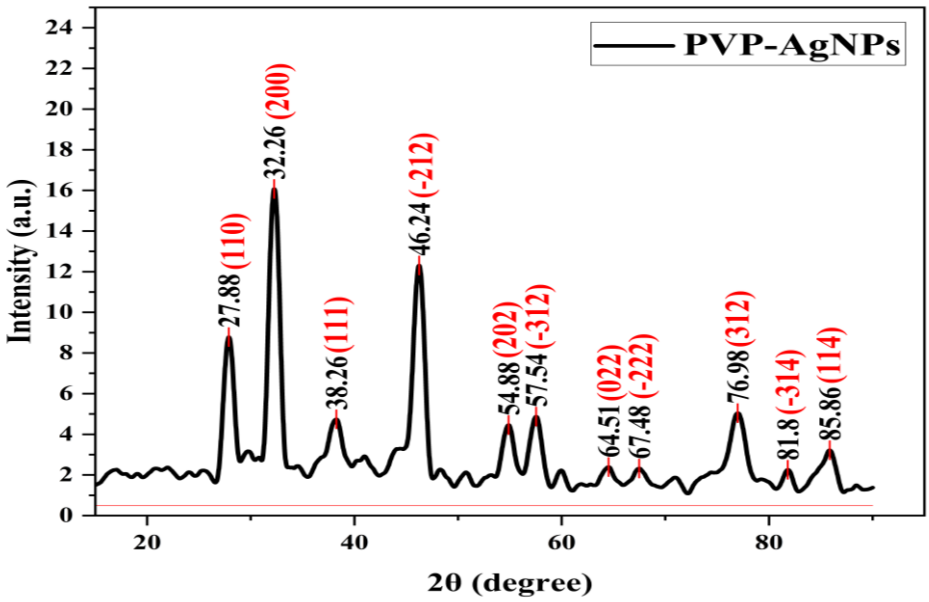


Figure 4: XRD Analysis of biosynthesized PVP-AgNPs.

XRD studies: X-ray diffraction (XRD) is a widely employed analytical method for the investigation of molecular and crystalline structures. It functions as a qualitative instrument for the identification of active compounds, the resolution of various molecular forms and the assessment of parameters such as crystallinity, isomorphous substitution and particle size. In this study, the XRD analysis was performed over a diffraction angle (2θ) range of 0˚ to 90˚ to validate the synthesis of PVP-AgNPs, as shown in figure 4. The resulting XRD pattern displays distinct diffraction peaks at angles of 27.88, 32.26, 38.26, 46.24, 54.88, 57.54, 64.51, 67.48, 76.98, 81.8 and 85.86˚ which indicate the monoclinic structure of silver crystals. These peaks correspond to the crystallographic planes (110), (200), (111), (-212), (202), (-312), (022), (-222), (312), (-314) and (114), aligning closely with the specifications outlined in JCPDS card number 01-074-1743, this verifies that silver nanoparticles have a monoclinic structure.

HR-TEM studies: The dimensions and morphology of the nanoparticles generated were evaluated using transmission electron microscopy. HRTEM images revealed that the silver nanoparticles (AgNPs) encapsulated in polyvinylpyrrolidone (PVP) displayed a spherical morphology, uniform distribution and homogeneity, with an average particle size of 14.36 nm. Figure 5(A, B, C) presents the TEM images of the synthesized CP/PVP-AgNPs. Figure 5(D) illustrates distinct ring patterns in the SAED pattern. Figure 5(B) presents the size distribution curve derived from the TEM analysis.

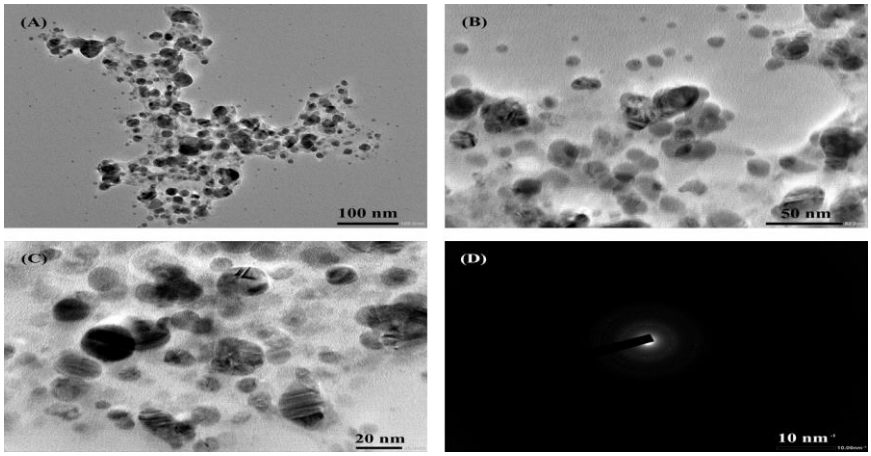


Figure 5: HR-TEM image of PVP-AgNPs observed at (A) 100 nm, (B) 50 nm, (C) 20 nm and (D) Selected area electron diffraction (SAED) pattern of PVP-AgNPs.

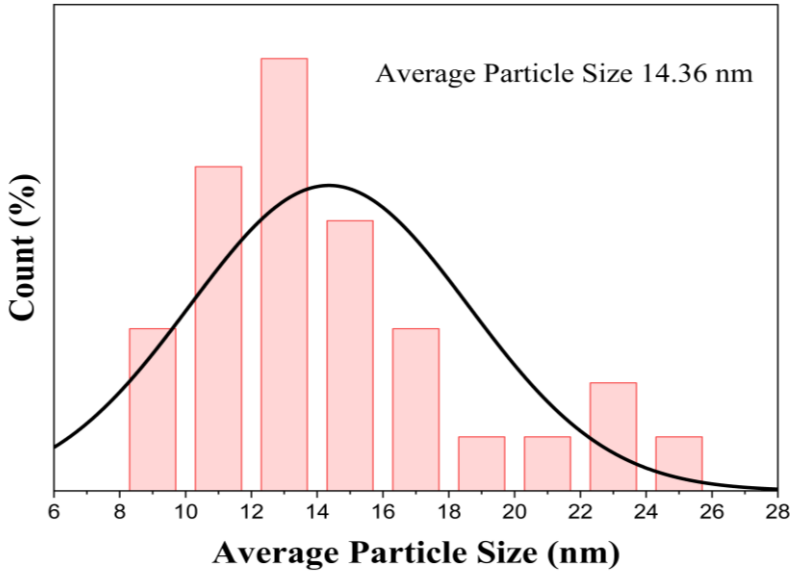


Figure 6: Size distribution curves from the TEM analysis and SAED pattern of PVP functionalized AgNPs.

Table 1
Antibacterial activity of plant extract, silver nanoparticles of *Calotropis procera* leaves and PVP-capped silver nanoparticles

Test Sample	Concentration (µL)	Zone of Inhibition (mm)			
		E. coli	*S. aureus*	*P. aeruginosa*	GPB
Plant Extract	100	18	16	17	19
Silver Metal Salt	100	20	19	20	21
AgNPs	100	23	21	22	23
Streptomycin	100	25	23	24	25
PVP-AgNPs	100	27	29	28	27

Antimicrobial activity: The agar well diffusion method, a recognized technique for assessing the inhibitory potential of antimicrobial agents, was employed to evaluate the antibacterial activity of the biosynthesized silver nanoparticles against both Gram-positive and Gram-negative bacterial strains. The inhibition zones for bacterial growth were measured as follows: *S. aureus* exhibited a zone of 29 mm, GPB showed 27 mm, *E. coli* recorded 28 mm and *P. aeruginosa* presented 27 mm. The findings indicate that in comparison to alternative pharmacological treatments, polymeric (PVP) encapsulated AgNPs demonstrate superior efficacy against both Gram-positive and Gram-negative bacteria[6]. All bacterial strains examined were significantly inhibited by PVP-AgNPs at a dose of 100 µL.

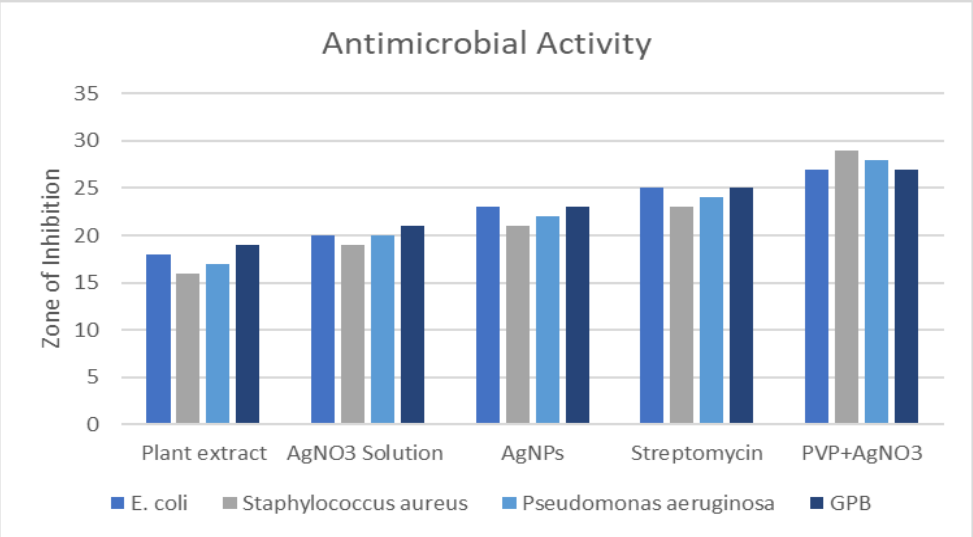


Figure 7: Zone of inhibition (In mm)

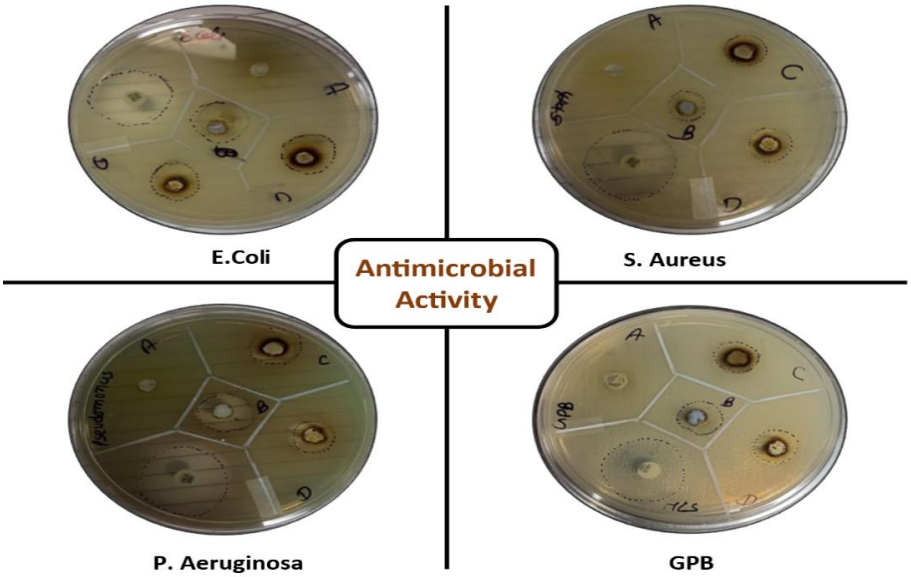


Figure 8: Antimicrobial activity of 1) *E. coli* 2) *Staphylococcus aureus* 3) *Pseudomonas aeruginosa* 4) GPB using *Calotropis procera* leaf extract and silver nanoparticles.

Together, plant extracts from *Calotropis procera* and silver nanoparticles show potential as antibacterial agents against harmful microbes, according to this study's results. In addition, studies have shown that antibacterial medicines containing AgNPs in PVP polymer are more effective than other therapies.

Conclusion

The development of antibacterial nanoparticles covered with polymers was approached in a way that was both environmentally friendly and financially viable. An extract from the leaves of *Calotropis procera* (Aakado) and a solution of silver nitrate ($AgNO_3$) were used to successfully synthesize silver nanoparticles (AgNPs). Adding polyvinylpyrrolidone (PVP) functionality to the produced AgNPs improved their biocompatibility and prevented the usage of hazardous or dangerous compounds. In order to validate the creation of AgNPs, UV-visible spectroscopy was used. After two hours, the color changed to dark brown and a significant peak at 468 nm was seen.

To identify the functional groups, present in the *Calotropis procera* extract that contributed to the biogenic synthesis of AgNPs and their polymeric coating, FTIR spectroscopy was employed. The monoclinic crystalline structure of the polymer-coated AgNPs was confirmed using XRD analysis. The spherical morphology was determined through the analysis of HR-TEM images, revealing particle sizes ranging from 10 to 100 nm, with an average measurement of 14.36 nm. Both the AgNPs and the AgNPs coated with polymers demonstrated notable antibacterial activity.

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