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# Studies on Green Synthesis of Silver Nanoparticles using Aqueous Leaf extract of *Sauropus androgynus* and their Antibacterial Activity

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**ABSTRACT: The synthesis of nanoparticles using environmentally friendly methods has attracted significant interest owing to their versatile applications and the need for sustainable approaches. Silver nanoparticles (SNPs) have emerged as a popular choice owing to their unique properties; however, they have drawbacks due to the involvement of hazardous chemicals. This study contributes to the field of environmentally friendly nanoparticle synthesis using *Sauropus androgynus* leaf extract as a natural reducing agent. This approach eliminates the use of hazardous chemicals that are typically involved in traditional synthesis methods, making it a more sustainable alternative. SEM, zeta potential analysis, UV-visible spectroscopy, and X-ray diffraction (XRD) were used to characterize the synthesized nanoparticles. The absorption peak at 420 nm in the UV-visible spectrum is evidence of the presence of SNPs. Spherical nanoparticles with an average size of 219 nm were discovered using SEM. An examination of zeta potentials revealed a negative zeta potential of -11.1 mV, demonstrating the stability of the nanoparticles. The crystalline nature of the nanoparticles was confirmed by X-ray diffraction (XRD) measurements, with an average crystalline size of 16.23 nm. The antibacterial activity of the synthesized nanoparticles was evaluated against both gram-positive (*Bacillus subtilis*) and gram-negative (*Escherichia coli*) bacteria at different concentrations. The results showed that *Sauropus androgynus* leaf extract-mediated SNP had antibacterial activity, and the extent of inhibition zones was concentration-dependent. This study addresses the challenges associated with green synthesis of SNPs and provides valuable insights into their characterization and antibacterial activity.**

**Keywords:** *Sauropus androgynus, Bacillus subtilis, Escherichia coli,* silver nanoparticles.

## INTRODUCTION

Nanotechnology is a field that investigates highly promising methods in materials science at the molecular level. Owing to the increasing significance of nanoparticles in various sectors, such as agriculture, medicine, electronics and etc, there has been an increase in interest in the development of environmentally benign methods for their synthesis in recent years (Koparde *et al.*, 2023; Shnoudeh *et al.*, 2019). Scientists have paid particular attention to silver nanoparticles (SNPs) because of their distinctive characteristics and potential uses. However, traditional techniques for producing SNPs frequently require dangerous chemicals and severe circumstances, endangering both the environment and human health (Yaqoob *et al*., 2020). Researchers have focused on creating alternative strategies that promote sustainability and environmental friendliness to address these challenges.

*Sauropus androgynus*

One such method is the green synthesis of silver nanoparticles, which uses natural sources, such as plant extracts, microbes, and proteins, to decrease silver ions and generate nanoparticles. This environmentally benign process has many benefits, such as the removal of hazardous chemicals, energy conservation, and the potential to produce SNPs on a wide scale (Alabdallah and Hasan 2021). Green-synthesized SNPs exhibit remarkable physicochemical properties such as size, shape, and surface chemistry, which can be tailored by modifying the synthesis parameters. This versatility opens exciting opportunities for their applications in various fields. For instance, the green-synthesized SNPs have shown promising antimicrobial, anti-inflammatory, and anticancer activities in the biomedical field (Sharifi-Rad *et al.,* 2020).

*Sauropus androgynusis* called a 'multigreen vegetable' because of its high nutritional value and vitamin quantity in comparison with other vegetable crops. It is a rich source of vitamins A and C, protein, calcium, and carbohydrates in comparison with other leafy vegetables (Anju *et al.,* 2022). In the current study, we used Chekurmanis (*Sauropus androgynus*) leaf extract to synthesize silver nanoparticles (SNPs) and evaluated their antibacterial effect against the growth of gram-positive and gram-negative bacteria.

## MATERIAL AND METHODS

*Sauropus androgynus* leaves, silver nitrate ($AgNO_3$), Nutrient Agar (Hi media, India), and agar type-1 (Hi media, India).

**Preparation of plant extract.** The *Sauropus androgynus* leaf extract was procured from the Karunya Institute of Technology and Sciences. The aqueous *Sauropus androgynus* leaf extract was prepared by combining the 5 g of powdered leaf with 100 ml of deionized water in a 250 ml Erlenmeyer flask. The mixture was then stirred at 80 °C for 90 min. The extract was filtered through Whatman No. 1 filter paper and stored at 4 °C for further use.

**Green synthesis of silver nanoparticles.** Silver nitrate ($AgNO_3$) of 1 mM was prepared and used. The nanoparticle solution is prepared by mixing *Sauropus androgynus* leaf extract and silver nitrate in a ratio of 1:9 v/v (extract: $AgNO_3$). The mixture is incubated in a water bath at 80 $^{o}$C for 45 minutes. The Ag-reduction was visible as the color changed from light yellow to brown (Yassin *et al*., 2022). The solution is centrifuged at 3000 rpm for 30 min. The pellet containing silver nanoparticles was washed twice with distilled water to remove excess silver ions. The pellets are placed in a hot air oven at 60 $^{o}$C for 3 hours (Bonnia *et al.,* 2018).

**Characterization of silver nanoparticles.** In this investigation, a UV-visible spectrophotometer (Hitachi U-2910 Spectrophotometer, Japan) was used to track the green production of silver nanoparticles (SNPs). The surface plasmon resonance (SPR) peaks of the synthetic SNPs, which were recorded between wavelengths of 200 and 800 nm, were measured using a spectrophotometer. This technique made it possible to characterize and assess the optical characteristics of green-synthesized SNPs. The characterization of green synthesized silver nanoparticles (SNPs) through the analysis of their size and morphology using a scanning electron microscope (JSM-6390 SEM). SEM provides high-resolution imaging to examine the structural features and surface characteristics of biosynthesized SNPs. The size was measured using Image J software, and the size distribution of SNPs in a liquid suspension was determined using the DLS approach. It provides details on the stability, aggregation behavior, and zeta potential of nanoparticles. A Malvern Zeta sizer was used to determine the crystal structure of the SNPs. It is possible to learn important details about the crystallinity, crystal size, and lattice characteristics of nanoparticles by examining the diffraction patterns created when X-rays interact with them. The standard JCPDS files were compared while they were investigated on Lab X XRD-6100 with a voltage of 40 kV and a current of 30 MA Cu Ka radiation (=1.5406) in the 2 range of 5-80 diffraction intensities. The average crystalline size of the nanoparticles was calculated using the Debye-Scherrer equation.

$$D = \frac{K\lambda}{\beta \cos(\theta)}$$

**Antibacterial activity.** The agar-well diffusion method was used to test the SNPs created from *Sauropus androgynus* leaf extract for antibacterial efficacy against two harmful bacteria, including one gram-negative bacteria, *E. coli,* and one gram-positive bacteria, *B. subtilis* microbe cultures were cultivated in nutrient broth one day before being put into nutritional agar media with 100 µl using an L-rod that was evenly disseminated. 6 mm-diameter wells were drilled onto the agar medium's surface using a borer. 10 µg, 20 µg, 40 µg, 60 µg, 80 µg, and 100 µg/ml, of synthesized silver nanoparticles/ml were each placed to a separate well

## RESULTS AND DISCUSSION

**UV-VIS spectroscopy.** In the current study, SNPs were synthesized using the leaf extract of *Sauropus androgynus*. The mixture of silver nitrate and leaf extract leads to a light yellow to dark brown preliminary confirmation of silver nanoparticles (SNPs) formed through the reduction of Ag+ ions using an aqueous leaf extract. A UV-vis spectroscopy test has been carried out for SNP, as shown in Figure 1. The UV-visible spectrum displays a characteristic absorption peak at a wavelength of 420 nm which is attributed to the surface plasmon resonance (SPR) of silver nanoparticles. This peak confirms the successful synthesis of silver nanoparticles and provides valuable information regarding their optical properties.

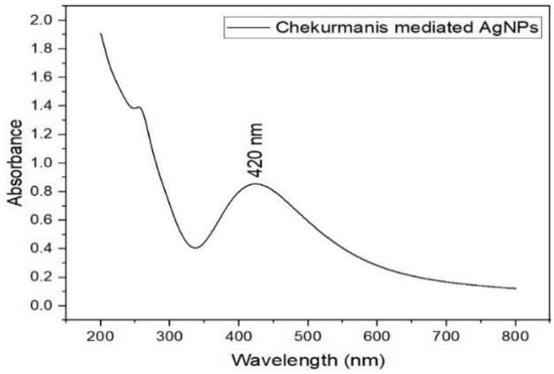


**Fig. 1.** Presence of green SNPs observed at 420 nm by UV-vis spectrum.

The green synthesis of silver nanoparticles (SNPs) using *Sauropus androgynus* leaf extract as reducing and capping agents. The peak confirms the successful formation of silver nanoparticles through the reduction of silver ions by the phytochemicals present in the leaf extract. The SPR phenomenon is highly dependent on the size, shape, and surface chemistry of the nanoparticles. The observed peak at 420 nm suggests that the synthesized nanoparticles Previous studies have described that SNPs exhibit an absorption peak in the range of 410–450 nm because of the SPR (Hamidi *et al.,* 2019; Heikal *et al.,* 2020).

**Scanning Electron Microscope (SEM).** The Scanning electron microscope (SEM) images of the silver nanoparticles are shown in Fig. 2. The size of the nanoparticles was determined using ImageJ software. The analysis revealed that the average particle size of the SNPs was measured to be 219 nm. The SEM analysis revealed that the silver nanoparticles obtained from the green synthesis method were spherical in shape.

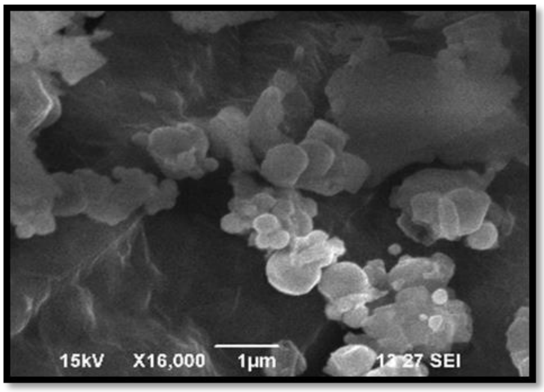


**Fig. 2.** SEM image of Chekurmanis-mediated SNP.

The uniform size and shape of the nanoparticles are crucial, as they can significantly influence their properties and reactivity. Periasamy *et al.* (2022) have reported that SNPs synthesized from Hibiscus rosasinensis have a spherical shape with a size of more than 200 nm in its leaf, flower, and bark.

**Zeta potential.** The zeta potential refers to the electric potential difference between the dispersion medium and the surface of the particles in a colloidal system. The results are shown in Fig. 4.

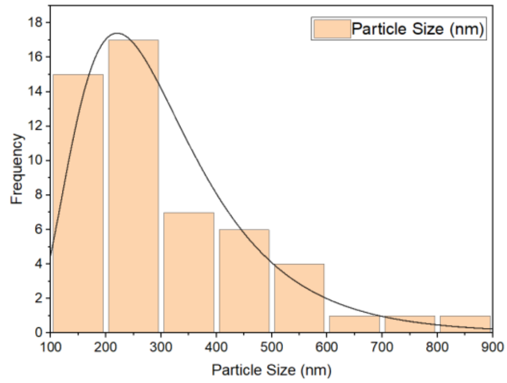


**Fig. 3.** Size distribution of SNPs**.**

Information states that the zeta potential of silver nanoparticles is -11.1 mV, with a zeta deviation value of -5.24 mV. The silver nanoparticles had a net negative charge on their surface, as evidenced by their negative zeta potential of -11.1 mV. This negative charge suggests a repulsive attraction between the particles, which helps the colloidal system remain stable and disperse. The standard deviation or variability in the zeta potential measurements is indicated by a zeta deviation value of -5.24. Raja *et al.* (2017) previously showed that the -17.2-mV negative zeta potential of SNPs produced from *Calliandra haematocephala* leaf extract demonstrated the stability of the silver nanoparticles.

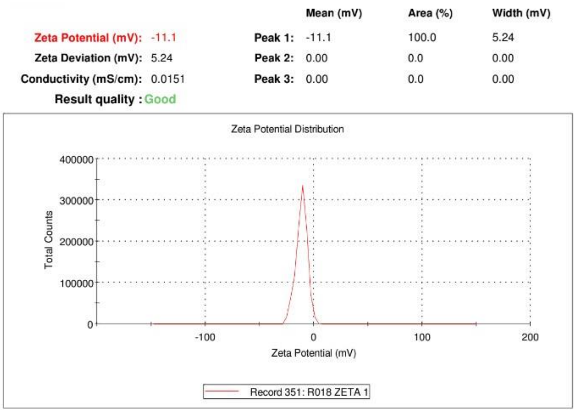


**Fig. 4.** Zeta potential result of synthesized SNPs.

**X-ray diffraction (XRD).** The X-ray diffraction (XRD) pattern obtained from the mediated SNPs exhibited distinct peaks at specific angles (2θ) of 38.41°, 46.49°, 65.10°, and 77.50°. These peaks correspond to the (111), (200), (220), and (311) crystal planes of face-centered cubic (fcc) silver, respectively. The indexing of the diffraction peaks was based on a comparison with the standard reference data from JCPDS file no. 04-0783, as reported by Anandalakshmi *et al.* (2016). The presence of well-defined diffraction peaks corresponding to the crystallographic planes of fcc Ag confirmed the crystalline nature of the synthesized SNPs. This indicated that the silver atoms within the nanoparticles were arranged in a regular, ordered manner, contributing to their structural stability and potential functionality.

The average crystalline size of the SNPs was determined using the Debye–Scherrer equation. This equation relates the size of the crystals (or nanoparticles) to the diffraction peak broadening observed in the XRD pattern. The calculated average crystallite size was found to be 16.23 nm, indicating that the SNPs possess nanoscale dimensions.

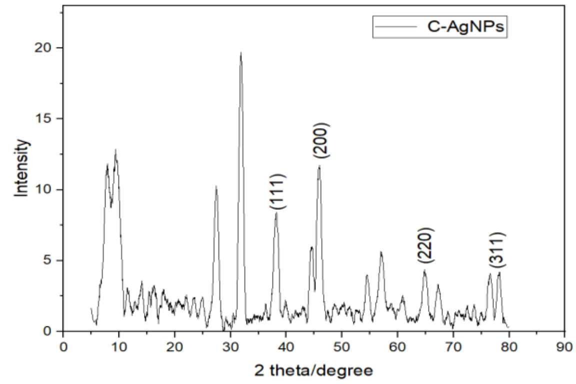


**Fig. 5.** XRD pattern of the silver nanoparticles synthesized *Sauropus androgynus.*

**Antibacterial Activity.** In the current study, gram-positive *B. subtilis* and gram-negative *E. coli* were used as test organisms to determine the antibacterial impact of produced silver nanoparticles. The inhibitory zones sizes and dependency on concentration varied between the effects of the drug onboth bacteria. For *B. subtilis*, the inhibition zone gradually increased as the SNP concentration increased, and maximum inhibition was observed at 100 µg/ml with 13.6mm. On the other hand, *E. coli* exhibited a similar trend with larger inhibition zones. The inhibition zone steadily increased with increasing concentrations of the SNPs, reaching a maximum of 23.5 mm at 100 µg/ml. These results suggest that *E. coli* is more sensitive to the substance, requiring higher concentrations to achieve the maximum inhibitory effect compared to *B. subtilis*.

**Table 1: Antibacterial activity of SNPs against *E. coli* and *B. subtilis*.**

| Microorganism | Zone of Inhibition in (mm) | |
|---|---|---|
| | Gram positive | Gram Negative |
| | ***B. subtilis*** | ***E. coli*** |
| **Concentrations** | | |
| 10 µg/ml | 7.2 | 10 |
| 20 µg/ml | 7.4 | 12.1 |
| 40 µg/ml | 9.1 | 14.2 |
| 60 µg/ml | 10.69 | 16.7 |
| 80 µg/ml | 11.9 | 18.2 |
| 100 µg/ml | 13.6 | 23.5 |

The results demonstrated concentration-dependent inhibition zones, with bigger inhibition zones occurring at greater nanoparticle concentrations. Compared to *B. subtilis*, the inhibitory impact was more pronounced against *E. coli*. It has been demonstrated that gram-negative bacteria are more vulnerable to the antibacterial effects of SNPs than gram-positive bacteria due to the thickness of the cell wall. Gram-positive bacteria's cell walls are 30 nm thick, in contrast to gram-negative bacteria's 3–4 nm thick peptidoglycan-based cell walls (Dakal *et al.*, 2016; Madivoli *et al.*, 2020). The nanoparticles likely to interacted with the bacterial cell membranes, leading to disruption and inhibition of bacterial growth.

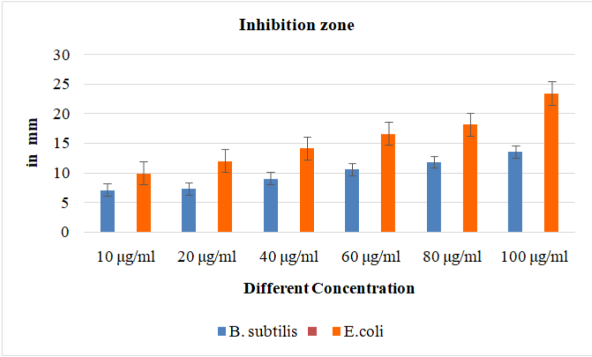


**Fig. 6.** Graphical representation of Inhibition zone of *B. subtilis* and *E. coli.*

## CONCLUSIONS

In conclusion, this study successfully demonstrated the environmentally friendly synthesis of silver nanoparticles (SNPs) using *Sauropus androgynus* leaf extract as a natural reducing and capping agent. The synthesized nanoparticles exhibited favorable physicochemical properties, including size, shape, stability, and crystallinity, which are crucial for their potential applications in various fields. Furthermore, the antibacterial activity of the silver nanoparticles was evaluated against two common bacterial strains, namely *B. subtilis* and *E. coli*. The results revealed significant inhibitory effects, indicating the potential of these nanoparticles for antimicrobial applications. The inhibition zones observed were larger at higher concentrations, suggesting a concentration-dependent antibacterial activity. Interestingly, the silver nanoparticles exhibited more pronounced activity against *E. coli* compared to *B. subtilis*. These findings underscore the promising prospects of utilizing *Sauropus androgynus* leaf extract-mediated silver nanoparticles as an effective antimicrobial agent. The green synthesis approach utilized in this study provides a sustainable and eco-friendly alternative to conventional methods involving hazardous chemicals.

## FUTURE SCOPE

Green synthesis of silver nanoparticles offers a sustainable and eco-friendly approach for their production. These nanoparticles can be further explored for environmental applications such as water purification, catalysis, and sensors. Their ability to inhibit bacterial growth can be utilized in developing antimicrobial coatings for various surfaces and materials.


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**Conflict of Interest.** None.

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