



# Green Synthesis of Silver Nanoparticles Using *Lawsonia inermis* Leaf Extract and their Antimicrobial Activity

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## ABSTRACT

This study aimed to synthesize silver nanoparticles (Ag NPs) using the aqueous leaf extract of *Lawsonia inermis* via a green, eco-friendly method and to test their antimicrobial activity. The novelty of this work lies in utilizing plant extract as a natural reducing and stabilizing agent without the use of harmful chemicals. Ag NPs were prepared using 2 mM AgNO<sub>3</sub> and confirmed by a UV–Vis absorption peak at 420 nm. The nanoparticles were grain-shaped, with an average size ranging from 13 to 61 nm, and exhibited a crystalline nature. All examined microorganisms, including two fungal species (*Candida Tropicalis* and *Candida albicans*) and four bacterial species (*Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Bacillus subtilis*), were significantly inhibited by the produced Ag NPs. The bacterial strain that exhibited the largest zone of inhibition was *Pseudomonas aeruginosa* (10 mm) at 100 µg/mL, followed by *Staphylococcus aureus* (9 mm) and *Escherichia coli* (8 mm). *Bacillus subtilis* showed the lowest susceptibility with a zone of inhibition of 7 mm. These results show the green-synthesised Ag NPs' broad-spectrum antibacterial activity and imply their applicability as potent antimicrobial agents.

**Keywords:** Nanoparticles; UV–Vis spectroscopy; FTIR, Lawsone; *Lawsonia Inermis*; Antimicrobial activity.

## 1. INTRODUCTION

Nanotechnology is the manipulation of materials at the nanoscale (1–100 nm), where they exhibit distinct optical, chemical, and biological properties compared with their bulk counterparts. Among engineered nanomaterials, silver nanoparticles (Ag NPs) have garnered considerable attention owing to their broad-spectrum antimicrobial activity and applications in medicine, drug delivery, wound dressings, and biomedical devices (Singh *et al.* 2020).

Conventional physical and chemical methods for synthesizing Ag NPs often require high energy input and toxic reducing agents, raising environmental and health concerns (Ahmed *et al.* 2019). Green synthesis using plant extracts has emerged as a sustainable and eco-friendly alternative. Plant-derived phytochemicals such as flavonoids, phenolic compounds, terpenoids, and quinones can reduce silver ions (Ag<sup>+</sup>) into silver nanoparticles while simultaneously acting as stabilizing agents. Although numerous plant species have been reported for Ag NP synthesis (Md. Arshad *et al.* 2020), there remains a need to identify medicinal plants with

strong bioactive compounds that can enhance nanoparticle stability and antimicrobial efficacy (Md. Abdul *et al.* 2018). In particular, systematic studies correlating phytochemical composition with nanoparticle characteristics and antibacterial performance are still limited (Rafique *et al.* 2021).

Silver nanoparticles have demonstrated remarkable effectiveness against a wide range of bacterial and fungal pathogens due to their unique physicochemical properties, including high surface area-to-volume ratio and enhanced reactivity. Green-synthesized Ag NPs have attracted significant interest because they combine the antimicrobial potential of silver with the biocompatibility and environmental sustainability of plant-mediated synthesis.

*Lawsonia inermis*, a medicinal plant widely used in traditional medicine, contains bioactive constituents such as lawsone (2-hydroxy-1,4-naphthoquinone), flavonoids, and phenolic compounds, which are known for their antimicrobial and antioxidant activities. These compounds can facilitate the efficient reduction of silver ions and improve the biological activity of the synthesized nanoparticles. However,

comprehensive evaluation of *L. inermis*-mediated Ag NP synthesis and their antibacterial activity against both Gram-positive and Gram-negative bacteria remains insufficiently explored.

Therefore, the present study aims to synthesize silver nanoparticles using the aqueous leaf extract of *L. inermis* through a green approach and to characterize and evaluate their antimicrobial activity.

## 2. EXPERIMENTAL METHODS

### 2.1 Preparation of *Lawsonia inermis* Leaf Extract

Fresh and healthy leaves of *Lawsonia inermis* were collected, washed thoroughly with running tap water two or three times, and rinsed with distilled water to remove surface impurities. The leaves were shade-dried at room temperature (25–30 °C) for 7–10 days and

ground into a fine powder using a mechanical grinder. For extract preparation, 20 g of leaf powder was mixed with 100 mL of double-distilled water (1:5 w/v ratio) and heated at 60 °C for 3 h under continuous magnetic stirring (500 rpm) (Sahar and El Rahman, 2019). After allowing the mixture to cool to room temperature, it was filtered using Whatman No. 1 filter paper. The filtrate was stored at 4 °C and used for nanoparticle synthesis within 48 h.

### 2.2 Green Synthesis of Silver Nanoparticles

The green synthesis of Ag NPs is depicted in Fig. 1. A 2 mM aqueous silver nitrate ( $\text{AgNO}_3$ ) solution was prepared using double-distilled water. For nanoparticle synthesis, 10 mL of the 2 mM  $\text{AgNO}_3$  solution was mixed with 30 mL of plant extract (1:3, v/v). The reaction mixture was maintained at 70 °C under constant stirring at 600 rpm for 2 h. The pH of the reaction mixture was adjusted to 8 using 0.1 M NaOH to facilitate the reduction of  $\text{Ag}^+$  ions (Xiaokun *et al.* 2025).

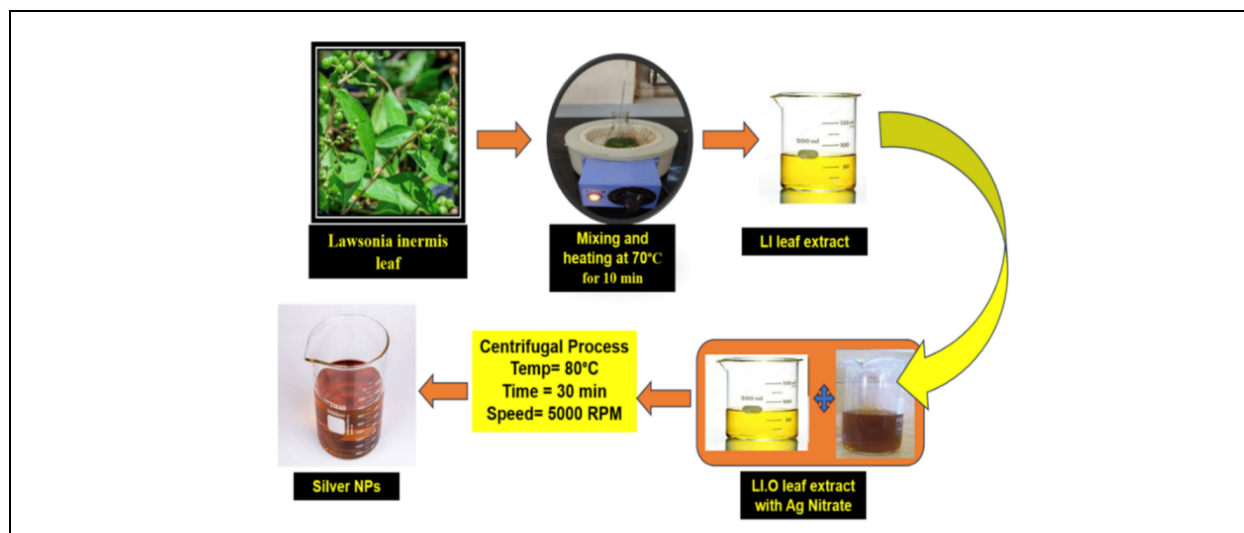


Fig. 1: Green synthesis of silver nanoparticles

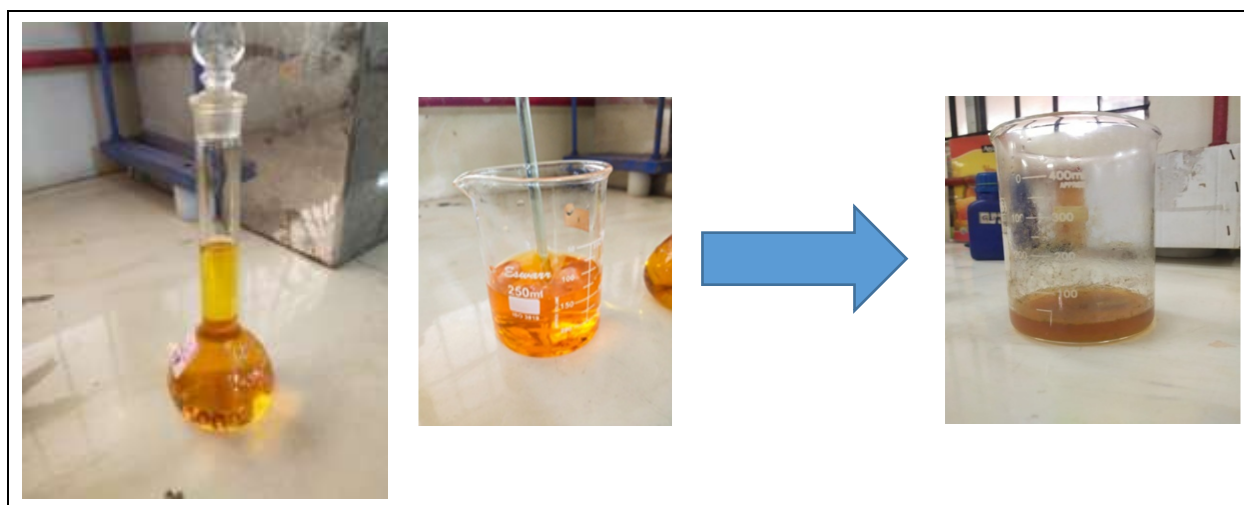


Fig. 2: Color change from orange to dark brown during Ag NP synthesis

Formation of silver nanoparticles was visually confirmed by a color change from orange to dark brown, indicating surface plasmon resonance (Fig. 2). The reaction mixture was allowed to cool to room temperature and incubated in the dark for 24 h to ensure complete reduction.

### 2.3 Purification of Silver Nanoparticles

The synthesized Ag NPs were separated by centrifugation at 10,000 rpm for 30 min. The pellet was washed three times with double-distilled water and once with ethanol to remove unreacted biomolecules and residual silver ions. The purified nanoparticles were dried in a hot air oven at 50 °C for 4 h and stored in airtight containers for further characterization and biological studies.

### 2.4 Microbial Strains

The antibacterial and antifungal activity of sample were exhibited against two gram positive bacterial strains *Staphylococcus aureus* (MTCC 87), *Bacillus subtilis* (MTCC 441) and two gram negative bacterial strains *Escherichia coli* (MTCC 443), *Pseudomonas aeruginosa* (MTCC 1688) and for fungal culture used in the study are *Candida albicans* (MTCC 183), *Candida tropicalis* (MTCC 230) were prepared as test organisms. All the bacterial strains were purchased from the Microbial Type Culture and Collection (MTCC) at Chandigarh, India and the fungal strains from National Chemical Laboratory (NCL), Pune, Maharashtra, India.

### 2.5 Antibacterial Activity (Disc Diffusion Method)

The disc diffusion method is used to evaluate the antibacterial activity of the sample. Ten ml of Mueller-Hilton agar medium was poured into sterile petri dishes (diameter 60 mm) and inoculated with test organism. Sterile filter paper discs loaded with various concentrations of sample of 60, 80 and 100 µg/ml were placed on the top of Mueller-Hilton agar plates. Filter paper disc loaded with 5 µg of amoxicillin was used as positive control. The plates were incubated at 37 °C for 24 hours and the zone of inhibition was recorded in millimeter and the experiment was repeated twice. (Singh *et al.* 2023).

### 2.6 Antifungal Activity (Disc Diffusion Method)

Disc diffusion method in order to test the antifungal activity of sample against test pathogens was carried out. In petri dishes (60 mm) filled with Sabouraud's dextrose agar (SDA) and seeded with a 0.3 ml of test organism, a sterile filter paper disc (diameter 6 mm, whatmann paper no.3) was placed. The sterile disc was impregnated with 10 µl of samples at varying concentration of 60, 80 and 100 µg/ml respectively. The

zones of growth inhibition around the disc were measured after 24 h of incubation at 37°C. while, Fluconazole was used as a positive control.

## 3. RESULTS AND DISCUSSION

### 3.1 Phytochemicals Investigation of Plant Extract

Qualitative phytochemical analysis of dried *L. inermis* leaf extract (Fig. 3) has shown the presence of various compounds. This shows that the ethanol extract contains carbohydrates, flavonoids, quinones, glycosides, and phenol compounds in good quantity.



Fig. 3: Phytochemical screening for *L. inermis* leaf extract

### 3.2 UV–Vis Spectroscopy

The formation of Ag NPs was confirmed by UV–Vis spectroscopy (Fig. 4). A characteristic surface plasmon resonance (SPR) peak was observed at 420 nm, which is typical for spherical Ag NPs. The sharp and symmetric nature of the peak indicates relatively uniform particle distribution (Femina *et al.* 2021). The absence of additional peaks suggests minimal aggregation. The intensity of the SPR peak increased with reaction time and stabilized after 24 h, confirming completion of reduction. The peak (420 nm) corresponds to nanoparticles in the size range of approximately 20–50 nm, consistent with SEM results.

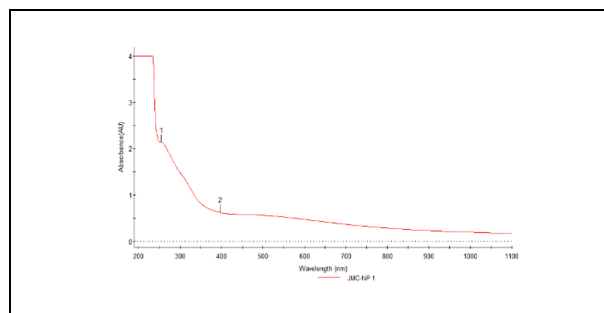


Fig. 4: UV–Vis spectrum of synthesized Ag NPs

### 3.3 Fourier Transform Infrared (FTIR) Spectral Analysis

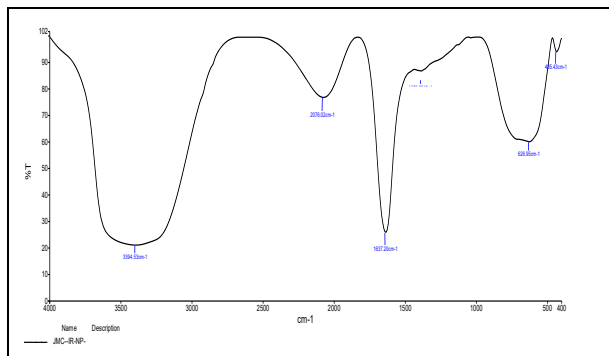


Fig. 5: FTIR spectrum of synthesized Ag NPs

FTIR spectrum of *L. inermis* leaf extract (Fig. 5) showed prominent peaks at 3394  $\text{cm}^{-1}$  (O–H stretching), 1637  $\text{cm}^{-1}$  (C=O stretching), and 1385  $\text{cm}^{-1}$  (C=C aromatic stretching), confirming the presence of phenols, flavonoids, and quinones such as lawsone (Dhand *et al.* 2021). After Ag NP formation, noticeable shifts and reduced intensities of the O–H and C=O peaks were observed, indicating their involvement in reduction and stabilization. The proposed mechanism involves:

- phenolic –OH groups donating electrons to reduce  $\text{Ag}^+ \rightarrow \text{Ag}^0$ ;
- quinone groups undergoing oxidation during reduction; and
- phytochemicals acting as capping agents, preventing aggregation through electrostatic stabilization and hydrogen bonding.

Thus, *L. inermis* phytochemicals function both as reducing and stabilizing agents.

### 3.4 Scanning Electron Microscopy (SEM)

SEM micrographs, shown in Fig. 6, revealed predominantly spherical nanoparticles with a size ranging from 13 to 61 nm. Particle size distribution analysis (measured from SEM micrographs,  $n = 100$  particles) showed an average particle size of  $34.5 \pm 8.2$  nm. Slight aggregation was observed, likely due to drying during sample preparation. The relatively narrow size distribution indicates controlled nucleation and growth during synthesis (Femina *et al.* 2021).

### 3.5 Energy Dispersive X-ray Analysis (EDAX / EDS)

EDAX spectrum (Fig. 7) showed a strong signal at 3.8 keV, confirming elemental silver. Quantitative analysis revealed 67.89% silver content, indicating efficient reduction of  $\text{Ag}^+$  ions. Minor peaks

corresponding to O, C, Ca, and Cl were detected, which may arise from plant biomolecules adsorbed on the nanoparticle surface. The presence of oxygen supports phytochemical surface capping, thereby enhancing nanoparticle stability (Auxilia *et al.* 2025a).

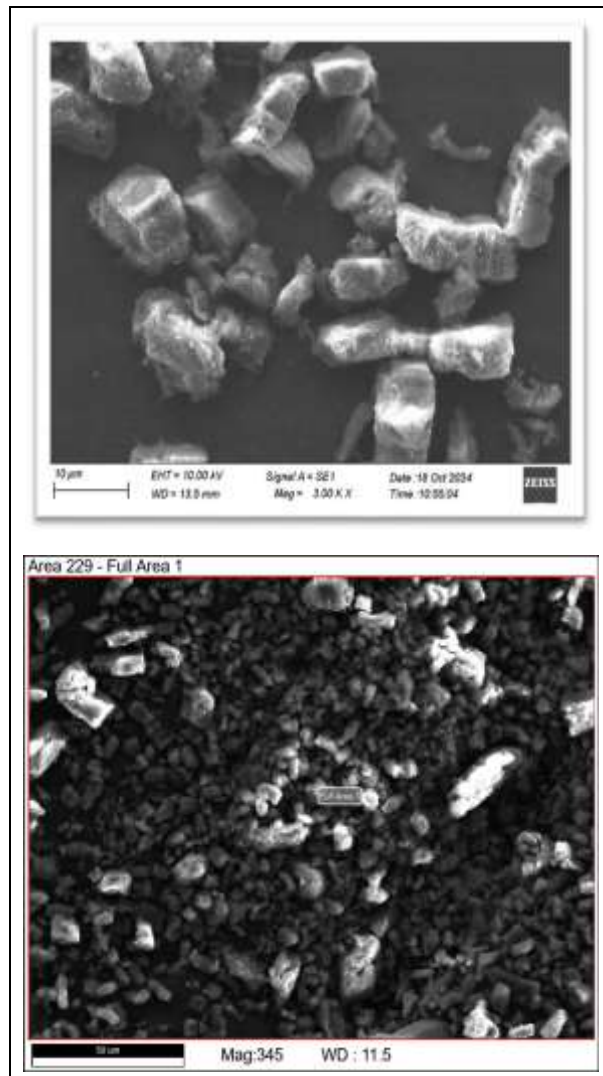


Fig. 6: SEM micrographs of biosynthesized Ag NPs showing predominantly spherical morphology with slight agglomeration

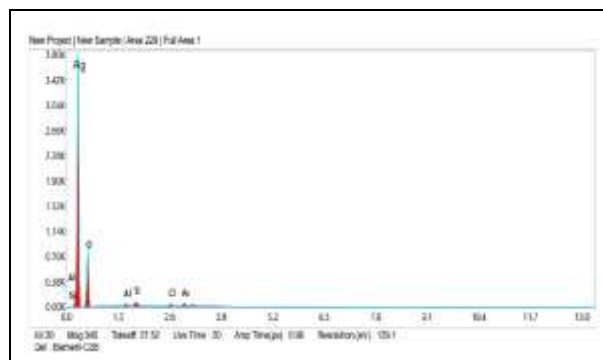


Fig. 7: EDAX spectrum of biosynthesized Ag NPs

### 3.6 X-ray Diffraction (XRD) Analysis

XRD analysis (Fig. 8) revealed characteristic diffraction peaks at  $2\theta$  values corresponding to (111), (200), (220), and (311) planes, confirming a face-centered cubic (FCC) crystalline structure of silver (JCPDS No. 04-0783) (Auxilia *et al.* 2025b).

The average crystallite size (D) was calculated using the Scherer equation:

$$D = 0.9\lambda/(\beta\cos\theta) \quad \dots (1)$$

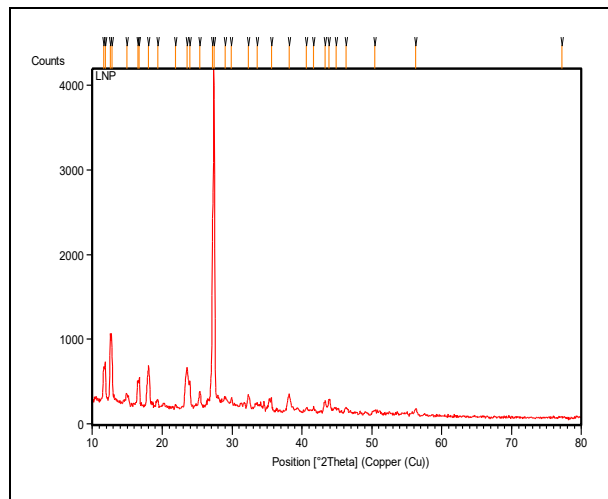


**Fig. 8: XRD pattern of Ag NPs showing characteristic peaks indexed to the (111), (200), (220), and (311) planes confirming FCC structure**

where  $\lambda$  is the Cu  $K\alpha$  radiation (0.15406 nm),  $\beta$  is the full width at half maximum (FWHM) in radians, and  $\theta$  is the Bragg's angle.

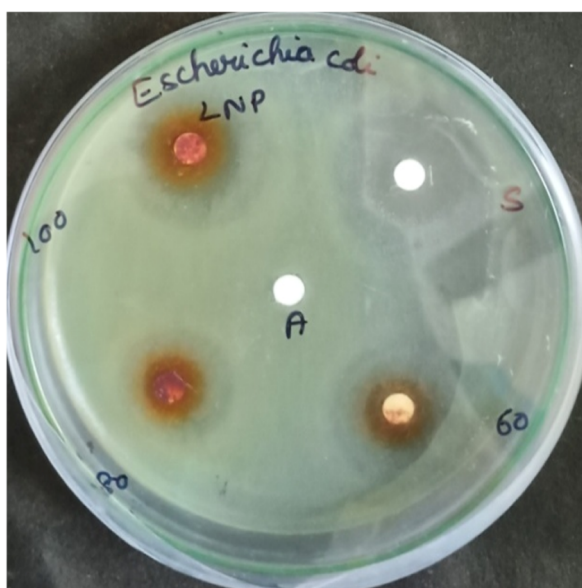
The calculated average crystallite size was 28.3 nm, which correlates well with the SEM findings.

### 3.7 Antimicrobial Activity

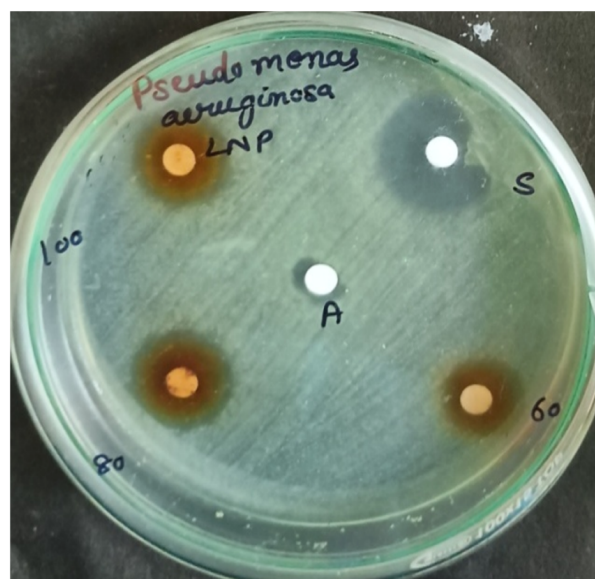
The synthesized sample exhibited significant concentration-dependent antibacterial activity against both Gram-positive (*S. aureus* and *B. subtilis*) and Gram-negative (*P. aeruginosa* and *E. coli*) bacteria. The highest activity was observed at 100  $\mu\text{g/mL}$ , with *P. aeruginosa* being the most sensitive organism. Although the activity was slightly lower than that of the standard antibiotic amoxicillin, the results demonstrate the potential of the synthesized material as an effective antibacterial agent. The absence of activity in the aqueous extract confirms that nanoparticle formation played a crucial role in enhancing the antimicrobial properties. The synthesized Ag NPs exhibited concentration-dependent antimicrobial activity (Table 1). Results are expressed as mean  $\pm$  SD ( $n = 3$ ) (Chika *et al.* 2024).

**Table 1. Antibacterial activity (zone of inhibition in mm)**

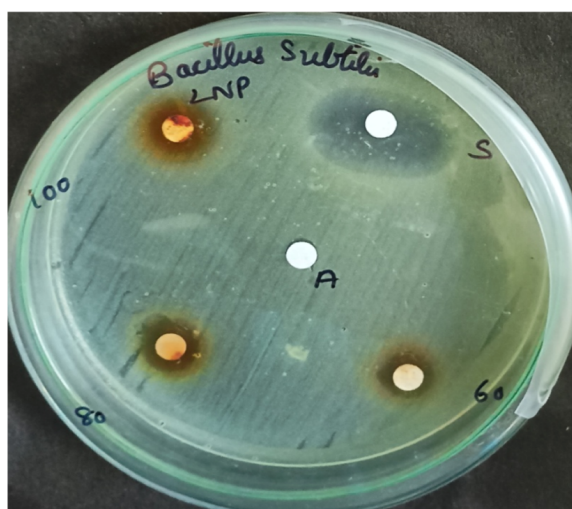
| Sample                 | Concentration( $\mu\text{g/mL}$ ) | <i>S. aureus</i><br>(mm) | <i>P. aeruginosa</i><br>(mm) | <i>B. subtilis</i><br>(mm) | <i>E. Coli</i><br>(mm) |
|------------------------|-----------------------------------|--------------------------|------------------------------|----------------------------|------------------------|
| Test Sample            | 60 $\mu\text{g/mL}$               | 6.0 $\pm$ 0.3            | 4.0 $\pm$ 0.2                | 4.0 $\pm$ 0.2              | 5.0 $\pm$ 0.3          |
| Test Sample            | 80 $\mu\text{g/mL}$               | 8.0 $\pm$ 0.4            | 7.0 $\pm$ 0.3                | 5.0 $\pm$ 0.2              | 6.0 $\pm$ 0.4          |
| Test Sample            | 100 $\mu\text{g/mL}$              | 9.0 $\pm$ 0.5            | 10.0 $\pm$ 0.5               | 7.0 $\pm$ 0.3              | 7.0 $\pm$ 0.3          |
| Aqueous Extract        | 10 $\mu\text{L/disc}$             | 0                        | 0                            | 0                          | 0                      |
| Amoxicillin (Standard) | 10 $\mu\text{L/disc}$             | 10                       | 11                           | 12                         | 10                     |



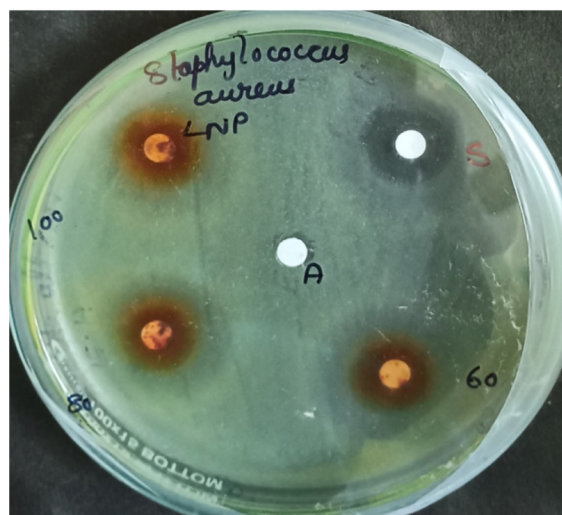
**ESCHERICHIA COLI**



**PSEUDOMONAS AERUGINOSA**



**BACILLUS SUBTILIS**



**STAPHYLOCOCCUS AUREUS**

**Fig. 9: Antibacterial activity (zone of inhibition in mm)**

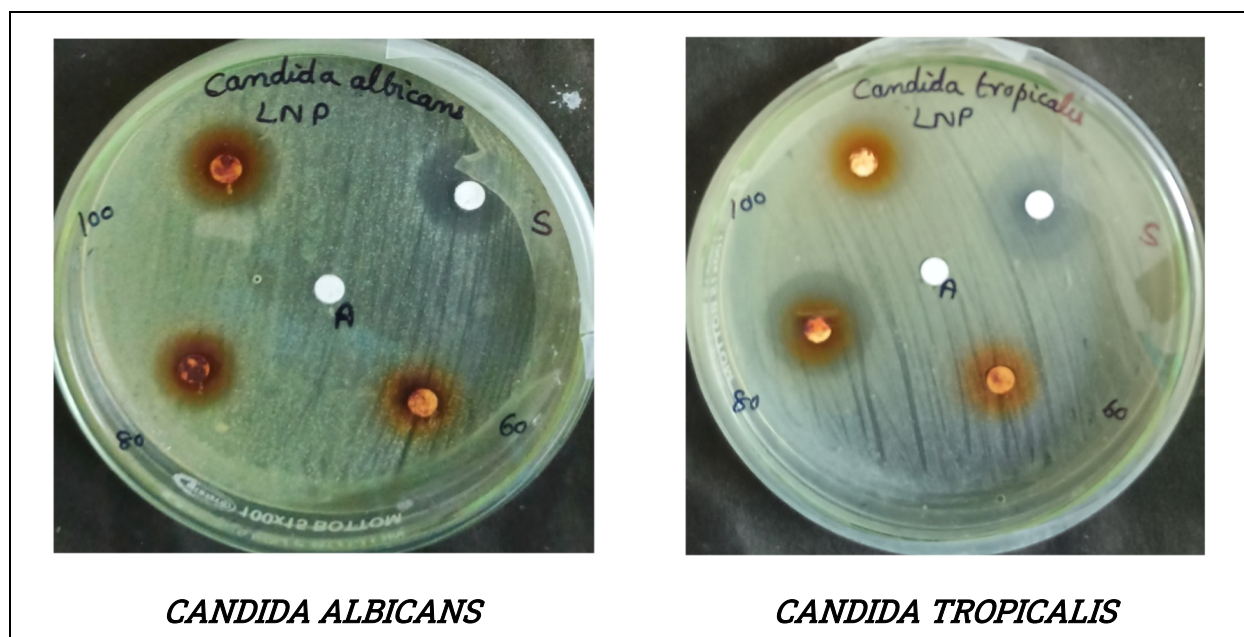


**Fig. 10: Antifungal activity (zone of inhibition in mm)**

The synthesized sample exhibited significant antifungal activity against both *Candida albicans* and *Candida tropicalis*, with activity increasing as the concentration increased. The highest inhibition zones were observed at 100 µg/mL, with *Candida tropicalis* showing greater susceptibility than *Candida albicans*. Although the antifungal activity was lower than that of the standard drug fluconazole, the results demonstrate the potential of the synthesized nanoparticles as an effective antifungal agent. The antifungal activity increased with concentration (Table 2) and showed statistically significant inhibition ( $p < 0.05$ ) (Tahir *et al.* 2019; Zhang *et al.* 2022).

**Table 2. Antifungal activity (zone of inhibition in mm)**

| Sample                 | Concentration(µg/ml) | Candida albicans (mm) | Candida tropicalis (mm) |
|------------------------|----------------------|-----------------------|-------------------------|
| Test Sample            | 60 µg/mL             | 4.0 ± 0.2             | 6.0 ± 0.3               |
| Test Sample            | 80 µg/mL             | 6.0 ± 0.3             | 8.0 ± 0.4               |
| Test Sample            | 100 µg/mL            | 8.0 ± 0.4             | 9.0 ± 0.5               |
| Aqueous Extract        | 10 µL/disc           | 0                     | 0                       |
| Fluconazole (Standard) | 10 µL/disc           | 10                    | 13                      |

#### 4. CONCLUSION

Silver nanoparticles (Ag NPs) were successfully synthesized using *Lawsonia inermis* leaf extract via a green synthesis approach. The formation of Ag NPs was confirmed by a characteristic surface plasmon resonance peak at 420 nm in the UV–Vis spectrum. FTIR analysis

indicated the involvement of phenolic and quinone functional groups in the reduction and stabilization of silver ions. SEM analysis showed predominantly spherical nanoparticles with sizes ranging from 13 to 61 nm (average ~30 nm), while EDAX confirmed silver as the major elemental component (67.89%). XRD analysis confirmed a crystalline face-centered cubic (FCC) structure, with an average crystallite size of approximately 28 nm, as calculated using the Scherrer equation.

The synthesized Ag NPs demonstrated concentration-dependent antimicrobial activity against both bacterial and fungal pathogens, with maximum inhibition zones of 9–10 mm at 100 µg/mL. Among the tested bacterial strains, *Pseudomonas aeruginosa* exhibited the highest susceptibility, whereas *Candida tropicalis* showed greater sensitivity among the fungal species. Statistical analysis ( $p < 0.05$ ) confirmed significant antimicrobial effects compared to the negative control, although the activity was slightly lower than that of the standard antimicrobial agents.

The results highlight the dual role of *Lawsonia inermis* phytochemicals as reducing and capping agents, facilitating the formation of stable and biologically active nanoparticles without the use of hazardous chemicals. The environmentally friendly synthesis process, combined with the nanoscale size, crystalline nature, and broad-spectrum antimicrobial activity of the Ag NPs, demonstrates the potential of this approach as a sustainable alternative to conventional nanoparticle synthesis methods.

Overall, the study confirms that *Lawsonia inermis* leaf extract is an efficient, cost-effective, and eco-friendly resource for the green synthesis of silver nanoparticles. The synthesized nanoparticles exhibit promising antimicrobial properties and may find applications in biomedical fields such as wound healing materials, antimicrobial coatings, drug delivery systems, and infection-control products. However, further investigations involving toxicity studies, nanoparticle stability assessment, minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination, mechanism-of-action studies, and in vivo evaluations are required to establish their safety and efficacy for practical biomedical applications.

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## CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

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