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Green Synthesis and Comprehensive Characterization of Silver Nanoparticles Using *Lawsonia inermis* Extract for Biological Applications

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This research focuses on the production, characterization, and application of silver nanoparticles (AgNPs) for biomedical research. An eco-friendly extracellular biosynthetic method was employed, utilizing aqueous leaf extracts of *Lawsonia inermis* (Henna) as reducing agents. The synthesized

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AgNPs were characterized using UV-Vis spectroscopy, showing an absorption peak at 424 nm. FT-IR analysis was conducted to identify chemical groups present in nanoparticles. TEM and SEM analyses revealed that AgNPs were spherical and ranged in size from 10 to 50 nm. *Staphylococcus aureus*, *Klebsiella, Salmonella*, and *Escherichia coli* were inhibited by the green-synthesised AgNPs. *Penicillium, Aspergillus niger*, *Aspergillus flavus*, and *Fusarium* showed antifungal activity, forming zones of inhibition. Furthermore, the Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) values were found to be 75 mg/ml (Escherichia coli ATCC25922) and the MIC value was 50 mg/ml for the same strain. These nanoparticles demonstrated significant anti-inflammatory activity and dose-dependent cytotoxicity against A549 lung cancer cells. As a result of the synthesized nanoparticles, strong antioxidant activity was observed. The results suggest that plants can be effectively used as a resource for the production of silver nanoparticles. Such particles have potential applications in numerous fields such as biomedical research and nanotechnology.

Keywords: Herbal plants; silver nitrate; Ag-NPs; SEM;TEM; FTIR; antimicrobial activity; anticancer potential.

1. INTRODUCTION

Nanotechnology involves manipulating materials at the atomic level (1 to 100 nanometers) to harness unique properties, applicable across various domains. It integrates knowledge from physics, chemistry, biology, and technology, focusing on Nano scale objects [1]. Recent advancements in nanotechnology have spurred breakthroughs in medicine, particularly in cancer therapy, owing to the distinctive attributes of nanoparticles such as small size, controlled drug release, and minimized toxicity [2,3]. With sizes akin to biomolecules like proteins and DNA, nanoparticles offer immense potential for integration into biotechnology [4-8]. Their high surface area-to-weight ratio enhances reactivity with other molecules, driving their utility across diverse fields [9,10].

Nanoparticles are broadly categorized into organic and inorganic. Inorganic nanoparticles, favored for drug delivery due to their ease of use, functionality, biocompatibility, and targeted drug release, are increasingly prominent [11-15]. Green synthesis methods, including those employing microorganisms, plant extracts or enzymes, and templates like DNA, align with principles of eco-friendliness and offer advantages such as utilization of reducing and capping agents [16,17,18].

Among nanoparticles, silver nanoparticles (AgNPs) are extensively employed in various applications such as biological product development, cancer therapy, and water treatment [19]. Silver is preferred for its potent antibacterial, antifungal, and anti-inflammatory properties [20,21]. The synthesis of AgNPs emphasizes high yield and uniformity [22]. Green synthesis of AgNPs presents advantages over physicochemical methods, being cost-effective, eco-friendly, scalable, and devoid of high energy, temperature, and toxic chemicals [23].

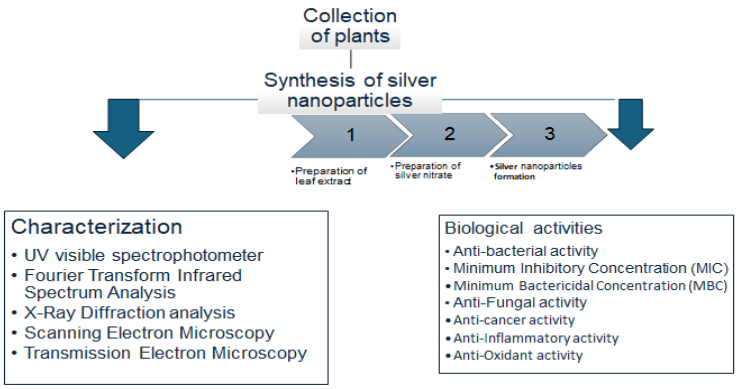


Fig. 1. Study objectives as depicted in the diagram

This study focuses on synthesizing AgNPs using *Lawsonia inermis* (Henna) plant, a biennial herb native to North Africa and southwest Asia, known for its ethnobotanical uses [24]. Henna contains lawsone, responsible for its orange-red dye, and is utilized in medicinal and cosmetic applications [25,26,27,28]. Our aim is to explore the biomedical applications of AgNPs synthesized from Lawsonia inermis.

1.1 Objectives

Lawsonia inermis extract is being used to synthesize AgNP in an eco-friendly and simple manner.. Characterization techniques include visual observation, UV-Vis spectroscopy, FTIR spectroscopy, XRD analysis, SEM, and TEM. Furthermore, antibacterial, antifungal, hydrogen peroxide scavenging, anti-inflammatory, and anticancer activities of the synthesized AgNPs will be evaluated (Figs. 12,13,14).

2. METHODOLOGY

2.1 Collection of Herbal Plant Sample

The leaves of *Lawsonia inermis* (Henna) plant (Fig. 2) were collected freshly from the home garden from Velagapuram, Chennai, Tamilnadu. The plant was identified as *Lawsonia inermis* (Henna) at the Department of Botany, Dwaraka Doss Goverdhan Doss Vaishnav college, Chennai 106.

Fig. 2. *Lawsonia inermis* (Henna)

2.2 Preparation of the Extract of *L. inermis*

Lawsonia inermis leaves were procured fresh and meticulously washed with distilled water to remove any impurities. 10 grams of washed and finely chopped leaves were placed in a 250 mL Erlenmeyer flask and 100 mL of sterile deionized water was added. After 10 minutes of gentle boiling at 60°C, the mixture was cooled. It was then cooled to room temperature and filtered using Whatman filter paper no. 1 to obtain a clear filtrated. Afterward, the filtrated was stored at 4°C for subsequent analyses [29].

2.3 Preparation of Silver Nitrate Solution ($AgNo_3$)

Silver nitrate (17 mg) of 99.9% purity was weighed and transferred to a 500 mL Erlenmeyer flask. Gently swirling distilled deionized water over the silver nitrate gradually dissolving the silver nitrate. Once the solid had completely dissolved, additional water was slowly added to bring the solution to the 100mL volume mark. [30] A solution of 1 mM silver nitrate was prepared and stored at 4°C in an amber-colored bottle.

2.4 Biogenic Synthesis of Silver Nanoparticles (Ag-NPs)

To initiate the synthesis process, 10 mL of the previously prepared aqueous plant extract was transferred into a 150 mL Erlenmeyer flask. Subsequently, 90 mL of a 1 mM silver nitrate ($AgNO_3$) solution was added to the flask, and the mixture was left at room temperature for the reduction process to occur. Throughout the reaction, the change in color was carefully monitored. To prevent photoactivation of $AgNO_3$, the entire process was conducted in darkness. The appearance of a brown coloration indicated the successful synthesis of silver nanoparticles [29].

2.5 Characterization of Synthesized AgNPs

In the following steps, the solution containing AgNPs was centrifuged for 10 minutes at 3000 rpm and the pellets were dried in an oven at 100°C for 24 hours. Following are the procedures used to characterize the purified AgNPs

- •Visual observation
- •UV-Visible spectral analysis
- •Fourier Transform Infrared spectrum analysis (FTIR)
- •X-Ray Diffraction analysis (XRD)
- •Scanning Electron Microscopy (SEM)
- •Transmission electron microscopy (TEM)

2.6 Visual Observation

The alteration in color within the reaction mixture was observed visually. The formation of silver nanoparticles in the solution containing 1 mM

$AgNO_3$ and the aqueous extract of *Lawsonia inermis* (Henna) plant sample was verified by the transition from a dark brown hue to a colloidal gray appearance.

2.7 UV-Visible Spectrophotometer

The optical characteristics of AgNPs and the formation of pure Ag+ ions were observed by analyzing the UV-Visible band of the reaction mixture. Samples were diluted with 4 mL of distilled water at regular intervals, and UV-Vis spectral analysis was conducted using a UV-Spectrophotometer (Systronics – 119) operating within a scanning range of 200-700 nm [29].

2.8 Fourier Transform Infrared (FTIR) Spectrum Analysis

To identify the biomolecules responsible for the reduction of Ag+ ions and the stabilization of AgNPs, FTIR spectral analyses were conducted using a Perkin Elmer Spectrum One instrument. In order to obtain quantitative information on the dried product sample, the sample was ground with KBr pellets and analyzed using FTIR spectroscopy, which operated at a resolution of 4 cm-1 within the range of 4500 to 400 cm-1 [31].

2.9 X-Ray Diffraction Analysis

A X-ray diffractometer (BRUKER AXS KAPPA APEX-II, Germany) was used to determine the crystallite size of the synthesized product. It operated at a voltage of 40 kV and a current of 30 mA, using CuKα radiation. Scans were conducted on a 2θ angle range of 10 to 80° at a scanning rate of 2° per minute.

2.10 SEM Analysis

Analyses were carried out using a Scanning Electron Microscope (SEM) machine manufactured by FEI, called the Quanta-200 MK II SEM. The sample was deposited in thin films onto copper grids coated with carbon. SEM grids were dried under a mercury lamp for five minutes after excess solution was removed with blotting paper.

2.11 TEM Analysis of Silver Nanoparticles

A JEOL JEM-1200EX electron microscope was used to perform the TEM observations. Deionized water is used to disperse the sample. The staining mat is coated with a thin drop of dispersion. With the carbon coated side facing upwards, the copper grid is inserted into the drop. The grid is removed after approximately ten minutes and air dried. UTHSCSA Image Tool version 3.00 was used to measure the particle size distributions [32].

3. BIOLOGICAL STUDIED OF SILVER NANOPARTICLES

3.1 Antibacterial Activity of Biologically Synthesized AgNPs

The antimicrobial properties of silver nanoparticles (AgNPs) synthesized using *L. inermis* was evaluated against several bacterial strains, including Staphylococcus aureus ATCC 25923, Klebsiella pneumoniae ATCC 700603, Salmonella typhi ATCC 13311, and Escherichia coli ATCC 25922, using the agar well diffusion method. Each bacterial strain was evenly spread onto separate plates of Muller Hinton agar using sterile cotton swabs. Wells of 4 mm diameter were created in the agar using a gel puncture tool. Subsequently, 20 µL of various substances, including plant extract, AgNO3, distilled water, AgNPs, and Ampicillin, were dispensed into each well. Following 24 hours of incubation, antimicrobial efficacy was measured by measuring the zones of inhibition. The experiments were conducted in triplicate to ensure reproducibility and reliability of the conclusions.

3.2 Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

It was determined that the crude leaf extracts displayed minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) at various concentrations (12.5 mg/ml, 25 mg/ml, 50 mg/ml, 75 mg/ml, and 100 mg/ml) against bacterial strains sensitive to the crude leaf extracts. This was accomplished through the broth dilution method followed by plating. Every test concentration was accompanied by a control tube. According to this definition, the minimum inhibitory concentration of the extract was determined by comparing it to the control tubes at which it inhibited visible bacterial growth. In order to determine the MBC of a test dilution, it was subcultured onto fresh drug-free solid medium, after which it was incubated for 18 to 24 hours. Identified as the MBC is the highest dilution on

agar medium that failed to yield individual bacterial colonies. These experiments were conducted meticulously to ensure accuracy and reliability, with controls incorporated for comparison.

3.3 Anti-Fungal Activity of Biologically Synthesized AgNPs

The same diffusion method used in agar wells was followed to study the antifungal activity of synthesized AgNPs. Sabouraud dextrose agar plates and fungi such as *Penicillium, Aspergillus niger, Aspergillus flavus, and Fusarium* were utilized for this study. Itraconazole was used as a control. An incubation period of 48-72 hours was followed by measurement of the zones of inhibition on the plates.

3.4 Anti-Cancer Activity of Biologically Synthesized AgNPs

As a part of the study, the A549 lung cancer fibroblast cell lines were cultured in 96-well microplates at a seeding density of 1 x 106 cells per well for 24 hours under 5% CO2 at 37 °C until 90% confluence was reached. After 24 hours, the medium was replaced and the cells were treated with a variety of silver nanoparticle concentrations (10, 25, 50, 75, and 100 l) and incubated again. A sugar-buffered saline solution (PBS, pH 7.4) was added to each well after the cells had been washed with phosphate-buffered saline (PBS, pH 7.4) and MTT solution (5 mg/mL) was added. A further four hours were spent at 37°C in the dark before the microplates were removed from the oven. A spectrophotometric detector at 570 nm was used to measure the absorbance of the formazan crystals after they had been dissolved in 100 mL of dimethyl sulfoxide (DMSO). Silver nanoparticles were assessed for their cytotoxic effects on A549 lung cancer cells by performing these procedures meticulously [33].

3.5 Antioxidant Activity of Biologically Synthesized AgNPs

Hydrogen peroxide scavenging capability of AgNPs (30% H2O2) was evaluated using a standard method). The following procedures were performed to prepare a solution of hydrogen peroxide (40 mM) in phosphate buffer saline (pH 7.4). Test samples of different concentrations (10, 20, 30, 40 and 50 g/ml) were prepared and 4 ml of each concentration were mixed with 0.6 ml of the previously prepared H2O2 solution after the concentration was determined. In order to determine the absorbance of the solution after 10 minutes, a blank solution containing phosphate buffer without hydrogen peroxide was compared to the solution. A UV-Vis spectrophotometer was used [34]. A formula is used to calculate the percentage of hydrogen peroxide scavenged by the test samples % scavenged $[H_2O_2]$ = 1- Abs (standard)/Abs (control) x100.

Where Abs control represents the absorbance of the control at 560nm (without extract); Abs sample represents the absorbance when extract is present at 560nm. The experiment was repeated in triplicate.

3.6 Anti-Inflammatory Activity of Biologically Synthesized AgNPs

There was no interruption of soap solution during any of the reactions carried out in clean test tubes. There were 5 mL of reaction mixture, consisting of 0.2 mL of egg albumin (from fresh hen's eggs), 2.8 mL of phosphate buffered saline (PBS, pH 6.8) and 0.2 to 2 mL of Biologically Synthesized Silver Nanoparticles in different concentrations, resulting in 0 to 10 mg/mL of final concentrations. Control samples were composed of a similar volume of double-distilled water. An incubator with a BOD was used to incubate these mixtures at 37°C for 30 minutes, followed by a heating cycle at 75°C for 10 minutes. The absorbance of the samples at 660 was measured using a vehicle as a blank after cooling. In order to test for absorbance, diclofenac sodium was used as a reference drug at a final concentration of ten milligrams per mL and treated similarly [35].

The percentage inhibition of protein denaturation was calculated by using the following formula:

% inhibition = 100 X (Vt / Vc - 1)

Where, Vt = absorbance of test sample,
Vc = absorbance of control.

A plot of percentage inhibition against test concentration was used to determine the extract/drug concentration for 50% inhibition (IC50).

4. RESULTS AND DISCUSSION

Nanotechnology is a multifaceted discipline encompassing the design, synthesis, characterization, and application of materials at the nanoscale, with wide-ranging implications across various scientific domains including chemistry, biology, physics, material science, and medicine.

Biological approaches have emerged as innovative methods for nanoparticle synthesis, facilitating the development of eco-friendly processes. Among these, plant extract-based biosynthesis has gained traction as a cost-effective and environmentally sustainable alternative to traditional chemical and physical methods. Plant extracts serve dual roles as both reducing and stabilizing agents in the synthesis process.

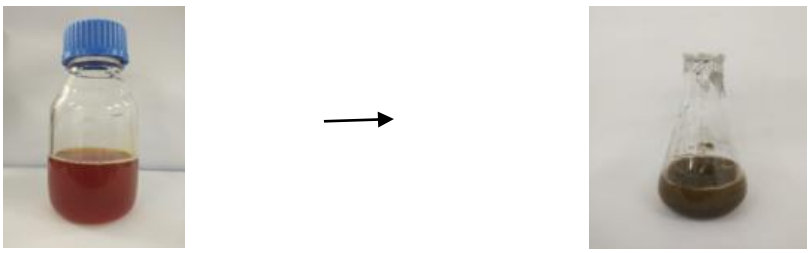
Silver nanoparticles and their assemblies have found diverse applications in fields such as sensing, peptide probing, antimicrobial and wound healing agents, optics, magnetism, electronics, and catalysis.

In this study, we utilized *Lawsonia inermis* plant extract for the synthesis of silver nanoparticles under ambient conditions, highlighting the potential of natural resources for nanomaterial production.

4.1 Visual Observation

A method for synthesizing silver nanoparticles by means of 1 mM $AgNO_3$ is shown in Fig. 3. The fresh suspension of leaves *Lawsonia inermis* of was dark brown in color. However, after addition of Ag $(NO_3)_2$ the suspension turned colloidal grey, roughly demonstrating that the formation of silver nanoparticles. It was confirmed by this color change that silver ions were reduced into Ag-NPs during the reduction process.

As a result of surface plasmon vibrations within the silver nanoparticles, silver nanoparticles exhibit a yellowish-brown color in aqueous solutions. Interestingly, when Gallic acid is employed as the reducing agent, silver nanoparticles assume a grayish color. Notably, gallic acid is a component found in Henna, while the primary constituent is lawsone. This observation suggests that both gallic acid and lawsone function as reducing agents in the synthesis process.



A B

Fig. 3. Color changes before (A) and after (B) the process of reduction of $AgNo_3$ to Ag NPs

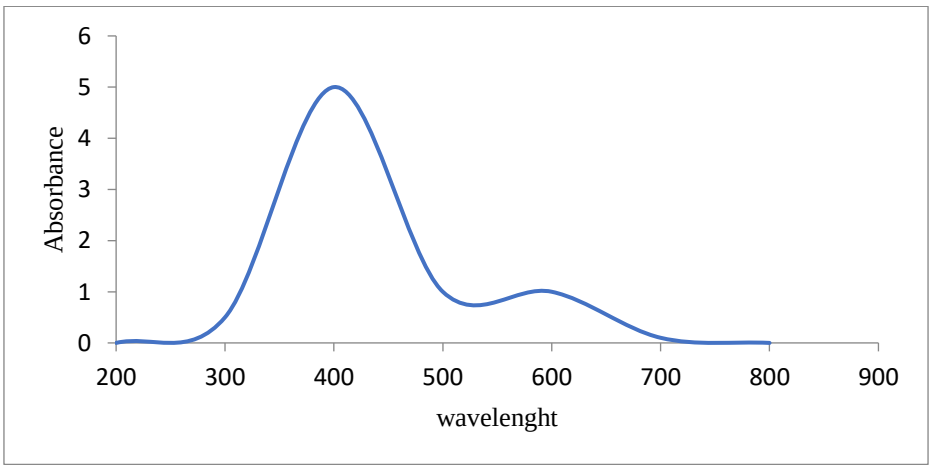


Fig. 4. UV–visible spectrum of biologically synthesized AgNPs using *Lawsonia inermis*

The reduction of silver ions is facilitated by various antioxidants and phytochemicals present in the plant extract. This phenomenon has been observed by multiple researchers, indicating the versatile nature of natural compounds in nanoparticle synthesis. [36,37].

4.2 Characterization of Synthesized AgNPs

Nanoparticle behavior, safety, biodistribution, and efficacy can be greatly influenced by their physico-chemical properties. Thus, it is necessary to characterize silver nanoparticles in order to evaluate their functional characteristics. Silver nanoparticles synthesized can be characterized using different methods [38]. In order to characterize the bio synthesized AgNPs in this study, UV-Visible spectral analysis, Fourier Transform Infrared spectrum analysis (FTIR), X-Ray Diffraction (XRD), Scanning Electron Microscopy (SEM) and Transmission electron microscopy (TEM).

4.3 UV-VIS Spectra Analysis

Aqueous extract of Lawsonia inermis was reduced with silver nitrate (AgNO3) in order to generate AgNPs and the results obtained have confirmed the formation of biological AgNPs in reaction mixtures. 420nm and 470nm were observed to be the broadest, strongest peaks in the UV Visible spectrum (Fig. 4). An absorption peak associated with silver nanoparticles' surface plasmon resonance (SPR) was observed at wavelength 424 nm, due to the dipole resonance of conducting electrons at the nanoparticle surface [38].

An effective method of characterizing nanomaterials is UV-Vis spectroscopy, which is relatively simple and inexpensive. By using this method, the light intensity reflected from a sample is measured and compared with the intensity reflected from a reference material. As UV-Vis spectroscopy is sensitive to the size, shape, concentration, agglomeration state, and refractive index near the surface of NPs, it is an important tool for identifying, characterizing, investigating, and evaluating the stability of NP colloidal solutions [39].

4.4 XRD Analysis

Silver nanoparticles were characterized using XRD based on their particle size and nature.

Based on the characteristic peaks in the XRD image for silver, the X-ray structural diffraction Angle of of the biologically synthesized AgNPs has been verified and validated. In order to determine the average grain size of the silver nanoparticles formed during bioreduction, Scherr's formula, d= (0.9λ × 180º) was used. An XRD pattern clearly illustrates the crystalline structure of silver nanoparticles

A number of techniques are available for analyzing nanoparticles, including X-ray diffraction (XRD). A crystal's structure, its chemical composition, its lattice parameters, and the size of its grains can be determined by XRD. In order to determine the particle composition, the position and intensity of the peaks can be compared to reference patterns available in the International Centre for Diffraction Data database (ICDD, previously known as Joint Committee on Powder Diffraction Standards, JCPDS) [39].

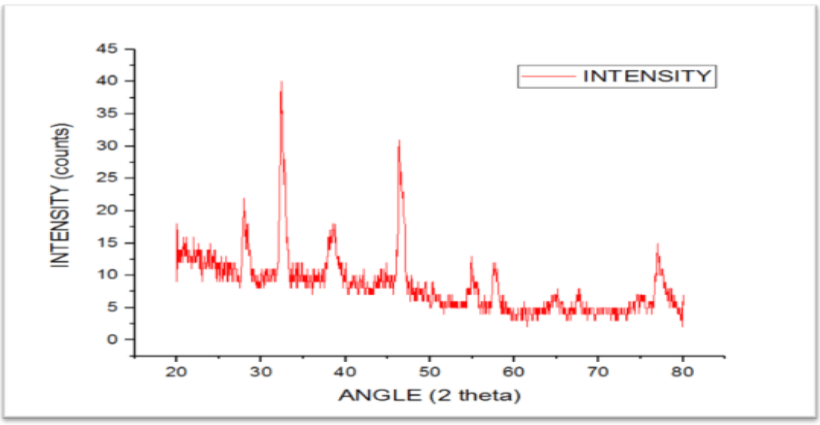


Fig. 5. XRD patterns of biologically synthesized AgNPs using *Lawsonia inermis*

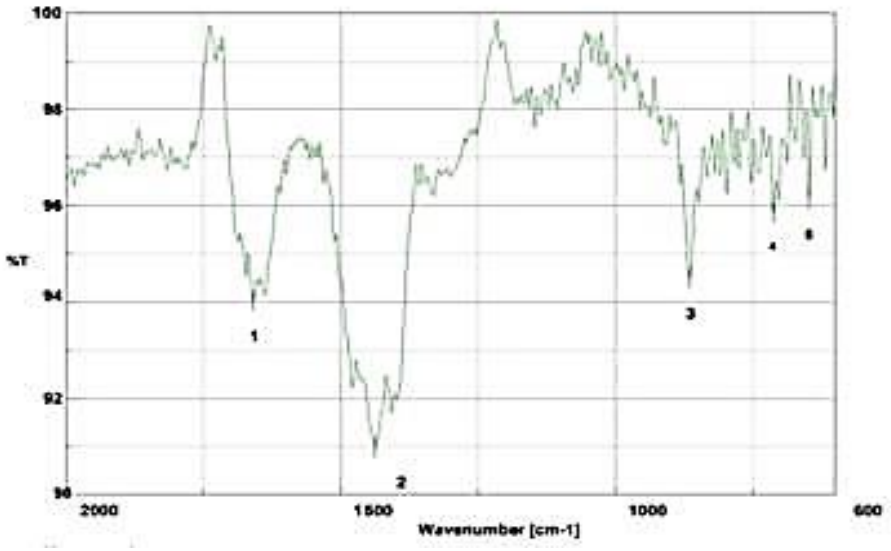


Fig. 6. FTIR Analysis of biologically synthesized silver nanoparticles

Table 1. FTIR Peak details

No	Position	Intensity
1	1659.45	94.0379
2	1437.67	90.9865
3	864.917	94.4949
4	712.569	95.8521
5	647.001	96.1199

4.5 FTIR Spectroscopy Analysis

Biomolecules responsible for reducing Ag+ ions and capping Ag-NPs were identified by FTIR measurements. Based on FTIR spectra (Fig. 6 & Table 1), Ag-NPs from Henna leaves extract were found to exhibit prominent bands at 1659.45, 1437.6,864.917,712.569, and 647.001 cm−1. The band at 1659 cm−1 may be a result of the stretching vibrations of C–N in the C–N of aliphatic and aromatic amines [40].

FTIR (Fourier transform infrared spectroscopy) is a technique for measuring electromagnetic radiation absorption at wavelengths within the mid-infrared region (4000–400 cm−1). Molecules that absorb IR radiation undergo dipole moment modifications in some way, causing them to become IR active. The position of bands on a spectrum can provide information regarding bond strength and nature as well as functional groups, providing information regarding the structure of molecules and their interrelationships

4.6 Scanning Electron Microscope Analysis

As illustrated in Fig. 7, SEM analysis was used to examine the morphological characteristics of silver nanoparticles synthesized. SEM results indicated that the majority of silver nanoparticles were spherical in shape.

In addition to enabling high-resolution imaging of surfaces, scanning electron microscopy is widely used for characterizing nanoscale materials. A SEM produces images by using electrons in the same way that a light microscope produces images by using visible light [39].

4.7 Transmission Electron Microscopy

To depict the shape, surface morphology and size of synthesized AgNPs, TEM was used. A TEM micrograph (Fig. 5) revealed polydispersed spherical nanoparticles ranging in size from 10-50nm.

TEM was used to determine the shape, surface morphology, and size of synthesized AgNPs. TEM micrograph (Fig. 5) revealed polydisperse spherical nanoparticles with sizes ranging from 10 to 50 nm.

In the field of nanoparticle size and shape analysis, transmission electron microscopy (TEM) has become the predominant technique due to its ability to provide Images of the sample

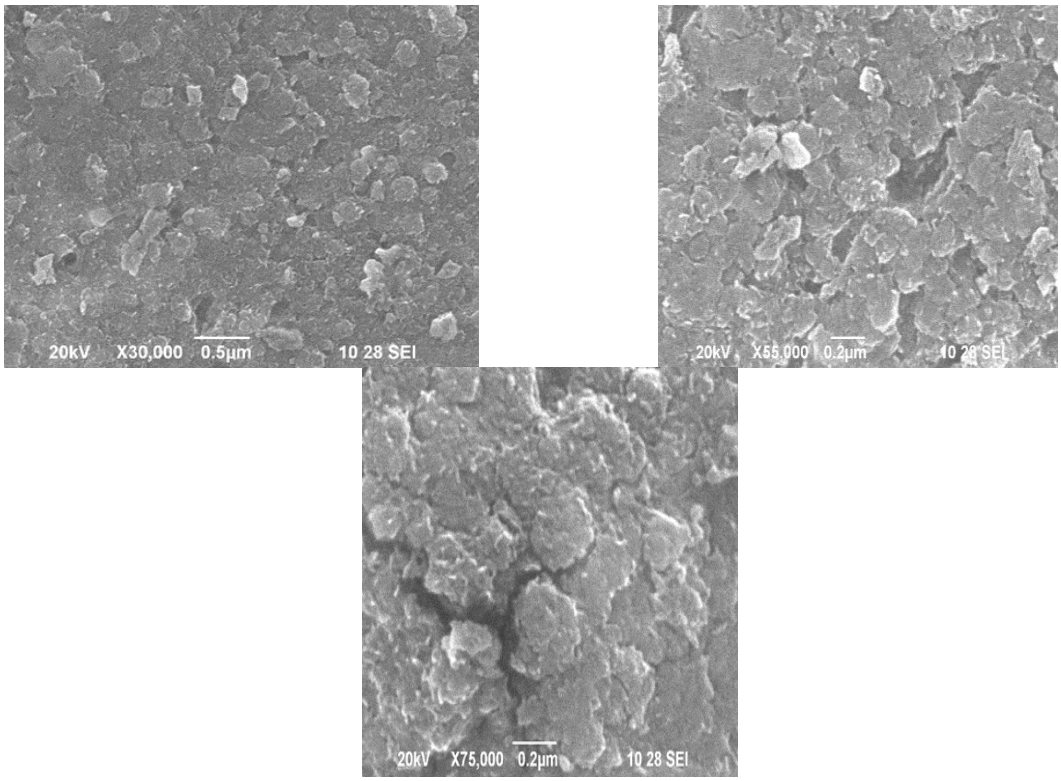


Fig. 7. SEM micrograph of biologically synthesized silver nanoparticles

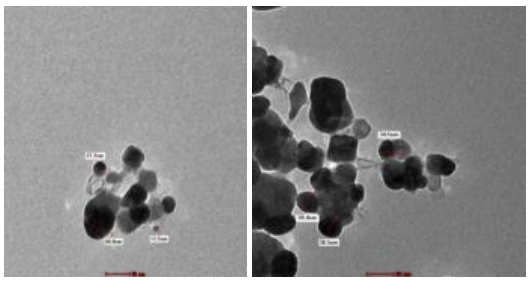


Fig. 8. TEM micrograph of biologically synthesized silver nanoparticles

in direct light, which allows the homogeneity estimation with the greatest accuracy of these nanoparticles. In spite of this, it is imperative to acknowledge that this method has certain inherent limitations. There are several challenges associated with measuring large numbers of particles, including the difficulty of quantifying them and the possibility of Orientation effects can lead to misleading images.

For analyzing extremely uniform samples, alternative techniques that analyze larger quantities of nanoparticles can yield more reliable results. For instance, small-angle X-ray scattering (SAXS) proves effective for larger and spherical nanoparticles, while X-ray diffraction (XRD) offers insights by leveraging the broadening of XRD reflections and applying the Scherrer formula. These complementary techniques enhance the comprehensive understanding of nanoparticle properties and behaviors [41,42].

5. BIOLOGICAL APPLICATIONS OF SYNTHESIZED SILVER NANOPARTICLES

5.1 Anti-Bacterial Activity

Assays were conducted to determine in vitro antibacterial activity of Ag-NPs derived from L. *inermis* leaves extract and the Ag-NPs and antibiotics were evaluated against bacterium *Staphylococcus aureus* ATCC 25923, *Klebsiella*

pneumoniae ATCC 700603, *Salmonella typhi* ATCC 13311 and *Escherichia coli* ATCC25922. At 37°C, the plates were incubated for 24 hours prior to observing the zone of inhibition (Fig. 6 and Table 2). As a result of the synthesized Ag-NPs, significant activity was demonstrated against all four test organisms. Unlike AgNO3 and aqueous plant extract, no significant activity was observed against these organisms using the synthesized Ag-NPs.

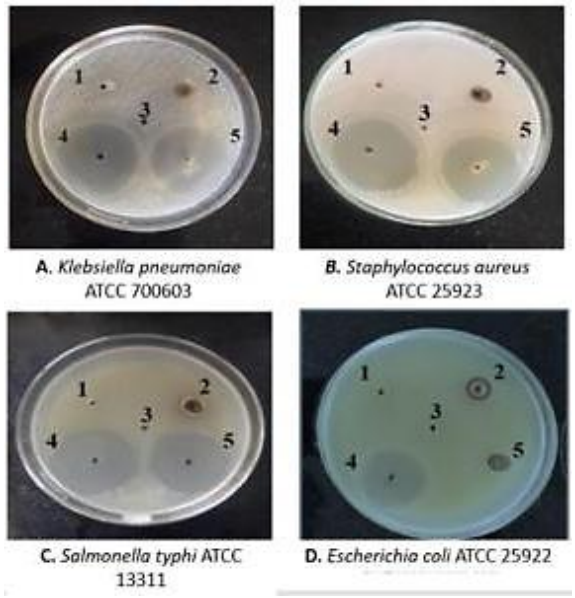


Fig. 9. Anti-bacterial activity of AgNPs against pathogen

(1. Plant extract; 2. $AgNO_3$; 3. Distilled water; 4. AgNPs [20µg]; 5.Ampicillin.)

It was found from the results (Table 2 & Fig. 6) that the synthesized AgNPs exhibited good antibacterial activity against all the tested pathogens. The larger zone of inhibition was 23 mm,20 mm 18 mm and 17 mm against *Klebsiella pneumoniae* ATCC 700603, *Salmonella typhi* ATCC 13311, *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC 25923 respectively. In testing against all pathogenic strains, the plant extract showed no activity. The standard drug, ampicillin with 1mg/mL was also tested along with AgNPs as positive control.

Alternative antibacterial agents such as nanoparticles demonstrate the ability to overcome the resistance of bacteria to antibiotics. Biosynthesized silver nanoparticles have been reported to have antimicrobial properties [43]. AgNPs have many advantages over conventional antimicrobial agents. In addition to their effectiveness against a wide range of microbes and parasites, they pose minimal toxicity to humans and can be used at very low concentrations [44]. Antibacterial activity of Ag-NPs has been attributed to several mechanisms in the literature. Several biomolecules, such as amino acids, proteins, carbohydrates, may be expelled by the disorganization of the cytoplasmic membrane after exposure to Ag-NPs [45]. Alternative antibacterial agents such as nanoparticles demonstrate the ability to overcome the resistance of bacteria to antibiotics. Biosynthesized silver nanoparticles have been reported to have antimicrobial properties [43]. AgNPs have many advantages over conventional antimicrobial agents. In addition to their effectiveness against a wide range of microbes and parasites, they pose minimal toxicity to humans and can be used at very low concentrations [44]. Antibacterial activity of Ag-NPs has been attributed to several mechanisms in the literature. Several biomolecules, such as amino acids, proteins, carbohydrates, may be expelled by the disorganization of the cytoplasmic membrane after exposure to Ag-NPs [45].

Table 2. Anti-bacterial activity of AgNPs against pathogen

Bacterial pathogen	Zone of inhibition in mm				
	Plant extract	$AgNO^3$	Distilled water	Ag-NPs	Standard
*Escherichia coli*ATCC25922	-	-	-	18mm	17mm
Klebsiella pneumoniae ATCC 700603	-	2mm	-	23mm	21mm
Staphylococcus aureus ATCC 25923	-	2mm	-	17mm	18mm
Salmonella typhi ATCC 13311	-	4mm	-	20mm	10mm

Probably the difference in the structure of cell walls between gram-negative and gram-positive bacteria determines the complex antibacterial activity against *Klebsiella pneumoniae* ATCC 700603, Salmonella typhi ATCC 13311, *Escherichia coli* ATCC25922 and *Staphylococcus aureus* ATCC 25923. [46,47].

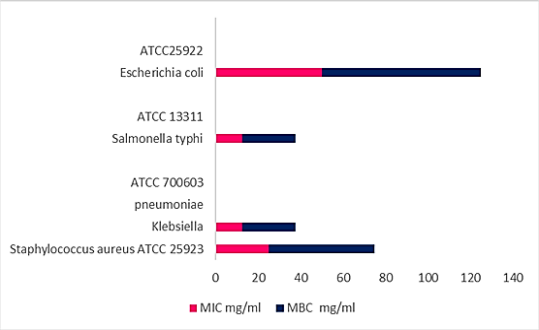


Fig. 10. MIC and MBC (mg/ml) values of AgNPs

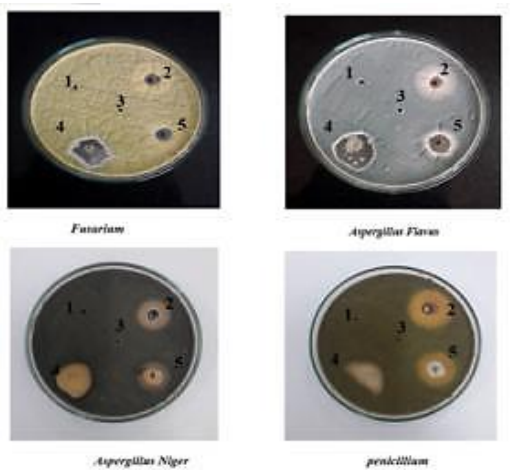


Fig. 11. Anti-fungal activity of AgNPs

1. Plant extract; 2. AgNO3; 3. Distilled water; 4. AgNPs [20µg]; 5. Itraconazole)

The results of this research could provide a novel platform for the development of new drugs to treat a variety of bacterial infections due to the noticeable antibacterial properties.

5.2 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC)

The MIC and MBC values (Fig. 10) were recorded for AgNPs against the susceptible bacteria. The MIC value ranged from the lowest of 12.5 mg/ml against *Klebsiella pneumoniae* ATCC 700603 and *Salmonella typhi* ATCC 13311 to highest of 50 mg/ml against *Escherichia coli* ATCC25922 .The array of MBC values found was 25mg/ml *(Klebsiella pneumoniae* ATCC 700603 and *Salmonella typhi* ATCC 13311) to 75 mg/ml (*Escherichia coli* ATCC25922).

5.3 Anti-Fungal Activity

Antifungal activity of Ag-NPs produced from L. inermis leaf extract. Ag-NPs and an antifungal antibiotic (itraconazole) were evaluated against *Penicillium*, *Aspergillus niger*, *Aspergillus flavus*, and *Fusarium*. An inhibition zone was observed after incubation at 25°C for 48-72 hours (Fig. 11). All four test organisms were significantly inhibited by synthesized Ag-NPs, while AgNO3 and aqueous plant extract were not significantly inhibited by synthesised Ag-NPs.

5.4 Anti-Cancer Activity of Biologically Synthesized AgNPs

Pharmacological trials require a careful examination of the cytotoxic effects of biological materials. Accordingly, we performed an MTT assay using the A549 lung cancer cell line in order to determine the cytotoxicity of AgNPs. Different concentrations of synthesized silver nanoparticles were tested for their cytotoxicity (10, 25, 50, 75 and 100 l). AgNPs were found to have a direct dose-response relationship in terms of both viability and cytotoxicity; cytotoxicity increased with increasing concentration (Fig. 12). The cytotoxicity of AgNPs was dose-dependent in A549 cells treated with AgNPs. Biosynthesized AgNPs inhibit 50% of A549 cell death at 50 g/mL in 24 h as determined by the inhibitory concentration (IC50). A549 cells were not significantly cytotoxic to biosynthesized AgNPs at lower concentrations, while A549 cells were significantly cytotoxic after increasing the concentration by 80 g/mL in 24 hours.

There is evidence that silver's cytotoxic effects are the result of dynamic physicochemical interactions between silver atoms and functional groups present in intracellular proteins. In addition, silver atoms interact with nitrogen bases and phosphate groups within DNA. [48]. Silver nanoparticles (AgNPs) also exhibit potential as antitumor agents due to their ability to prevent tumor development. AgNPs appear to induce cytotoxic effects on cells, inhibiting tumor progression without harming healthy cells.

Furthermore, research by Zolghadri and colleagues highlights that silver nanoparticles

induce a significant reduction in hydrophobicity within bovine hemoglobin, triggering a transition from alpha helices to beta sheets. This structural alteration leads to partial unfolding and aggregation of the protein, further elucidating the mechanistic underpinnings of silver nanoparticle-induced cytotoxicity. [49,50].

5.5 Anti-Inflammatory Activity of AgNPs

In this study, we investigated the in vitro anti-inflammatory effect of silver nanoparticles (AgNPs) against egg albumin denaturation. UV spectroscopic analysis indicated maximum absorption in the range of 220-240nm. The concentrations of AgNPs within the range of 4 mg/mL to 10 mg/mL of the mixture inhibited the denaturation of protein (albumin) in a concentration-dependent manner. A concentration-dependent inhibition of protein denaturation was observed using diclofenac sodium from 2 mg/mL to 10 mg/mL (Fig. 13). IC50 values for diclofenac sodium and AgNP extract confirmed that the impact of diclofenac sodium was greater. IC50 values for AgNPs were 6 mg/mL and for diclofenac sodium were 2 mg/mL.

The anti-inflammatory activity of AgNPs was evaluated in vitro through the use of a protein denaturation bioassay. During inflammation, proteins lose their secondary and tertiary structures as a result of exposure to chemicals or stress. The development of anti-inflammatory drugs requires the prevention of protein denaturation. This mechanism has been implicated in the stabilization of heat-treated albumin by certain non-steroidal anti-inflammatory drugs, providing evidence of its importance.

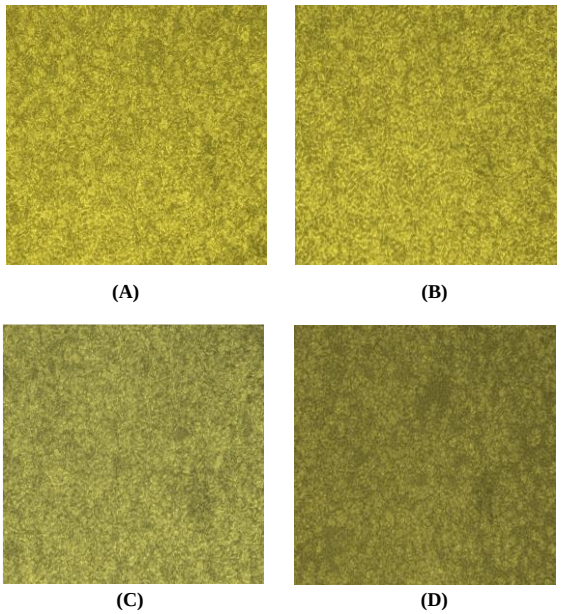


Fig. 12. Images of cytotoxicity effect of biologically synthesized silver nanoparticles against A549 Lung cancer cells: Silver nanoparticles at different concentration of such as (A).10, (B) 25, (C)50, (D)75 and (E) 100 µL and control

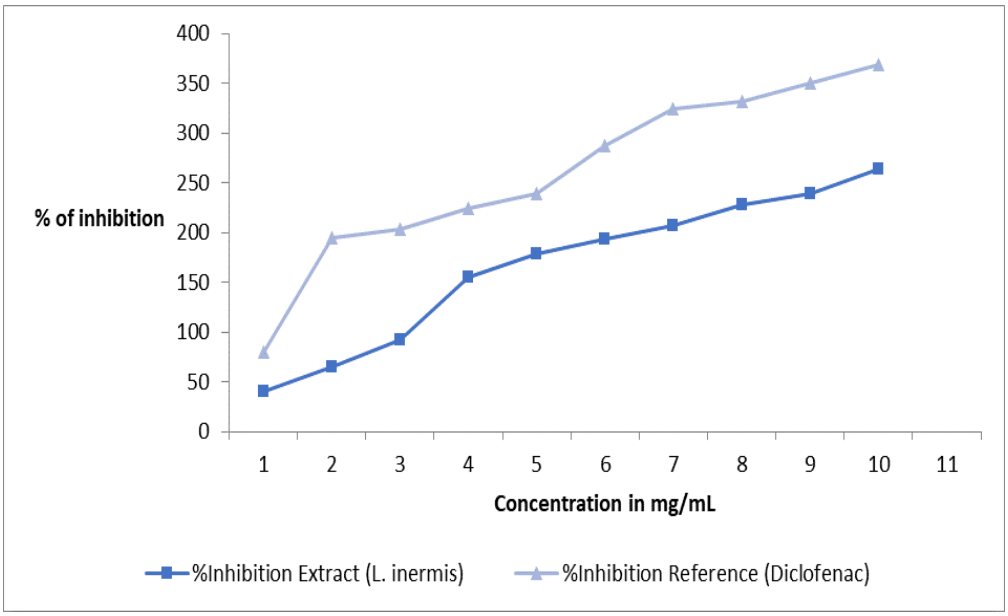


Fig. 13. *In vitro* anti-inflammatory effect of AgNPs

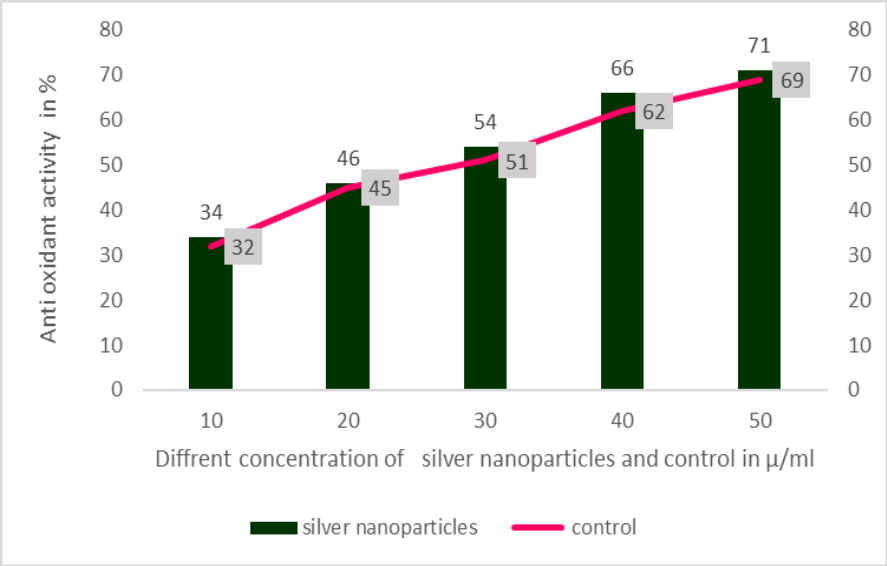


Fig. 14. Antioxidant activity of AgNPs

The results of our experiment clearly demonstrate that AgNPs exhibit anti-inflammatory effects against protein denaturation in vitro. For an understanding of how these compounds act as anti-inflammatory agents, further comprehensive research is necessary.

AgNPs were evaluated in vitro using a protein denaturation bioassay. When proteins are denaturated, they lose their tertiary structure and secondary structure due to excessive external stresses, such as strong acids or bases, concentrated inorganic salts, organic solvents, or heat. A denatured protein loses most of its biological function. A well-documented cause of inflammation is the denaturation of tissue proteins [51,52]. An anti-inflammatory drug development strategy should include agents that prevent protein denaturation.

Nonsteroidal anti-inflammatory drugs have been reported to be capable of stabilizing heat-treated albumin (preventing denaturation) [53]. Consequently, it is evident from the results of the current experiment that AgNPs are able to inhibit the denaturation of protein in vitro in an effective manner. For a full understanding of its anti-inflammatory properties, further research is required.

5.6 Antioxidant Activity of AgNPs

The experimental results of antioxidant activity of AgNPs were given in Fig. 14. Test samples are measured for their ability to scavenge hydrogen peroxide according to the formula below [54].

$$\%\text{Scavenged} = \frac{\text{abs of the control} - \text{abs of the sample}}{\text{abs of the control}} x\ 100$$

At a concentration of 50 grams/ml, silver nanoparticles (AgNPs) showed the highest level of hydrogen peroxide scavenging activity, reaching 71%, while at a concentration of 10 grams/ml there was the lowest level, 34%. The radical scavenging activity of the test samples increased with increasing concentrations. This enhanced radical scavenging activity is attributed to the strong oxidant capacity, electron-donating properties, and presence of capping agents on the surface of AgNPs. [55].

Hydrogen peroxide, though not highly reactive itself, acts as a weak oxidizing agent biologically. In the presence of metal ions in living systems, it can transform into the highly reactive hydroxyl radical, thereby initiating and propagating lipid peroxidation, contributing to cellular toxicity. This underscores the significance of AgNPs in mitigating oxidative stress by effectively scavenging hydrogen peroxide radicals. [56].

6. CONCLUSION

In conclusion, this study presents a straightforward, cost-effective, and efficient method for synthesizing silver nanoparticles using the *L. inermis* (Henna) plant, employing a

green chemistry approach. The eco-friendly synthesis route proved to be simpler compared to conventional physicochemical methods. Validation through visual observation and characterization techniques including UV-Visible spectrophotometry, characterization of silver nanoparticles provided clear evidence that AgNP is synthesized.

The synthesized AgNPs exhibited significant antibacterial properties against a variety of pathogens including *Staphylococcus aureus, Klebsiella, Salmonella*, and *Escherichia coli.* Additionally, the AgNPs demonstrated noteworthy in vitro antioxidant activity, highlighting their potential as effective antioxidants. Furthermore, the study revealed the anti-inflammatory properties of AgNPs, evidenced by their ability to inhibit protein denaturation.

Furthermore, the investigation explored the potential therapeutic utility of green-synthesised AgNPs against tumor cell lines A549 by using the MTT assay, indicating that the nanoparticles may be used for the treatment of lung cancer in the future. Further research is required to understand the mechanisms by which AgNPs operate as antibacterial, antifungals, anti-inflammatory agents, and anticancer agents, as well as to evaluate their potential toxicities.

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 writing or editing of manuscripts.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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