

## Green Synthesis of Silver Nanoparticles

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### Abstract

Over the years, there have been numerous attempts to develop new green synthesis technologies. Precious metals like copper, zinc, titanium, magnesium, silver, gold, and platinum are used to make nanoparticles. They have drawn a lot of interest due to their versatility as theragnostic agents. Significant antibacterial efficacy against *Escherichia coli* and *Staphylococcus aureus*, as well as antifungal activity against *Trichosporon seinieli* and *Candida albicans*, has been demonstrated by silver nanoparticles (Ag NP). AgNPs function in both drug delivery and successfully inducing the death of cancer cells. With the addition of Enterobacteriaceae cell filtrate (*Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*) to AgNO<sub>3</sub> solution, the silver ions are rapidly reduced within 5 minutes. The Temperature, pH, and AgNO<sub>3</sub> concentration all influence the size of AgNPs generated using *Escherichia coli*, *Klebsiella pneumoniae* and *Enterobacter cloacae*, all of which create AgNPs. Since pH is essential for the effective synthesis of nanoparticles, this element increases the reactivity of plant extract with silver ions. Silver nanoparticle dilutions of 10,20,40,80,160 g/ml were prepared and used in this analysis. 200µl of bacterial suspension was inoculated into each test tube containing varying amounts of AgNPs and a comparable volume of Muller Hinton Broth (MHB) and incubated for 24 hours at 37°C.

**Keywords:** Green Synthesis; Fourier-transform infrared spectroscopy; Transmission electron microscopy; Silver nanoparticle

### Introduction

Nanotechnology has become one of the largest and most active areas of research, offering unique properties and broad applications in various fields such as agriculture, food, and biomedicine. Compared to larger particles in bulk solids, nanoparticles have completely new or improved properties, and these new properties are based on variations in specific properties such as particle size, morphology and distribution. Nanoparticles are made of precious metals such as silver, gold, platinum, copper, zinc, titanium, and magnesium. They have received considerable attention because of their multifunctional theranostic ability. Nanoparticles have various structures with shapes such as rods, spheres, tubes, hollow spheres, and blood platelets. There were different types of nanoparticles like semiconductors, core-shell particles, polymers, metal and metal oxides. These types of metal nanoparticles have exceptionally high physical and chemical properties. AgNPs have the properties like large surface area to volume ratio (silver is a powerful bactericidal metal as it is non-toxic to animal cells and highly toxic to bacteria, have antioxidant and antimicrobial properties and AgNPs are used in coatings or inlays for medical purposes. In addition to their medical use, AgNPs are also used in clothing, Paints, Food, Electronics, and Other Areas AgNPs are used worldwide to manufacture a wide range of products including aerosols, water filters, water treatment, detergents, refrigerators, paints, cosmetics, washing machines and electronic products, because of its high antimicrobial properties [1-6].

In recent years the increase in antibiotic resistance of microbes posed a serious threat to the health sector. Nanoparticles are a promising antibacterial agent candidate due to their small size and high surface to volume ratio, which assures a broad assault area on the bacterial surface. Silver functions as a highly efficient antibacterial. Several tests were carried out on silver nanoparticles to investigate their antimicrobial activity. It showed significant antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and antifungal activity against *Trichosporon seinieli* and *Candida albicans*. AgNPs are one

of the most important Materials in nanomedicine. In the treatment of bacterial skin infections, silver nanoparticles (Ag NP) have been utilized as an antibacterial agent for topical administration. In other situations, Ag NPs have drawn a lot of attention due to their possible application in cancer treatment. AgNPs successfully trigger cancer cell death and also have a role in medication delivery. The development of new chemical and physical methods has led to environmental pollution as the chemical processes used to synthesize the materials generate a large no. of hazardous by-products. Whereas the green synthesis method does not require the use of high energy, pressure or temperature and toxic chemicals for the production.

The Biological methods include the synthesis of nanomaterials from extracts of plants, bacteria, fungi, etc. Plant extracts comprising leaves, fruit, bark, roots, flowers, rhizoids and latex, are utilized for nanoparticles synthesis. These nanoparticles have different morphological features including size, shape, and dispersion that are more efficient than those synthesized by chemical or physical processes. Hence, using green plants for nanoparticle biosynthesis process is an exciting method that is compatible with pharmaceutical and biomedical applications as no toxic chemicals are used for nanoparticle synthesis. When the nanoparticles are produced with an extract method, the extract is added at room temperature to a metal salt solution and the reaction is finished within a few minutes. With this process, nanoparticles were produced from silver, gold, and other

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metals in the amount of silver and the released silver. Silver is inactive in metallic form, but reacts with moisture in the skin and wound fluids and ionizes. Silver ions are highly reactive and stick to tissue proteins this leads to structural changes in the bacterial cell wall and the cell nuclear membrane and ultimately to deformations and Cell death. The use of plants to synthesize nanoparticles prevents the release of large amounts of toxic chemicals in solid, liquid, and gaseous forms into the environment and also eliminates the adhesion of toxic substances to synthesized nanoparticles [7-9].

The size, stability, morphology as well as physical and chemical properties of nanoparticles, plays an important role in their applications. Tools are needed to control the size and shape of metallic nanostructures and improve their specific uses, much like metals developed for macroscopic devices. The size and shape of the nanoparticles can be controlled by maintaining several reaction parameters such as temperature, Ph, the concentration of the metal solution, and concentration of reducing agents, the particles obtained were analyzed and characterized by UV-Vis spectroscopy, Scanning electron microscope (SEM) and Transmission electron Microscope (TEM), X-ray diffraction (XRD) and Fourier transform spectroscopy (FTIR).The antibacterial activity of AgNPs was investigated by performing Agar well diffusion test and MIC.

## Green synthesis

### Using Bacteria:

Bacteria are one of the best bioagent for nanoparticle synthesis due to their remarkable ability to reduce heavy metal ions. Bacteria are known to produce inorganic materials either intracellularly or

extracellularly. As a result, they could be used as biofactories to produce gold and silver nanoparticles. Shahverdi et al. demonstrated fast production of Ag NPs (within 5 minutes) using culture supernatants of K. pneumonia, E. coli, and Enterobacter cloacae. Saravanan et al. have reported an extracellular production of Ag NPs using B. megaterium culture supernatant in the presence of Aq solutions of silver ions in minutes. With the addition of Enterobacteriaceae cell filtrate (Escherichia coli, Klebsiella pneumonia, and Enterobacter cloacae) to AgNO<sub>3</sub> solution, the silver ions are rapidly reduced within 5 minutes. Saifuddin et al. used a combination of B. subtilis culture supernatant and microwave irradiation in water to show extracellular production of Ag NPs (5–50 nm). Bacillus flexus formed anisotropic nanoparticles with spherical (12 nm) and triangular (61 nm) dimensions. For the manufacture of AgNPs utilizing Bacillus cereus, an incubation time of 3–5 days at room temperature is required. The interaction of silver ions with bacteria influences the size and structure of AgNPs generated by microorganisms.

The Temperature, pH, and AgNO<sub>3</sub> concentration all influence the size of AgNPs generated using Escherichia coli, Klebsiella pneumoniae and Enterobacter cloacae, all of which create AgNPs. Green synthesis based on bacteria is adaptable, affordable, and suited for large-scale manufacturing. The biggest disadvantage of utilizing bacteria as nano factories is the sluggish synthesis rate and the restricted variety of shapes and sizes accessible when compared to standard chemical synthesis methods. As a result, fungi-based nano factories and chemical reactions involving plant-based materials have been studied [10-14] (Table 1).

**Table1:** Bacteria based synthesis of AgNPs.

s.no	Article/Author	Particle Characterization	Operating Conditions	Particle Characterization	Particle Characteristics
1	Synthesis of Gold and Silver Nanoparticles Using Purified URAK. Colloids and Surfaces B: Bio-interfaces.	Fibrinolytic URAK enzyme produced by Bacillus cereus NK1	1 mM, 24 hr without NaOH and 5 min with NaOH, 37°C.	TEM XRD UV-Vis	Size- 50 - 80 nm Shape--spherical. Structure-FCC
2	Extracellular Biosynthesis of Silver Nanoparticles Using Cell Filtrate of Streptomyces sp. ERI-3.	Aqueous cell filtrate of Streptomyces sp. ERI-3	1 mM, 28°C, 48 hr., Dark. Shaken	UV-Vis TEM SEM XRD	10 - 100 nm Spherical
3	The Antibacterial and Anti-Biofouling Performance of Biogenic Silver Nanoparticles by Lactobacillus fermentum.	Lactobacillus fermentum.L	10 g/L, 24 hr, 30°C, 10 g/L, Shaken, 6 min at 5000 rpm and 10 min at 6000 rpm	UV-Vis TEM XRD	6 nm spherical FCC
4	Biogenic Synthesis of Antimicrobial Silver Nanoparticles Caped with L-Cystine.	Reducing Agent	1 or 5mM, 30°C, 24 hr, Ratio of AgNO <sub>3</sub> : L-cysteine = 1:5, Shaken, 10 min at 1851 g	UV-Vis TEM FTIR	Size--5 nm
5	Biosynthesis, Purification and Characterization of Silver Nanoparticles Using Escherichia coli.	E. coli supernatant	1 - 10mM, , 20°C - 90°C, 24 hr, pH: 5 - 12, 10 min at 10000 rpm Stati	UV-Vis TEM FTIR DLS	10 - 90 nm Spherical Crystalline
6	Rapid Synthesis of Silver Nanoparticles Using Cultural Supernatants of Enterobacteria: A Novel Biological Approach.	K. pneumonia (Enterobacteria)	1mM, 5 min, Room temperature.	UV-Vis EDS TEM	Average: 52.25 nm Spherical.
7	Biosynthesis of Silver and Gold Nanoparticles Using Brevibacterium casei.	Brevibacterium casei	1mM, 24 hr, 37°C, 1 g, Shaken, 30 min at 16000 g	UV-Vis XRD FTIR TEM	10 - 50 nm. Spherical. FCC
8	Biosynthesis of Silver Nanoparticles from Staphylococcus aureus and Its Antimicrobial Activity against MRSA and MRSE.	Supernatant of Staphylococcus aureus	1 mM AgNO <sub>3</sub> , 5 min	AFM UV-Vis	160 - 180 nm Nature-PD
9	Production and Structural Characterization of Crystalline Silver Nanoparticles from Bacillus cereus Isolate.	Bacillus cereus PGN1 cells	1 mM, 10 g/100 ml, 12hr, 37°C, 15 min at 15000 rpm. Shaken	UV-Vis FTIR TEM XRD	4-5 nm Spherical FCC. Nature-MD.
10	Intensified Biosynthesis of Silver Nanoparticles Using a Native Extremophilic Ureibacillus thermosphaerius Strain.	supernatant of Ureibacillus thermo sphaerius	1 - 100 mM, 24 hr, 60°C - 80°C, Dark, 15 min, 13000 rpm Static	UV-Vis DLS XRD FTIR	10 - 100 nm Spherical FCC
11	Biosynthesis of Silver Nanocrystals by Bacillus licheniformis.	Bacillus icheniormis cells	1 mM, 24 hr, 37°C, 30 min at 15000 rpm. Shaken	UV-Vis SEM EDX XRD	50 nm Crystalline

12	Green synthesis of silver nanoparticles using <i>Rhodobacter sphaeroides</i> .	<i>Rhodobacter sphaeroides</i>	1mM, 5g, 30°C, 72hr, 10min at 4000g	UV-vis XRD TEM and HRTEM	3–15nm Spherical crystalline
13	Intracellular and extracellular biosynthesis of silver nanoparticles by using marine bacteria <i>Vibrio alginolyticus</i> .	<i>Vibrio alginolyticus</i>	1M, 24-48hr, 37°C, 7500rpm for 15min	UV-Vis SEM EDX	50–100 nm; Spherical
14	Intra/extracellular biosynthesis of silver nanoparticles by an autochthonous strain of <i>Proteus mirabilis</i> isolated from photographic waste.	<i>Proteus mirabilis</i>	1mM, 24hr, 37°C, 7000rpm for 30 min	UV-Visible Spectroscopy, TEM and EDS	10–20 nm; spherical
15	Synthesis and characterization of bactericidal silver nanoparticles using cultural filtrate of simulated microgravity grown <i>Klebsiella pneumoniae</i> .	<i>Klebsiella pneumoniae</i>	1mM, 72hr, 35°C, 10000rpm for 30 min	UV-Vis FTIR TEM	15–37 nm; spherical
16	In vitro antiplatelet activity of silver nanoparticles synthesized using the microorganism <i>Gluconobacter roseus</i> : an AFM-based study.	<i>Gluconobacter roseus</i>	1mM, 24hr, 37°C, 7000rpm for 12hr	UV-Vis TEM EDS FTIR	10 nm
17	Synthesis, optimization, and characterization of silver nanoparticles from <i>Acinetobacter calcoaceticus</i> and their enhanced antibacterial activity when combined with antibiotics.	<i>Acinetobacter calcoaceticus</i>	1mM, 72hr, 40°C, 6000rpm for 10min	UV-Vis XRD TEM SEM	8–12 nm; spherical
18	Biosynthesis of silver nanoparticles using <i>Bacillus thuringiensis</i> against dengue vector, <i>Aedes aegypti</i> (Diptera: Culicidae).	<i>B. thuringiensis</i>	1mM, 72hr, 37°C, 5000rpm for 10min	UV-Vis SEM EDX XRD	43.52–142.97 nm
19	Mechanistic antimicrobial approach of extracellularly synthesized silver nanoparticles against gram positive and gram negative bacteria.	<i>Exiguobacterium</i> sp.	1mM, 24hr, 30°C, 8000g for 10min	UV-Vis FTIR TEM XPS	5–50 nm; spherical
20	Green synthesis of silver nanoparticles using keratinase obtained from a strain of <i>Bacillus safensis</i> LAU 13	<i>B. safensis</i> LAU 13	1mM, 2hr, 30°C, 10000rpm for 20 min	UV-Vis FTIR TEM XRD	5–30 nm; spherical
21	Microbial synthesis of silver nanoparticles by <i>Bacillus</i> sp.	<i>Bacillus</i> sp.	3.5mM, 7days, 27°C, 10,000rpm for 10min	TEM EDX	5–15 nm
22	Synthesis of anisotropic silver nanoparticles using novel strain, <i>Bacillus fexus</i> and its biomedical application.	<i>B. fexus</i>	1mM, 24hr, 30°C	UV-Vis FTIR AFM XRD EDAX	12 & 65 nm; spherical and triangular
23	Biosynthesis of silver nanoparticles using a probiotic <i>Bacillus licheniformis</i> Dahb1 and their antibiofilm activity and toxicity effects in <i>Ceriodaphnia cornuta</i> .	<i>B. licheniformis</i> Dahb1	1mM, 24hr, 37°C, 5000g for 10 min	UV-Vis TEM XRD	18.69–63.42 nm Spherical.
24	Biosynthesis of silver and gold nanoparticles using thermophilic bacterium <i>Geobacillus stearothermophilus</i> .	<i>Geobacillus stearothermophilus</i>	0.01M, 48hr, 27°C, 5000rpm for 10 min	UV-Vis TEM XRD	5–35 nm; spherical
25	comparative study of morphology, reactivity and stability of synthesized silver nanoparticles using <i>Bacillus subtilis</i> and <i>Catharanthus roseus</i> .	<i>B. subtilis</i>	1mM, 24hr, 27°C	UV-Vis XRD EDAX	Triangular, hexagonal

### Using Fungi:

Fungi, like bacteria, have been studied in the biological creation of metallic nanoparticles because of their high binding capacity and metal bioaccumulation ability, high tolerance and intracellular absorption. The switch from bacteria to fungus as a technique of generating natural nano factories has the advantage of simplified biomass handling and downstream processing. Fungus is known to release far greater amounts of proteins than bacteria, which increases the productivity of this biosynthetic technique; also, fungi might be utilized to produce enormous numbers of metal nanoparticles. Polydispersed spherical AgNPs with sizes ranging from 17-33 nm were produced with *Helminthosporium tetramera* cell-free filtrate and had considerable antibacterial activity.

Fungi are easier to work within a laboratory setting than bacteria. Fungus employs a distinct process to produce nanoparticles; fungi release enormous amounts of enzymes that are utilized to decrease Ag ions that stimulate the synthesis of metal nanoparticles. *Bipolaris nodulosa* was used to create silver nanoparticles with spherical, semi

pentagonal, and hexahedral structures (10–60 nm). Silver ions are reduced by the enzymes present on the surface of *Verticillium*, and cells were observed to grow even after the formation of AgNPs.

Thus, the biomimetic conduit towards plant species has been established using the microbially aided syntheses of AgNPs. Enzymes found in microbes are responsible for the reduction of silver ions, which results in the formation of AgNPs. Higher amounts of silver ions are toxic to these species. As a result, when employed in biomedical applications, nanosilver generated by microorganisms presents several challenges (Table 2).

### Using Plants:

Plant components such as leaves, stems, roots, shoots, flowers, barks, seeds, and their metabolites have been effectively employed for the efficient production of nanoparticles. The Protocol for nanoparticle synthesis includes that the section of the plant of interest is collected at the accessible places and rinsed in tap water twice/three, completely, to eliminate the two epiphytes and the necrotic plants. These are clean and fresh sources, then pulverized by the household blender for 10-15 days

**Table2:** Fungi based synthesis of AgNPs.

s.no	Article/Author	Reducing Agent	Particle Characterization	Particle Characterization	Particle Characterization
1	Biosynthesis of Silver Nanoparticles Using Aqueous Extract from the Compactin Producing Fungal Strain.	Penicillium brevicompatum	1 mM, 25°C, 72 hr Shake	UV-Vis TEM XRD FTIR	Size—17.8 nm Structure—FCC
2	Silver-NanoBiohybrid Material: Synthesis, Characterization and Application in Water Purification.	Mycelia of Rhizopus oryzae	1 to 5 mM, 72 hr, 30°C, 0.2 g/25 ml. pH—2 to 8, Shaken	UV-Vis FTIR HRTEM EDAX	15 nm spherical FCC
3	Extracellular Biosynthesis of Silver Nanoparticles Using the Filamentous Fungus Penicillium sp.	Aqueous cell filtrate of Penicillium Sp. fungi	1 mM, 24 hr, room temp, dark 50 ml/50 ml, Agitated, Lyophilized	UV-Vis FTIR AFM	52 -104 nm
4	Extracellular Biosynthesis of Functionalized Silver Nanoparticles by Strains of Cladosporium cladosporioides Fungus.	Cladosporium clado sporioides	10 ml, 27°C, 78 h Shaken	UV-Vis TEM XRD FTIR	Average: 35 nm Spherical FCC
5	Fusarium solani: A Novel Biological Agent for the Extracellular Synthesis of Silver Nanoparticles.	Fusarium solani	1mM, Static, 10 min, 10,000 g, room temperature.	UV-Vis TEM FTIR	5 - 35 nm Spherical
6	Studies on Silver Nanoparticles Synthesized by Marine Fungus, Penicillium fellutanum Isolated from Coastal Mangrove Sediment.	Penicillium fellutanum	0.5 - 2.5 mM, 48 hr, 40°C, dark, pH: 7.5. Shaken.	TEM UV-Vis	5 - 2.5 nm Spherical
7	Extracellular Biosynthesis of Silver Nanoparticles Using the Fungus Fusarium semitectum.	Fusarium semitectum	1 mM AgNO <sub>3</sub> , 27°C, 48 hr Shaken.	UV-Vis FTIR XRD TEM	10 - 60 nm Spherical crystalline
8	Fungal Based Synthesis of Silver Nanoparticles—An Effect of Temperature on the Size of Particles.	Trichoderma viride	1 mM AgNO <sub>3</sub> , 40°C, dark, Shaken.	UV-Vis TEM FTIR XRD	80 - 100 nm Plate like Crystalline
9	Green synthesis of protein capped silver nanoparticles from phytopathogenic fungus Macrophomina phaseolina (Tassi) Goid with antimicrobial properties against multidrug-resistant bacteria.	Macrophomina phaseolina	1mM AgNO <sub>3</sub> , 28°C, dark, 72hr	UV-Vis XRD TEM SEM AFM	5–40 nm; spherical
10	Biomimetic synthesis and characterisation of protein capped silver nanoparticles.	Cladosporium cladosporioides	1mM AgNO <sub>3</sub> , 37°C, dark shaken	UV-Vis XRD TEM AFM	10–100 nm
11	Biological synthesis of silver nanoparticles using the fungus Aspergillus favus.	P. sajor-caju	0.1-10mM AgNO <sub>3</sub> , 37°C, dark, 10 days	UV-Vis XRD TEM FTIR	30.5 ± 4.0 nm spherical
12	Biosynthesis of silver nanoparticles by fungus Trichoderma reesei (a route for large-scale production of AgNPs).	T. reesei	1mM AgNO <sub>3</sub> , 28°C, dark, 120hr	UV-Vis TEM FTIR	5–50 nm
13	Tailoring shape and size of biogenic silver nanoparticles to enhance antimicrobial efficacy against MDR bacteria.	T. viride	1mM AgNO <sub>3</sub> , 30°C-40°C, 48hr-72hr	UV-Vis DLS TEM FTIR	2–5 nm; spherical 40–65 nm; rectangular
14	Green synthesis and characterization of silver nanoparticles using ascomycota fungi Penicillium nalgiovense AJ12.	P. nalgiovense AJ12	1mM AgNO <sub>3</sub> , 25°C, dark, shaken	UV-Vis DLS TEM FTIR	25 ± 2.8 nm; spherical
15	Green synthesis of highly stabilized nanocrystalline silver particles by a non-pathogenic and agriculturally important fungus T. asperellum.	Trichoderma asperellum	1mM AgNO <sub>3</sub> , 25°C, dark, shaken	UV-Vis XRD FTIR	13–18 nm; nanocrystalline
16	green synthesis of silver nanoparticles using Aspergillus terreus.	A. terreus	1mM AgNO <sub>3</sub> , 27°C, 24hr, shaken	UV-Vis XRD TEM	1–20 nm; spherical

and shaded. About 10 g dry powder is cooked in 100 mL of deionized distilled water for plant broth production. The resultant infusion is next carefully filtered until there is no precipitate on the broth. Following the addition of a few ml of plant extract to a 10 M AgNO<sub>3</sub> solution, the reduction of pure Ag(I) ions to Ag (0) may be observed at regular intervals by monitoring the solution's UV-visible spectra [15-19].

Beg et al. recently reported green production of Ag NPs from Pongamia pinnata seed extract. A maximum absorption at 439nm confirmed the development of nanoparticles. The well-distributed nanoparticles with an average size of 16.4 nm exhibited a zeta potential of 23.7 mV, indicating dispersion and stability. The biosynthesis of AgNPs from the fruit extract of Piper longum has also been accomplished. The nanoparticles produced were spherical, with an

average size of 46nm as assessed by SEM and a dynamic light scattering (DLS) analyzer. The extract's polyphenols are thought to work as a silver nanoparticle stabilizer. In vitro, both the fruit extract and the stabilized nanoparticles demonstrated antioxidant capabilities. The nanoparticles were discovered to be more effective against pathogenic bacteria than P. longum flower extract.

The comparatively high quantities of steroids, carbohydrates, saponin and flavonoids work as reducing agents, and Phytochemicals act as capping agents, providing silver nanoparticle stability. The produced nanoparticles were discovered to be spherical and of average size approximately 7–17 nm. The XRD technique revealed that these nanoparticles had a crystalline structure with a face-centered cubic shape. Using tea as a capping agent, 20–90 nm AgNPs



with the crystalline structure were produced. The reaction temperature and tea extract dose influenced the production efficiency and pace of nanoparticle formation. According to TEM, the size of spherical AgNPs ranges from 5-20nm. Silver nanoparticles revealed a progressive change in color of the extracts to yellowish-brown when treated with callus extract of the *Sesuvium portulacastrum* L plant, as the strength of the extract increased over the incubation period. Because of its quick, non-pathogenic, eco-friendly affordable protocol and provision of a one-step methodology for biosynthesis processes, the use of plants as a production assembly of silver nanoparticles has piqued the interest of researchers. The stabilization and reduction of silver ions through the use of biomolecules such as enzymes, proteins, alkaloids, amino acids, phenolics, polysaccharides, tannins, saponins and vitamins that are present in plant extracts with medicinal properties and are environmentally friendly but have chemically complex structures [20-24] (Table 3).

### Preparation of Plant extracts:

Plant leaves were purchased fresh. They were carefully washed under running water and dried in the shade for 2-3 weeks before being ground to a fine powder with a mixer. The plant sample (5 g) was boiled for 15 minutes in 50 ml of distilled water at 60 ° C. Until filtering, the mixtures were cooled on Whatman No. 1 filter film, and the filtrates were used to produce silver nanoparticles.

### Preparation of 1 mM AgNO<sub>3</sub> solution

A precise conc. of 1 mM AgNO<sub>3</sub> solution was prepared by dissolving 0.169 g AgNO<sub>3</sub> in 1000 ml of double distilled water and stored in an Amber-colored bottle to avoid oxidation of Silver.

### Synthesis of silver nanoparticles using plant leaf extract:

Aqueous AgNO<sub>3</sub> (1mM) solution was freshly prepared and used

Table 3: Plant based synthesis of AgNPs.

S.No	Article/Author	Reducing Agent	Operating Conditions	Characterization	Particle Characterization
1	Biosynthesis of silver nanoparticles using <i>Capparis spinosa</i> L. leaf extract and their antibacterial activity	Leaf extract of <i>Capparis spinosa</i>	0.01M AgNO <sub>3</sub> , 15min, room temperature,static	UV FTIR SEM TEM XRD	Size:20-25nm Shape: spherical Structure: crystalline
2	Facile green synthesis of silver nanoparticles using <i>Berberis vulgaris</i> leaf and root aqueous extract and its antibacterial activity	Leaf and Root extract of <i>Berberis Vulgaris</i>	10mM of AgNO <sub>3</sub> , 24 hrs, room temp, shaker	UV XRD DLS TEM	30 to 70 nm Spherical variable
3	Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity	Leaf extract of Neem	5 ml of leaf extract, 1 mM silver nitrate, 45 min, 10-50°, shaken.	XRD SEM FTIR Optical absorption PL	402-407 nm Spherical FCC
4	Green synthesis of silver nanoparticles using <i>Gymnema sylvestre</i> leaf extract and evaluation of its antibacterial activity	Leaf extract of <i>Gymnema sylvestre</i>	5ml of leaf extract, 0.1mM of silver nitrate, 100°C for 2 hours dried, centrifuged.	UV XRD TEM FTIR	20 to 30 nm Spherical Cube or crystal
5	Antioxidant and antibacterial activity of silver nanoparticles biosynthesized using <i>Chenopodium murale</i> leaf extract	<i>Chenopodium murale</i> leaf extract	1ml of leaf extract, 0.01mM of silver nitrate, room temperature, 24 hours, static.	UV TEM	30 to 50 nm Spherical FCC
6	Green synthesis of silver nanoparticles using <i>Azadirachta indica</i> aqueous leaf extract	<i>Azadirachta indica</i> aqueous leaf extract	1mM silver nitrate, 5ml leaf extract, Room temperature, incubated.	UV TEM DLS Photoluminescence	436 to 446 nm Spherical Crystal
7	Green synthesis of silver nanoparticles using olive leaf extract and its antibacterial activity	Olive leaf extract	9ml olive extract, 1ml of Silver nitrate, room temperature, shaker.	UV XRD SEM TGA	20-25 nm Spherical FCC
8	Green biosynthesis of silver nanoparticles using <i>Torreya nucifera</i> and their antibacterial activity	<i>Torreya nucifera</i> leaf extract	1 mM Silver nitrate, 10ml of the aqueous solution, incubated at 20° C, shaker.	XRD FT-IR	10 to 125 nm Crystalline FCC
9	Green biosynthesis of silver nanoparticles using <i>Calliandra haematocephala</i> leaf extract, their antibacterial activity, and hydrogen peroxide sensing capability	<i>Calliandra haematocephala</i> leaf extract	10 ml of leaf extract, 1 mM silver nitrate, water bath, 80° C for 10 minutes, static	UV SEM XRD EDS FT-IR	414 nm Crystalline FCC
10	Green synthesis of silver nanoparticles using extract of <i>Parkia speciosa</i> Hassk pods assisted by microwave irradiation	<i>Parkia speciosa</i> Hassk extract	10ml of extract, 1mM AgNO <sub>3</sub> , heated microwave at 300W for 4min, static.	UV SEM TEM FT-IR	271-273 nm Spherical FCC
11	Green synthesis of silver nanoparticles using aqueous extract of saffron ( <i>Crocus sativus</i> L.) wastages and its antibacterial activity against six bacteria	Extract of saffron ( <i>Crocus sativus</i> L.)	0.45 mM AgNO <sub>3</sub> , 5 mL of extract and centrifuged at 8 000 r/min for 10 min, static, dried.	UV TEM FTIR XRD	12–20 nm Spherical Crystalline

12	Synthesis of silver nanoparticles using fresh bark of <i>Pongamia pinnata</i> and characterization of its antibacterial activity against gram-positive and gram-negative pathogens	<i>P. pinnata</i> fresh bark extracts.	1mM AgNO <sub>3</sub> , 10 ml extract and kept at room temperature.	UV TEM SEM XRD EDAX	5-55 nm Spherical FCC
13	Green synthesis of silver nanoparticles using <i>Capsicum frutescence</i> and its intensified activity against <i>E. coli</i>	Fruit Extract of <i>Capsicum frutescence</i>	1 mM silver nitrate, 10 ml of fruit extract, 27°, shaker at 150 rpm.	UV XRD SEM	385– 435 nm Crystalline FCC
14	Synthesis of Pomegranate Peel Extract Mediated Silver Nanoparticles and its Antibacterial Activity	Pomegranate Peel Extract (Fruit)	1mM AgNO <sub>3</sub> , 5 ml of filtrate, 24 hours incubation with intermittent shaking.	UV FT-IR SEM	5-50 nm Spherical crystal
15	Mangrove plant, <i>Rhizophora mucronata</i> (Lamk, 1804) mediated one-pot green synthesis of silver nanoparticles and its antibacterial activity against aquatic pathogens	Fresh leaf buds of <i>R. mucronate</i> .	1mM AgNO <sub>3</sub> , 10 mL of the leaf extract of <i>R. mucronate</i> , 15 psi pressure at 121°C for 5 minutes.	UV XRD FT-IR HRTEM	4 nm Crystalline FCC
16	Biosynthesis of silver nanoparticles and its antibacterial activity using seaweed <i>Urospora sp.</i>	Seaweeds were collected from the rocky shore.	1 mM AgNO <sub>3</sub> , at 70°C in dark condition at constant stirring (magnetic stirrer), centrifuged at 5000g for 20 min.	UV XRD FT-IR HRTEM	20 to 30 nm Spherical crystal
17	Retracted: Green Synthesis of Silver Nanoparticles Using <i>Polyalthia longifolia</i> Leaf Extract along with D-Sorbitol: Study of Antibacterial Activity	<i>Polyalthia longifolia</i> Leaf Extract.	3 mL of extract, 40 mL of AgNO <sub>3</sub> solution, room temperature (25°C), and 60°C.	UV FT-IR TEM	50 nm and 35 nm Crystalline FCC
18	Synthesis of Silver Nanoparticles from the Aqueous Extract of Leaves of <i>Ocimum sanctum</i> for Enhanced Antibacterial Activity	Aqueous Extract of Leaves of <i>Ocimum sanctum</i> .	10 mL of aqueous extract, 90 ml AgNO <sub>3</sub> , room temperature, 30 minutes.	UV TEM XRD	18 nm Crystalline static
19	Antibacterial Activity of Silver Nanoparticles Synthesized by Bark Extract of <i>Syzygium cumini</i>	Bark Extract of <i>Syzygium cumini</i>	1 mM AgNO <sub>3</sub> and extract, a ratio of 9: 1 stored under dark conditions	UV SEM AFM	20 to 60 nm Spherical crystal
20	Photo-Irradiated Biosynthesis of Silver Nanoparticles Using Edible Mushroom <i>Pleurotus florida</i> and Their Antibacterial Activity Studies	Fresh Edible Mushroom <i>Pleurotus florida</i>	0.001 M AgNO <sub>3</sub> solution, double filtered mushroom extract allowed to react at room temperature.	UV AFM FESEM TEM FT-IR XRD	20 ± 5 nm Crystal Crystalline
21	Characterization and Antibacterial Activity of Biosynthesized Silver Nanoparticles Using the Ethanolic Extract of <i>Pelargonium sidoides DC</i>	Extract of <i>Pelargonium sidoides DC</i> (Root)	80 ml 1mM AgNO <sub>3</sub> , 20ml ethanolic plant extract solution, at room temperature, 2hr.	UV XRD SEM TEM SPB FT-IR EDS	11 to 90 nm Spherical FCC
22	Biosynthesis of Silver Nanoparticles Using <i>Cucumis prophetarum</i> Aqueous Leaf Extract and Their Antibacterial and Antiproliferative Activity Against Cancer Cell Lines	<i>Cucumis prophetarum</i> Aqueous Leaf Extract (Leaf)	aqueous leaf extract of <i>C. prophet arum</i> was added to silver nitrate solution, for 3 hours at room temperature, shaker.	UV DLS XRD SEM FTIR EDAX	30–50 nm Spherical FCC
23	Green synthesis of silver nanoparticles using <i>phlomis</i> leaf extract and investigation of their antibacterial activity	<i>phlomis</i> leaf extract (leaf)	5.0 ml extract, 0.01 M of AgNO <sub>3</sub> , room temperature.	UV XRD TEM SEM FT-IR	25 nm Spherical Crystalline
24	Characterization of silver nanoparticles by green synthesis method using <i>Pedaliium murex</i> leaf extract and their antibacterial activity	<i>Pedaliium murex</i> leaf extract (Leaf)	0.01M AgNO <sub>3</sub> , 1-5ml leaf extract, 20 minutes at room temperature, shaker.	UV XRD DLS TEM FTIR SEM EDAX	430 nm Crystalline FCC
25	Antibacterial activity of silver nanoparticles synthesized by using whole plant extracts of <i>Clitoria ternatea</i>	whole plant extracts of <i>Clitoria ternatea</i>	10ml extract, 50ml 1mM AgNO <sub>3</sub> , constant stirring at 50–60° C, incubated at room temperature for 40 hours.	UV XAS TEM SEM	20-30 nm Spherical crystalline
26	Antimicrobial activity of Silver Nanoparticles Synthesized by using Medicinal Plants	Leaves of <i>Svensonia hyderabadensis</i> and the stem barks of <i>Boswellia</i> , <i>Shorea species</i>	1mM AgNO <sub>3</sub> , plant extract was added to make upto 200ml, 25 min, 18,000rpm, 95°C.	UV	30-40 nm Crystal FCC

27	Green synthesis and characterization of monodispersed silver nanoparticles obtained using oak fruit bark extract and their antibacterial activity	the oak fruit bark extract	0.001M AgNO <sub>3</sub> , 10 ml extract at room temperature.	UV XRD TEM	20–25 nm Spherical FCC
28	Green synthesis of silver nanoparticles using carob leaf extract and its antibacterial activity	carob leaf extract (leaf)	5 ml extract, 0.001M AgNO <sub>3</sub> , stirring magnetically at room temperature.	UV XRD SEM FTIR AAS	5 to 40 nm Spherical Crystalline and FCC
29	Biosynthesis of silver nanoparticles using stem bark extracts of <i>Diospyros montana</i> and their antioxidant and antibacterial activities	stem bark extracts of <i>Diospyros montana</i>	1 mM AgNO <sub>3</sub> solution and extract in 1:9 proportions and kept at room temperature for 30 min.	UV SEM TEM FTIR DPPH	28 nm Crystalline FCC
30	Biosynthesis of Silver Nanoparticles by Bamboo Leaves Extract and Their Antimicrobial Activity	Bamboo Leaves Extract (Leaf)	5 ml extract, 5 ml of 3 mM AgNO <sub>3</sub> heated at 65°C with continuous stirring.	UV EDX TEM XRD	400-450 nm Spherical or non-spherical Crystalline
31	Biogenic synthesis of silver nanoparticles using guava ( <i>Psidium guajava</i> ) leaf extract and its antibacterial activity against <i>Pseudomonas aeruginosa</i>	<i>Psidium guajava</i> leaf extract (Leaf)	20 ml of 1 mM of AgNO <sub>3</sub> , 0.2 ml of leaf extract and stirred for 10 min at 30°	UV TEM	10–90 nm Spherical FCC
32	Green and rapid synthesis of silver nanoparticles using <i>Borago officinalis</i> leaf extract: anticancer and antibacterial activities	<i>Borago officinalis</i> leaf extract (Leaf)	10 ml extract, 1mM AgNO <sub>3</sub> , 65 °C. The dried AgNPs were finally obtained after filtration, centrifugation and lyophilization.	UV SPR TEM XRD SAED	30 to 80 nm spherical, hexagonal, and irregular FCC
33	Green biosynthesis of silver nanoparticles using <i>Quercus brantii</i> (oak) leaves hydroalcoholic extract	<i>Quercus brantii</i> (oak) leaves hydroalcoholic extract.	1mM AgNO <sub>3</sub> as substrate, concentrated and freeze-dried plant extract, incubated at room temperature, pH 7, Reaction vol 50ml.	TEM DLS	mean size of 6 nm Spherical poly-dispersed
34	Green synthesis and characterization of silver nanoparticles using banana peel extract and their antimicrobial activity against representative microorganisms	banana peel extract	1 ml extract, 1 mM AgNO <sub>3</sub> at pH 4.5, incubated at 30°C for 5 min.	UV XRD FTIR SEM	23.7 nm Spherical crystallinity
35	Green synthesis of silver nanoparticles using <i>Rheum palmatum</i> root extract and their antibacterial activity against <i>Staphylococcus aureus</i> and <i>Pseudomonas aeruginosa</i>	<i>Rheum palmatum</i> root extract	5 ml extract, 2mM AgNO <sub>3</sub> solution, at room temperature, 24hr	TEM SEM FTIR DLS	121 - 2 nm hexagonal and spherical Crystalline
36	Green Synthesis of Silver Nanoparticles, Their Characterization, Application, and Antibacterial Activity	<i>Sansevieria trifasciata</i> <i>Impatiens balsamina</i> <i>Pelargonium graveolens</i>	3 ml filtrated Plant extract, 0.001M AgNO <sub>3</sub> , heated 75°C, 24 hr	UV TEM AFM	3 to 15 nm Spherical FCC
37	Green biosynthesis of silver nanoparticles using <i>Curcuma longa</i> tuber powder	<i>Curcuma longa</i> tuber powder extract	20 ml Plant extract, 0.001M AgNO <sub>3</sub> , 24 hours, at room temperature (25°C).	UV XRD TEM SEM EDXRF FTIR	6.30 ± 2.64 nm Crystalline FCC
38	<i>Ipomea carnea</i> -based silver nanoparticle synthesis for antibacterial activity against selected human pathogens	<i>Ipomea carnea</i> against selected human pathogens.	The leaf extract (1, 2.5 and 10% v/v) was mixed with enough 1 mM AgNO <sub>3</sub> at room temperature.	UV DLS AFM TEM FTIR	30 to 130 nm Spherical FCC
39	Antibacterial Activity of Synthesized Silver Nanoparticles from <i>Tinospora cordifolia</i> against Multi Drug Resistant Strains of <i>Pseudomonas aeruginosa</i> Isolated from Burn Patients	<i>Tinospora cordifolia</i>	1 mM AgNO <sub>3</sub> , 15 ml extract, 15-20 minutes at 70-75°C.	UV FTIR TEM EDX XRD	9 ± 36 nm Crystal Crystalline
40	Low-cost and eco-friendly synthesis of silver nanoparticles using coconut ( <i>Cocos nucifera</i> ) oil cake extract and its antibacterial activity	coconut ( <i>Cocos nucifera</i> ) oil cake extract	4 ml extract, 96 ml 1 mM AgNO <sub>3</sub> , incubated for 8 h in a rotary shaker (180 rpm) at 26°C.	TEM	10–70 nm Spherical crystal
41	Rapid Biosynthesis of Silver Nanoparticles Using <i>Cymbopogon Citratus</i> (Lemongrass) and its Antimicrobial Activity	<i>Cymbopogon Citratus</i> (Lemongrass)	1 mM AgNO <sub>3</sub> and extract in 1:4 ratio under aseptic conditions. The pH 8.0, incubated at 37°C for 24 hours.	UV TEM EDX NTA	20-40 nm Spherical FCC
42	Green Biosynthesis of Silver Nanoparticles Using <i>Callicarpa maingayi</i> Stem Bark Extraction	<i>Callicarpa maingayi</i> Stem Bark Extraction	100 ml extract solution, 100 ml 0.01M AgNO <sub>3</sub> , at room temperature (25°C) for 48hr	UV XRD TEM SEM EDX FTIR	12.40 ± 3.27 nm Crystalline FCC

43	Nanoscience and Nanotechnology/ Biosynthesis of Silver Nanoparticles using <i>Olea europaea</i> Leaves Extract and its Antibacterial Activity	<i>Olea europaea</i> Leaves Extract (leaf)	5 ml extract 0.001 M AgNO <sub>3</sub> at room temperature.	UV TEM SEM XRD FTIR	10 nm Spherical FCC
44	Synthesis of silver nanoparticles from <i>Sargassum tenerrimum</i> and screening phytochemicals for its antibacterial activity	<i>Sargassum tenerrimum</i> and screening Photochemical	1 mM AgNO <sub>3</sub> 5 ml seaweed extract, gradually heated at 90°C for 20 mins.	UV FTIR TEM DLS	20 nm Spherical Crystal
45	Biosynthesis and Characterization of Silver Nanoparticles Using Leaf Extract <i>Abutilon indicum</i>	Leaf Extract <i>Abutilon indicum</i>	10 ml Plant extract 1mM AgNO <sub>3</sub> at room temperature.	UV FTIR SEM	50-100 nm Crystal Crystalline
46	Plant-mediated synthesis of silver nanoparticles using parsley ( <i>Petroselinum crispum</i> ) leaf extract: spectral analysis of the particles and antibacterial study	<i>Petroselinum crispum</i> leaf extract	20mM AgNO <sub>3</sub> Extract added drop wise to maintain total concentration 10mM, 24 hr, 10000 rpm for 30min.	UV XRD DLS TEM FTIR	30 nm Spherical FCC
47	Sunlight-induced rapid and efficient biogenic synthesis of silver nanoparticles using aqueous leaf extract of <i>Ocimum sanctum</i> Linn. with enhanced antibacterial activity	leaf extract of <i>Ocimum sanctum</i>	5ml extracts of (10%, 7%, 5%, and 3%) ,45 mL of 0.001M AgNO <sub>3</sub> to make upto 50ml, kept Under sunlight.	UV TEM	10-20 nm Crystal FCC
48	Biosynthesis of Silver Nanoparticles using <i>Garcinia mangostana</i> Fruit Extract and their Antibacterial, Antioxidant Activity	<i>Garcinia mangostana</i> Fruit Extract	1mM AgNO <sub>3</sub> , 10ml fruit extract, boiled for 15 minutes at 80°C.	UV TEM	30 to 50nm Spherical FCC
49	<i>Coleus aromaticus</i> leaf extract mediated synthesis of silver nanoparticles and its bactericidal activity	<i>Coleus aromaticus</i> leaf extract	90 ml 1mM AgNO <sub>3</sub> 10 ml plant extract, incubated at room temperature for 10 min.	UV XRD FTIR TEM SEM	40-50 nm Crystal Crystalline
50	Biosynthesis of silver nanoparticles using lemon leaves extract and its application for an antimicrobial finish on fabric	lemon leaves extract	5ml extract, 45 ml 0.002 M AgNO <sub>3</sub> , room temperature in dark. 1 hr	UV TEM SEM FTIR AFM XRD	30-50 nm Spherical FCC
51	Waste-grass-mediated green synthesis of silver nanoparticles and evaluation of their anticancer, antifungal and antibacterial activity	Waste-grass-mediated green extract	15ml extract, 1.2, 5, 5 mM AgNO <sub>3</sub> , kept in dark at 28°C	Western blot	4-34 nm Crystal Crystalline

to synthesize AgNPs. Each aqueous plant extract (10 ml) was mixed with 1 mM aqueous AgNO<sub>3</sub> solution (90 ml) (for reduction of Ag<sup>+</sup> ions into Ag<sup>0</sup>) and incubated overnight at room temperature in the dark (to prevent the photo-activation of AgNO<sub>3</sub>). The resulting reddish-brown solution serves as an indicator for the processing of AgNPs. The solutions derived from Silver nanoparticles have been purified for 15 minutes by repeated centrifugation at 10000 rpm. For the additional settlement of particles, the supernatant was then transferred to a clean dry beaker, and a repetitive centrifuge was used with a microfuge to dry and purify silver nanoparticles. The samples thus collected were dried and utilized for further characterization in an incubator. Silver nanoparticles were therefore produced in one green step.

#### The effect of various silver nitrate concentrations on Ag<sup>+</sup> bioreduction:

From a 1M solution of AgNO<sub>3</sub>, various amounts of silver nitrate (1mM, 2mM, 3mM, 4mM, and 5mM) were prepared. 5 ml of the plant extract was mixed with 20 ml of solution from each AgNO<sub>3</sub> concentration solution. After leaving the mixed solutions at 27°C (Room temperature) for one day, the maximum values were recorded using a UV-Vis Spectrophotometer.

#### The impact of pH on Ag<sup>+</sup> bioreduction:

Since pH is essential for the effective synthesis of nanoparticles, this element increases the reactivity of plant extract with silver ions. The pH of the 1mM AgNO<sub>3</sub> solution with plant extract was retained by adding a 1M sodium hydroxide solution. The impact of pH on the synthesis of

AgNPs was investigated using reaction mixtures with varying pH (5, 6, 7, 8, and 9).

#### Time impact on Ag<sup>+</sup> bioreduction:

25 mL of *Syzygium* aqueum leaf extract was blended with 75 ml of 1mM AgNO<sub>3</sub> solution. The effect of time on silver nanoparticle synthesis was estimated from 30 minutes to 18 hours.

#### Phytochemical screening of plant extract

Phytochemical screening of the plant extracts was carried out by following standard procedures for different Phytoconstituents present in *Syzygium* aqueum leaf extract were performed as follows:

#### Hager's Test for Alkaloids –

A few drops of Hager's reagent were used for the test solution (saturated picric acid solution). The development of yellow precipitates confirms the presence of alkaloids.

#### Flavonoid assay (Shindo's assay):

A few magnesium turnings and a few drops of concentrate hydrochloric acid were applied to 2ml of the test solution before boiling for 5 minutes. The existence of flavonoids was denoted by the appearance of red pigment.

#### Test for Phenols:

A few drops of ferric chloride solution are applied to 2ml of the



test solution to conduct the phenol test. The expression of phenols was shown by a red color.

#### **Test for Tannins:**

Tannins were determined by combining the test solution with a simple lead acetate solution. Formation of a white precipitate confirms the presence of Tannins.

#### **Characterization of biosynthesized silver nanoparticles(AgNPs):**

##### **UV-Visible spectroscopy:**

Ultraviolet-visible spectroscopy, also known as ultraviolet-visible(UV-Vis) spectrophotometry, is a form of absorption spectroscopy that operates in the UV-visible spectral range. This means that visible and neighboring light (near UV and near-infrared (NIR)) are used. The observable field has a significant impact on the color perception of the chemicals involved. Molecules undergo an electrical transformation in this part of the electromagnetic spectrum. Absorption refers to a substance that absorbs light at a given wavelength. UV-Vis spectroscopy was used to monitor the color variations of the mixture over time. The UV-Vis spectrum was monitored at 54 wavelengths ranging from 200-700 nm. on a UV spectrophotometer.

UV-visible spectroscopy is utilized in this work to characterize metal nanoparticles and to comprehend surface Plasmons and electronic transitions in metal nanoparticles. To begin characterizing the synthesized silver nanoparticles, a tiny aliquot of material was placed in a UV-Visible spectrophotometer of absorption spectra at 300-700 nm. The UV-visible spectrophotometer is used to calculate the absorption spectrum of metal nanoparticles distributed in the water. The sample keeping cells were quartz cuvettes (cells) with a route length of 10 mm. Before employing UV radiation, the cuvettes are thoroughly cleaned with water, acetone, and dried.

##### **Fourier Transform Infrared Spectroscopy (FTIR):**

The technique is based on the fact that compounds and groups of compounds vibrate with characteristic frequencies. The molecule exposed to IR rays absorbs infrared energy at the characteristic frequency. Throughout FTIR analysis, a point in the sample is exposed to a modulated infrared (IR) beam. The resulting spectral pattern is then compared and analyzed to known signatures of materials identified in the FTIR-library. Silver nanoparticles and aqueous extracts were mixed with potassium bromide (KBr) and processed into a thin KBr disk under a pressure of 7845 KPa for 2 minutes, and all spectra were measured in the range of 4000 to 400  $\text{cm}^{-1}$ . The resultant spectrum is typical of the organic compounds contained in the sample. Without sample preparation, the method may instantly generate FTIR spectra of solid, semi-solid, and liquid materials in any shape. It can work with a minimum sample size of 15, minimal alignment, kinematic mount, and fast sample change. FTIR gives information on chemical bonding in a substance. Because the band intensities are related to the chemical concentration of the molecule, qualitative estimates are also achievable [25-28].

##### **Scanning Electron Microscopy (SEM):**

SEM is a high magnification microscope that creates images of the synthesized sample using a directed scanned electron beam. Thin films of the synthesized nanoparticles were made on a carbon-coated aluminum grid by simply dropping a small amount of sample onto the grid. An additional solution was made. It was removed using blotting paper and then the film was placed under a mercury-lamp for 5 minutes. EM images were observed at different levels of magnification. It's kind

of an electron Microscope that images a sample in a raster scan pattern by scanning an electron beam. The electrons interact with the atoms present in the sample and generate signals which contain information about the surface topography, chemical content, and crystalline structure of the synthesized nanoparticles. Data is gathered across a predetermined region of the sample's surface, and a 2-dimensional picture displaying spatial variation in these characteristics is created. The crystals were analyzed and separated using SEM to determine the sizes and forms of the materials.

##### **Transmission Electron Microscopy (TEM):**

TEM is a microscopy technique in which an electron beam is passed through a highly thin specimen, interfering with it as it travels. The association of electrons emitted through the specimen creates an image, which is focused and magnified onto an imaging system. The morphology of the nanoparticles was studied using high-resolution pictures taken with a transmission electron microscope (TEM) set to 300kV. AgNPs were sonicated for 5 minutes before analysis, and a drop of properly diluted sample was put onto a carbon-covered copper grid. Blotting paper was used to remove excess solution. The liquid portion was then allowed to dry at ambient temperature.

##### **Dynamic Light Scattering (DLS) and Zeta potential :**

The particle size distribution of silver was determined using DLS measurements, and the stability of the synthesized AgNPs was evaluated using zeta potential analysis. Both measurements were taken with Zeta-sizer Nano series compact dispersion spectrometer.

##### **Antibacterial activity of biosynthesized silver nanoparticles (AgNPs):**

In recent years, antibiotic resistance has been a major public health issue. Unlike traditional and limited spectrum antibiotics, metallic silver nanoparticles have a deadly impact on a wide variety of bacteria and do not allow pathogens to build resistance. Biosynthesized Silver nanoparticles can be utilized as a powerful tool to control harmful infections caused by microorganisms at extremely low concentrations and as a preventative agent. While silver ions or silver salts have antibacterial properties, the mechanisms for the action of silver nanoparticles are not yet completely characterized. Nutrient agar and nutrient broth were used for the sub-culturing of the bacterial isolates. Mueller-Hinton agar was used for the bacterial sensitivity screening. The antibacterial screening of the synthesized nanoparticles was done by agar well diffusion and MIC.

##### **Agar well diffusion method:**

The antibacterial behavior of the synthesized nanoparticles was tested using the nutrient agar well diffusion system defined by Schillenger and Luke (1989). The nutrient agar medium was inoculated with 0.1mL of a fresh overnight nutrient broth culture of *Staphylococcus aureus* and poured onto sterile Petri plates. Six 6mm diameter wells were punched in each plate using a borer, and the plates were dried for 5 minutes. After keeping the plate at 27°C for 2 hours to enable diffusion of the controls and samples into the nutrient agar medium, the well-known antibacterial medication Ofloxacin was used as a positive control for silver nanoparticles synthesized. The plates were incubated for 24 - 48 hours at 37°C. The test organism's exposure to any of the synthesized samples was demonstrated by a clear halo around the well. The diameters of the halos were determined with a translucent plastic ruler to calculate the degree of sensitivity. They were then tested to see whether they inhibited bacterial development. In each case, the diameters of inhibition zones were represented in millimeters of sensitivity.

### Minimum Inhibitory Concentration (MIC):

Silver nanoparticle dilutions of 10,20,40,80,160 g/ml were prepared and used in this analysis. 200µl of bacterial suspension was inoculated into each test tube containing varying amounts of AgNPs and a comparable volume of Muller Hinton Broth (MHB) and incubated for 24 hours at 37°C. The technique also contained a positive control (tube containing only bacterial suspension and nutrient media without nanoparticles) and negative control (tube containing nanoparticles and nutrient medium without bacterial suspension) [29, 30].

### Conclusion

Many attempts have been made over the previous decades to create novel technologies for green synthesis. Live organisms offer a great potential for nanomaterial synthesis which may be used in various areas and in particular in healthcare. Organisms from basic to very sophisticated eukaryotes can be utilized to produce the appropriate size and form of nanoobjects. Prokaryotes are simplest of biomass forms and are easier to modify genetically to generate more desirable synthesis ingredients. However, in comparison to others, the culture of bacteria and manufacturing at a big scale remains challenging. Bacteria have therefore been explored as initial nano fabrics in the development of noble metal nanoparticles as a first attempt. The poor synthesis rates of fungus and algae were nonetheless determined by the restricted size and shape distribution available. Fungi offer an appropriate choice for big green nano production. They are easy to handle and exude vast quantities of enzymes essential to reduce downstream processing. Filamentous metal tolerance, strong binding capacity, and intracellular uptake are also found. Nevertheless, it is considerably more difficult for eukaryotes to manipulate genes to press certain enzymes to accelerate synthesis.

Most recently, several studies were undertaken on potential plant extracts. Due to their simple availability, comfort of mind, and cost-effectiveness, the numbers of publications published in this sector have risen exponentially during the previous few years. In addition, plants contain the most effective phytochemicals and hence improve the pace of synthesis. The distribution in size and form of the nanoparticles derived from TEM investigations reveals that several factors impact their morphologies, including the plant extract origin, solution pH, and reaction temperature. Green-produced silver nanoparticles have unrivaled uses that have important features of nanotechnology. For the production of nanoparticles that use plants, it can be of benefit to other biological entities, which can take time to use microorganisms to maintain their culture and lose their potential for nanoparticles synthesis. However, it remains the subject of research to achieve the uniform size and form distribution of AgNPs.

Regardless of the manufacturing process, AgNPs are used as antibacterial agents, electrochemical sensors, and biosensors in the fields of medicine, healthcare, agriculture, and biotechnology, and are highly bactericidal against both Gram-positive and Gram-negative pathogens. Antibiotics are effective against many drug-resistant bacteria. As a ready-made medicine, they can be used to treat many infections. Silver nanoparticles in drug delivery systems generally increase solubility, stability, and biodistribution, thereby increasing their effectiveness. In the presence of nanoparticles, drug uptake has increased many times, so AgNPs can be utilized as a drug delivery system. Many papers on silver nanoparticles synthesis utilizing plant extracts like the one just described have been published. There is still a need to uncover the capability of natural reductions to create silver nanoparticles that have not previously been examined, which can be economically feasible, economical, and environmentally favorable.

Therefore, the utilization of plant extract for synthesis in the next decades can have an incredible effect.

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### Contribution of authors

All authors have invested their time, effort and knowledge into designing, drafting and editing this manuscript. The final draft was agreed upon mutual satisfaction after having gone through multiple rigorous revisions.

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