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Green Synthesis of Silver Nanoparticles: A Comprehensive Review For Sustainable Nanotechnology Applications

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Abstract: An eco-friendly and biocompatible alternative to the conventional chemical and physical synthesis methods is the green synthesis of silver nanoparticles (AgNPs). The synthesis of AgNP through plant, microbial, algal and enzyme is critically examined in this comprehensive overview. While nucleation and growth kinetics are governed by reaction parameters such as pH, temperature and extract concentration, information derived from spectroscopy, electron microscopy and computational modelling identifies phenolic, amino and protein functionalities to dominate reduction and stabilization. Green AgNPs have a wide range of functional variety in biomedical, environmental and catalytic applications. They have strong antibacterial, anticancer, photocatalytic and sensing properties. Although there are still challenges with reproducibility, mass production and long-term durability, comparative studies show advantages in energy efficiency, safety and environmental compatibility. Predictive control over particle qualities may be possible through the combination of experimental optimization with artificial intelligence (AI) and machine learning (ML). To ensure eco-friendly and industrially scalable applications, the emerging fields include hybrid synthesis techniques, life-cycle-oriented risk assessment and regulatory coordination. The integration of AI-based design and green chemistry is a ground-breaking approach to sustainable nanotechnology.

Keywords: Ag Nanoparticles, Green Synthesis, Sensors, Environment, Energy

1. Introduction

AgNPs or silver nanoparticles continue to play a significant role in nanotechnology and nanoscience. They possess unique physical and chemical characteristics. These consist of broad-spectrum antibacterial action, a high surface-to-volume ratio, a flexible shape and a distinct surface plasmon resonance. AgNPs are beneficial in a variety of fields because of their characteristics. For instance, they support in biomedicine by combating cancer, viruses and bacteria. They also aid in environmental remediation by trapping heavy metals and breaking down contaminants. Catalysis, optical and electrical devices, and sensing and diagnostics are additional applications. According to recent research, AgNPs work better when their size and shape are controlled. This results in reduced toxicity for medical applications, improved optical behavior in devices and higher catalytic efficiency [1].

AgNPs have historically been created by scientists through physical and chemical processes. Chemical reduction, thermal procedures, photochemical methods, laser ablation and evaporation-condensation are examples of these traditional techniques. Agents such as citrate, hydrazine, or sodium borohydride are used in chemical reduction. In general, these techniques provide good control over the purity, size distribution and form of the particles. But there are a lot of issues with these conventional methods. They frequently employ capping agents and hazardous reagents. They need a lot of energy and materials and produce dangerous byproducts. Extreme reaction conditions, like high pressure or temperature, are necessary for them. Potential hazards to the environment and human health are increased by this. Additionally, consistent outcomes and large-scale production are frequently still challenging [2-4]. Strong reductants, for example, rapidly create new particles but frequently produce surfaces that are defective or lack control over the final shape. Biological usages may be harmed by residual stabilizers. They require a number of purifying procedures [2].

The green or biosynthetic method provides a sustainable solution to these constraints. Natural resources are used in this process. These include biomass wastes, microorganisms (such as bacteria, fungi, and algae), plants and biomolecules. These substances stabilize the nanoparticles and function as agents to decrease the silver. Typically, green synthesis operates at gentler temperatures and pressures. It eliminates or drastically cuts down on the use of hazardous substances. Renewable feedstocks are used in this process. The finished product frequently has reduced ecotoxicity and improved biocompatibility. Recent research emphasizes the importance of phytochemicals, including alkaloids, terpenoids, flavonoids, and phenolics. They cover and stabilize the nanoparticles in addition to converting silver ions (Ag+) into silver atoms (Ag0). This coating has an impact on the liquid's ultimate size, shape and stability over time [5, 6]. Additionally, extracts from biomass by-products, agri-food waste, agricultural waste, and even extracellular polymeric compounds from algae are now used by researchers. By doing this, trash is converted into useful products and sustainability is enhanced [3, 7].

The comparison of green synthesis and conventional methods are given in Table 1. Green synthesis still faces a number of obstacles despite significant advancements. Controlling particle size and shape consistently across many biological sources is challenging. The precise molecular mechanisms and dynamics of the stabilization and reduction processes must be thoroughly understood. It is still difficult to scale up lab-based production to industrial levels. When using these nanoparticles in the environment or inside the body, we need to make sure they are safe, stable, and have a long shelf life. We also need to develop uniform procedures. This will enable reliable comparison of data from various labs. It is essential to address these problems. The full potential of green-synthesized AgNPs can only then be utilized in useful, real-world applications.

This paper offers a thorough and up-to-date assessment of the environmentally friendly production of silver nanoparticles. It looks at five important aspects. It first examines the biological origins and the function of the capping or reducing agents. Second, it looks at the mechanistic findings, such as the value of kinetic investigations and modelling.

Table 1. The Advantages Vs. Disadvantages Of Conventional And Green Synthesis Methods

Parameter	Conventional Methods	Green Methods	References
Cost	Equipment- or reagent-intensive, use of exotic reducing agents or high-purity chemicals increases cost.	Lower reagent cost (natural extracts, waste biomass, microbes) and often simpler setups.	[8]
Toxicity / Environmental Impact	Typically involve toxic solvents, reducing agents (e.g. hydrazine, sodium borohydride), stabilizers with potential environmental hazard; release of chemical byproducts.	Biocompatible capping agents, minimal toxic byproducts, less chemical waste.	[9]
Energy Input	Often require high temperature, high pressure, or specialized physical equipment (laser ablation, evaporation, high-temperature furnaces).	Typically ambient or mild temperature/pressure; reactions proceed under milder conditions.	[10]
Scalability	Well-developed industrial-scale chemical/physical nanoparticle synthesis exists; easier scale-up of chemical reactors; well-known processes.	Scalability is more challenging due to batch-to-batch variability, biological growth constraints, purification bottlenecks.	[11]
Reproducibility / Control	Good reproducibility, well-characterized reagents, tight control over parameters; narrow size distribution achievable.	Greater variation due to biological variability (seasonal, species differences), less precise control over reducing/capping agent concentrations.	[12]

Third, it talks about how synthesis parameters alter the physical and chemical properties as well as characterisation techniques. Fourth, it addresses functional applications that are both new and old. Fifth, it provides viewpoints on current difficulties, safety issues, scalability problems and upcoming trends. This study aims to assist academics and practitioners in the safe, efficient, and sustainable deployment of AgNPs in multidisciplinary nanotechnology by aggregating the results of previous investigations.

2. Principles of Green Synthesis of Silver Nanoparticles

The green synthesis of silver nanoparticles (AgNPs) rests upon the merging of principles from green chemistry, materials science, and biology to achieve environmentally benign, cost-effective, and scalable nanoparticle fabrication. At its core, green nanotechnology aims to reduce or eliminate the use or generation of hazardous substances, minimize energy usage, favor renewable resources, and design safer chemicals and processes that are benign by design. As applied to AgNPs, this means replacing harsh chemical reductants (e.g. sodium borohydride, hydrazine) and stabilizers (e.g. thiols, strong synthetic polymers) with safer biological or biomimetic molecules, operating under mild temperature, pressure, and pH conditions, and achieving control over nanoparticle size, shape, crystallinity, surface chemistry, and stability to suit downstream applications. Figure 1 displays the Schematic description of green synthesis of Silver Nanoparticles.

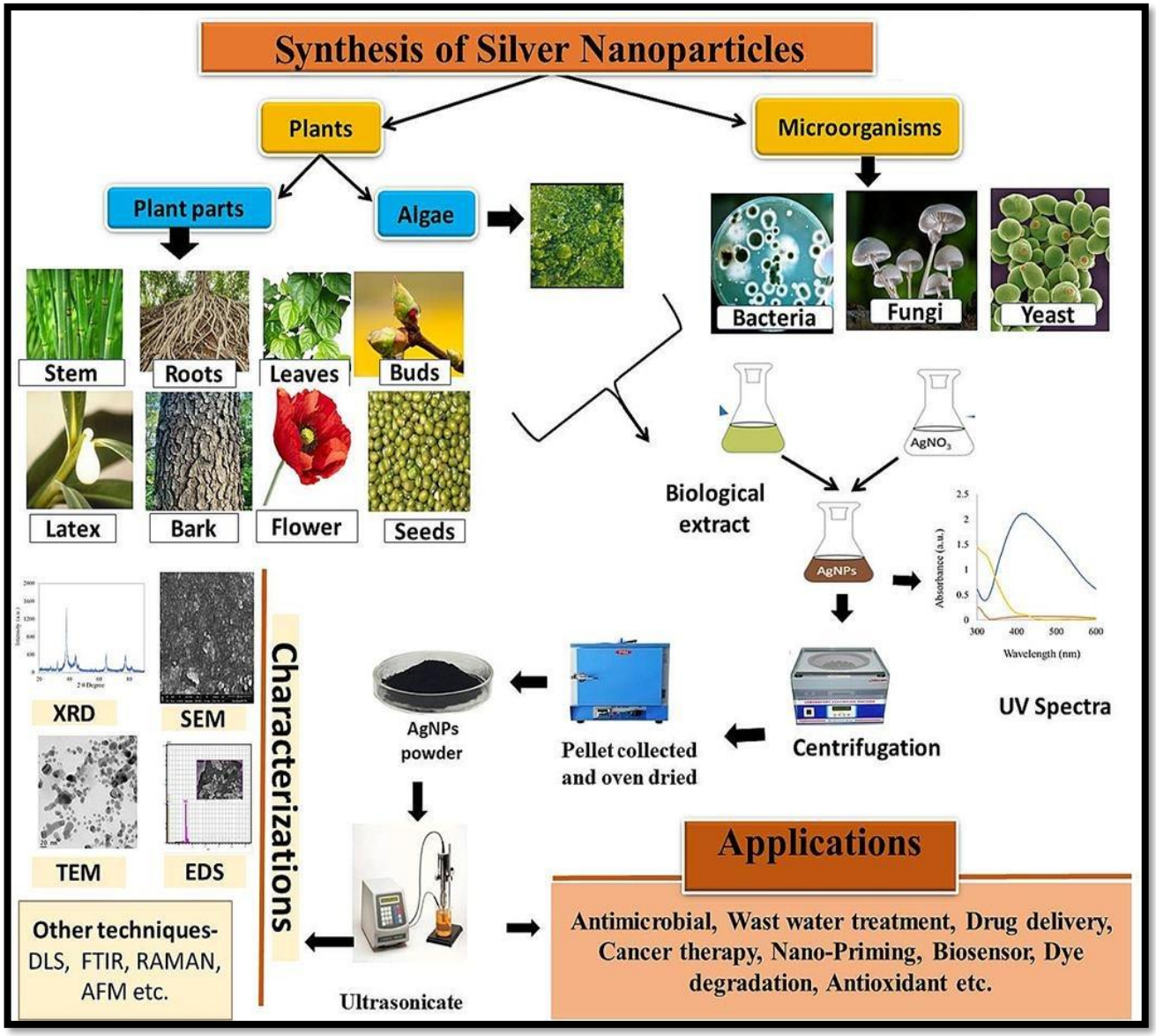

Figure 1. Schematic explanation of green synthesis of Silver Nanoparticles [13]

Fundamental to green synthesis are eco-friendly reducing and stabilizing agents. Biological entities—plant extracts (leaves, flowers, seeds, bark, roots), microorganisms (bacteria, fungi, algae), or biomolecules (proteins, polysaccharides, enzymes, flavonoids, phenolics)—serve dual roles: reducing Ag^{+} to Ag^{0}, and then stabilizing (capping) the formed AgNPs to prevent aggregation and to tailor surface chemistry. For example, a study using *Lavandula angustifolia* leaf extract demonstrated that flavonoids and proteins in the extract reduce silver ions and simultaneously cap the nanoparticles, producing ~20 nm spherical AgNPs with good colloidal stability [14]. Similarly, the buds of *Syzygium nervosum* rich in polyhydroxy and carboxyl functional groups were effective in both reduction and stabilization without extra chemical additives, yielding AgNPs with good stability and enhanced catalytic and antimicrobial performance [15].

These elements have a significant impact on the formation and stability of silver nanoparticles, or AgNPs, during green synthesis. The amount of biological extract employed, temperature, pH level, concentration of silver salt (such as AgNO3), and reaction time all have a significant impact. Exposure to light or radiation can occasionally be significant as well. The AgNPs' initial formation, growth, final shape and stability are all governed by these characteristics. For instance, green tea leaf extract was used in one study published in ACS Omega. Researchers discovered that superior nanoparticles were produced by boosting the pH from 5 to 11 and the temperature from 25 to 65 degrees Celsius. The resultant AgNPs had more constant sizes and more homogeneous spherical forms. This implies that at higher pH and temperatures, the silver declines more quickly and stabilizes better [16]. Leaf extract from Litchi chinensis was employed in another study. It demonstrated that in order to achieve optimal results, scientists need to modify the extract volume, temperature, and concentration of silver ions. A complete conversion of the silver salt and little size variation were considered ideal circumstances. Additionally, variations in the surface plasmon resonance (SPR) peaks demonstrated how pH influences surface stability and charge. At higher pH values (8–11 versus 2–6), the SPR peaks moved toward red. This alteration occurs as a result of the capping molecules' altered shape or the screening of the surface charge [17].

Mechanistically, green synthesis proceeds typically in two overlapping stages: nucleation and growth. In the nucleation stage, silver ions are reduced quickly by reducing agents to Ag atoms; these atoms aggregate to form nuclei; thereafter growth of these nuclei proceeds via further reduction, aggregation or surface diffusion. The rate of reduction versus rate of nuclei stabilization (i.e., capping) determines size, shape, and polydispersity. Strong reducing agents (or high temperature) favor rapid nucleation which can produce many small nuclei but often require effective capping to avoid uncontrolled growth and aggregation. Weak or slow reduction (lower temperature/pH or lower reducing agent concentration) tends to produce fewer nuclei that grow larger. The functional groups present in green reducing/stabilizing agents (e.g. –OH, –COOH, $–NH_2$, phenolic –OH, etc.) bind to the nanoparticle surface and provide steric and/or electrostatic stabilization. For example, *Syzygium nervosum* extract containing polyhydroxy and carboxyl groups was shown to cap AgNPs via these moieties, maintaining dispersion and activity over time [15]. Major green reducing/stabilizing agents used for AgNP synthesis are listed in Table 2.

An additional important parameter is light or irradiation (including sunlight or artificial light). Some green synthesis protocols incorporate light induction, which can accelerate reduction or alter shape through photochemical mechanisms. Also, storage conditions (temperature, light exposure, presence of oxygen) post-synthesis affect long-term stability: aggregation, oxidation, changes in SPR peak – these are influenced by how well the capping agents stabilize surface and prevent oxidative attack [18]. When comparing green synthetic strategies with conventional (chemical/physical) methods, green methods tend to offer lower toxicity, fewer by-products, milder reaction conditions, better biocompatibility, use of renewable feedstocks, and sometimes cost savings. However, in many cases, conventional methods still allow finer control over uniformity, narrower size distributions, and sometimes higher crystallinity (depending on method) because chemical reductants and synthetic capping/stabilizers are well understood and optimized. Thus, green methods frequently require more careful optimization of parameters, deeper understanding of mechanistic pathways, and standardized reporting (extract composition, reaction conditions etc.) to reach comparable precision and reproducibility.

3. Biological Sources for Green Synthesis

3.1 Plant-Mediated Synthesis

Plant-mediated synthesis remains one of the most explored and promising green routes for

producing silver nanoparticles (AgNPs) due to its simplicity, cost-effectiveness, and the dual reducing + stabilizing action provided by phytochemicals. Plants offer a broad array of bioactive compounds—flavonoids, phenolics, terpenoids, alkaloids, tannins, and more—that can both reduce Ag^+ ions to metallic Ag^0 and cap the nanoparticles to stabilize them, influencing size, shape, surface charge, stability, and downstream biofunctionality.

Recent studies demonstrate diverse plant parts (leaves, buds, seed extracts, flowers) used as reducing/capping agents, with notable control over nanoparticle size and morphology. For instance, Withania coagulans leaf extract was used to synthesize AgNPs with an average size ~26.6 nm (SEM/HRTEM), highly crystalline structure (XRD), and zeta potential of -21.4 mV, indicating moderate colloidal stability; the phytochemicals involved (hydroxyl, carbonyl, flavonoids) were clearly identified using FTIR spectra, confirming their role in reduction and stabilization [30]. Figure 2 depicts the HR-TEM images (figure. 2a - 2d) and Particle distribution size (figure. 2e) of the Ag-NPs. Figure 2f shows the Workflow for green synthesis of Ag-NPs using *W. coagulans* leaf extract. AgNPs with outstanding antimicrobial, antifungal and catalytic activity were also produced by Syzygium nervosum bud extracts, which are abundant in terpenoids, flavonoids and polyhydroxy/carboxylic groups. The functional groups in charge of capping and stabilizing were also identified and linked to the stability of the nanoparticles over time [15].

Table 2. Major green reducing/stabilizing agents used for AgNP synthesis

Agent	Advantages	Limitations	Reference
Whole plant extracts (leaf/fruit/seed/bark) — complex phytochemical mixtures	Readily available, low cost, provide both reducing (polyphenols, terpenoids) and capping biomolecules; facile room-temp syntheses; often yield biofunctionalized AgNPs suited for antimicrobial/biomedical uses.	Batch-to-batch variability (plant chemotype, season, extraction), complex composition complicates mechanistic control and reproducibility; may need post-synthesis purification for biomedical use.	[19]
Flavonoids (e.g., quercetin, apigenin) — isolated polyphenols	Act as stoichiometric electron donors for $Ag^+ \rightarrow Ag^0$ reduction and also provide steric/π-stacking stabilization; allow better mechanistic control than crude extracts; can impart antioxidant/biological functionality.	Isolated compounds increase cost vs crude extract; may require auxiliary stabilizers for long-term colloidal stability at neutral pH.	[20]
Simple antioxidants (e.g., ascorbic acid / vitamin C)	Fast, well-controlled reduction; water-soluble and biocompatible; easy scale-up; predictable kinetics.	Produces AgNPs that may require additional capping agents to prevent aggregation; pure chemical (although biocompatible) — sometimes considered "green" when combined with benign stabilizers.	[21]
Reducing sugars / carbohydrates (glucose, sucrose, oligosaccharides)	Mild reducing ability, multiple hydroxyls act for weak reduction and capping; often produce stable, biocompatible glucose-capped AgNPs suitable for biomedical targeting.	Slower reaction kinetics; reduction potential weaker than strong polyphenols — sometimes requires heating or longer reaction time; surface coverage may be less robust under ionic strength changes.	[22]
Polysaccharides (chitosan, starch,	Dual role: reducing (under certain conditions) and excellent	Variable molecular weight/composition affects NP	[23]

cellulose derivatives, maltodextrin, dextran)	steric/electrostatic stabilization; chitosan adds inherent antimicrobial activity; polysaccharide capping increases colloidal stability and biocompatibility.	size & dispersibility; some require prior chemical modification; viscosity can complicate downstream processing.	
Microcrystalline cellulose / food anti-caking agents (as stabilizers)	Inexpensive, food-grade stabilizers that can extend colloid shelf life and assist flavonoid-mediated reductions at neutral pH; safe for food/medical contexts.	Do not reduce Ag^{+}; require a reducing co-agent (flavonoid/polyphenol); may alter surface accessibility for further functionalization.	[20]
Proteins (serum albumin, plant proteins, silk sericin)	Strong electrostatic/steric stabilization, biocompatible coronas, can direct size and shape; used in drug delivery/biomedical AgNPs due to favorable protein corona.	Protein conformation & glycation state affect stability and biological identity; possible immunogenicity depending on source; reproducibility depends on protein purity.	[24]
Microbial exopolysaccharides (EPS)	EPS from bacteria/fungi act as reducing and capping agents, producing stable, often bioactive AgNPs; EPS can impart wound-healing or anti-biofilm properties.	Production and purification of EPS at scale can be resource-intensive; EPS composition variability affects NP reproducibility.	[25]
Algal / cyanobacterial extracts	Marine/freshwater algae provide pigments (chlorophylls, carotenoids), polysaccharides and phenolics that reduce and stabilize; sustainable marine biomass sources with unique surface chemistries.	Seasonal and location variability; potential co-extracted salts/metal ions require purification; ecological sourcing considerations (overharvesting).	[26]
Enzymes (e.g., laccases, keratinases)	Enzymatic reduction provides high selectivity and can proceed under mild conditions; enzymes can enable controlled nucleation and greener processing (biocatalysis).	Enzymes are sensitive to temperature/pH; cost and enzyme stability/immobilization are scale-up challenges; reaction rates can be slower vs chemical reducers.	[27]
Food-waste / agro-industrial extracts (tea, coffee, fruit peels, nutshells)	Valorizes waste streams, low cost, large supply; many waste extracts are rich in polyphenols and pigments that reduce and cap AgNPs — attractive for circular economy approaches.	Chemical complexity & contaminants (pesticides) can vary; safety certification needed for biomedical uses; reproducibility issues across waste batches.	[28]
Glycosaminoglycans / sulfated polysaccharides (e.g., alginate, carrageenan)	Provide ionic stabilization and can template anisotropic growth; often biocompatible and useful in biomedical hydrogels with embedded AgNPs.	Strong ionic character can be sensitive to salt concentration and pH; batch quality (degree of sulfation) affects results.	[29]
Polyphenol-enriched fractions (tea catechins, tannins, gallic acid)	Very strong reducing potential; produce small, often monodisperse AgNPs with intrinsic bioactivity (antioxidant/antimicrobial).	High reactivity may cause rapid nucleation leading to broad polydispersity unless conditions controlled; adsorption of tannins can alter surface chemistry.	[21]

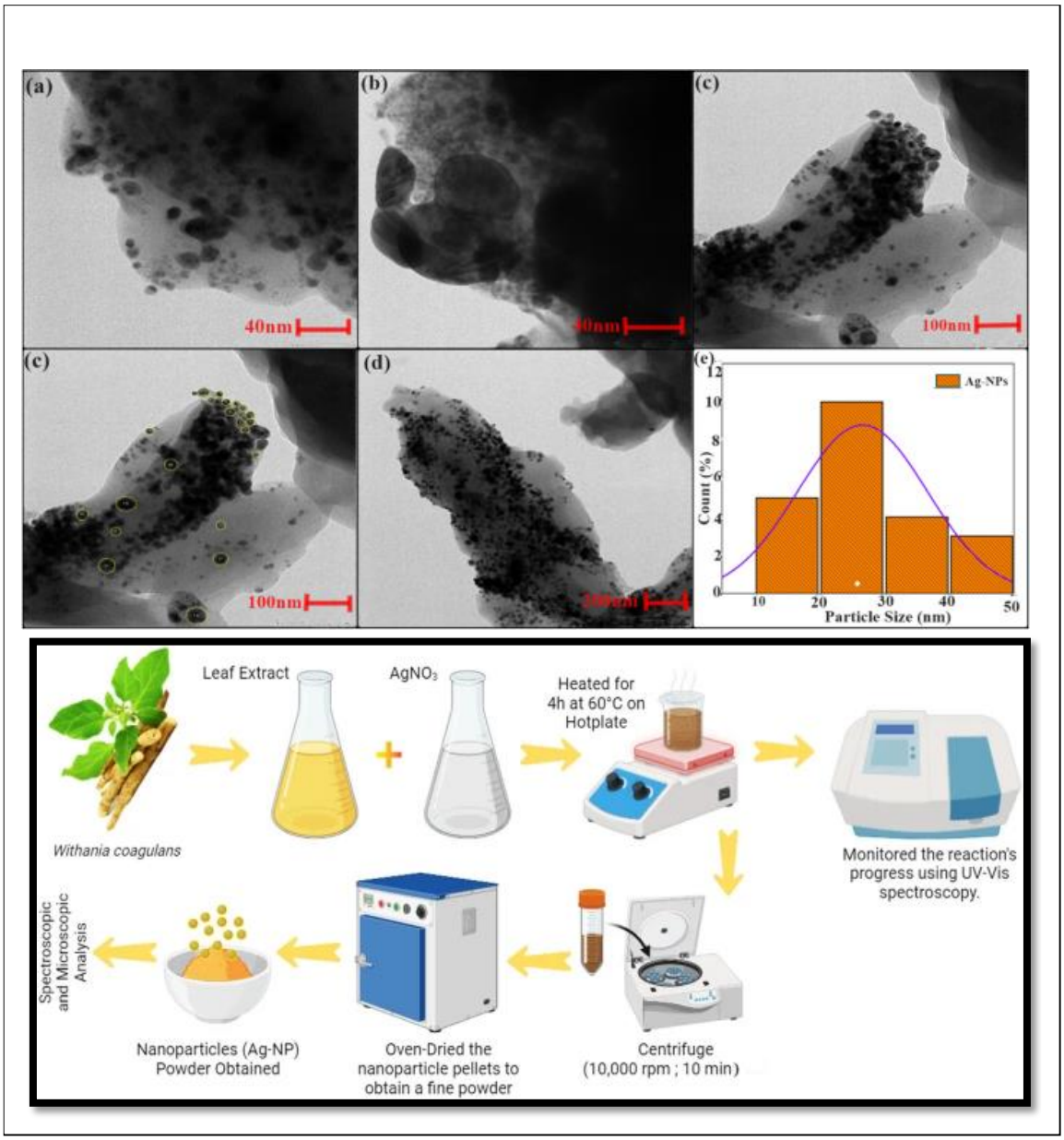

Figure 2. HR-TEM images of Ag-NPs at various magnifications (a-d) and e Particle distribution size of the Ag-NPs. (f) Workflow for green synthesis of Ag-NPs using W. coagulans leaf extract [30]

In order to enhance yield and antioxidant content, another recent study used response-surface approach to optimize AgNP synthesis via saw palmetto seed phenolic extract (SPS-phenolic extract). In comparison to the extract alone, the resultant AgNPs exhibited significantly enhanced antibacterial efficacy against both Gram-positive Staphylococcus aureus and Gram-negative Escherichia coli, with sizes ranging from approximately 11.2 to 38.3 nm and a strong negative zeta potential (≈ -32.8 mV) [31]. These examples show how important nanoparticle properties are influenced by plant selection, plant part, extract concentration, and experimental strategy (e.g., optimization). Comparative analysis of different plant-mediated studies shows that leaves are the most frequent source of extracts, likely due to ease of formulation and relatively high content of reducing/stabilizing phytochemicals. Buds, seeds, and flowers are less common but, in some cases, yield smaller, more monodisperse particles, possibly because they contain more specialized secondary metabolites. For example, *Psidium guajava* leaf extract yielded almost spherical AgNPs of ~12 nm, with strong antimicrobial coatings on textiles, whereas other plants in larger-leaf extract studies produced larger average sizes (>20 nm) with broader size distributions [32].

Phytochemical profiling is increasingly emphasized in recent literature, not just to confirm presence of potential reducing/stabilizing agents but to quantify them and correlate them with nanoparticle outcomes (size, morphology, stability, bioactivity). In the *Withania coagulans* study, total phenolic content

(TPC), total flavonoid content (TFC), antioxidant activity (DPPH, FRAP) etc., were measured for both extract and the resulting AgNPs; these parameters showed that while extract had higher TPC/TFC, AgNPs often displayed enhanced antioxidant capacity, suggesting synergistic effects between metallic silver core and the capping phytochemicals [30].

Important plant-mediated synthesis advances also address stability (colloidal, thermal), reproducibility, and biological safety. Silver nanoparticles (AgNPs) produced from saw palmetto seed extract demonstrated promising outcomes. TGA study verified the exceptional thermal stability of these particles. Additionally, their zeta potential was sufficiently negative. They are prevented from clumping together by this negative charge [31]. Another work synthesized AgNPs using Withania coagulans leaf extract. These particles exhibited mild cytotoxicity, according to safety tests such as hemolysis and brine shrimp assays. However, for many medical uses, this amount of toxicity is frequently tolerable [30]. Finally, plant-based synthesis has been increasingly popular in recent years. It is no longer merely a fundamental concept. These days, researchers employ more streamlined, comparative, and quantitative techniques. The ideal plant species, the appropriate plant portion, and the ideal extract concentration are now frequently the focus of scientific attention. The structure, optical characteristics, surface chemistry, stability and biological activity of the particles are also thoroughly investigated. However, there are still a few major issues. Results from many studies are hard to standardize. This entails standardizing the preparation of the extract and the reporting of its chemical constituents. It is still difficult to control the variance in particle size (polydispersity). More effort is also needed to scale up production while maintaining product consistency and biosafety.

3.2 Microbe-Mediated Synthesis

A diverse and biologically rich toolset for the environmentally friendly synthesis of silver nanoparticles (AgNPs) is provided by microbial systems, which include bacteria, fungi (including yeasts and marine fungi) and actinomycetes. Their metabolic, enzymatic and extracellular biomolecular machinery can stabilize the resulting nanoparticles and reduce silver ions, which makes them useful. Compared to plant extracts, they frequently produce narrow size distributions, unusual morphologies, higher yields and possibly more controllable processes. Particularly, microbial mediated synthesis can be executed either intracellularly (within cells) or extracellularly (in culture supernatant or secreted biomolecules), with the latter being more favourable for downstream processing, scalability and safety.

In recent years, many studies have demonstrated successful extracellular biosynthesis of AgNPs using fungal endophytes. For example, *Penicillium oxalicum* was used to synthesize AgNPs that exhibited potent antioxidant and antibacterial activity (Figure 3).

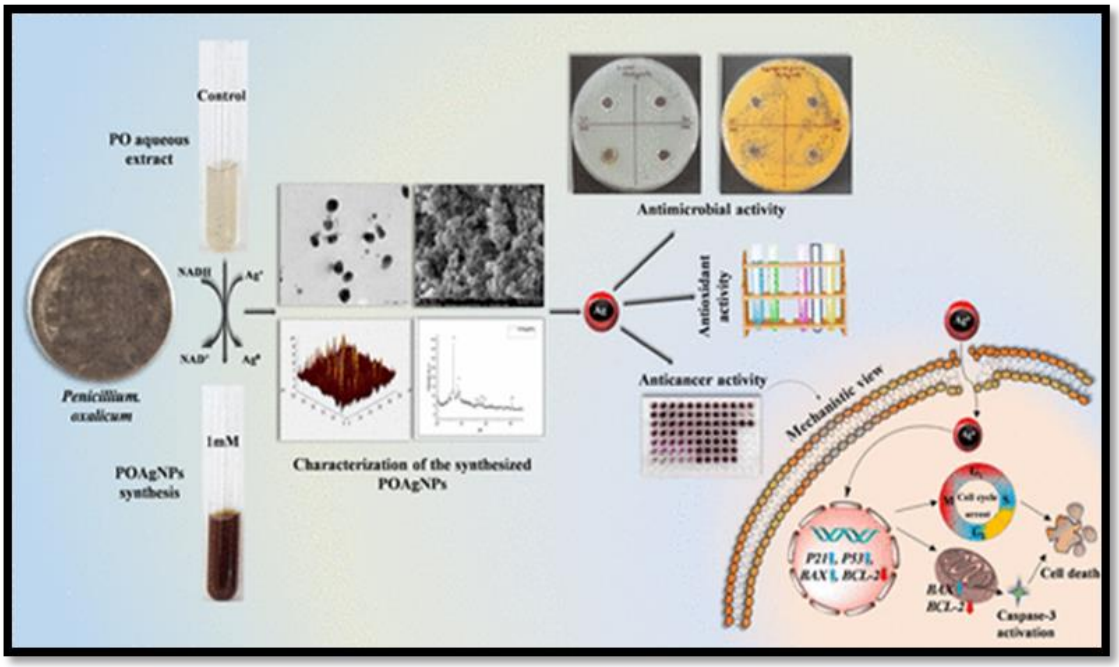

Figure 3. Biosynthesis of AgNPs using Penicillium oxalicum for antioxidant and antibacterial applications [33]

The nanoparticles were spherical, with diameters in the range of ~20-40 nm, and their activity was linked to fungal enzymatic reduction and secreted proteins/peptides acting as capping agents [33]. Marine fungi also play a crucial role: a study using marine fungal strains achieved AgNPs with strong antimicrobial activity against human pathogens; parameter control (pH, salt concentration) and fungal strain selection allowed tuning of both stability and bioactivity [34]. Additionally, endophytic bacteria and actinomycetes isolated from plant tissues have been shown to produce AgNPs extracellularly, with substantial antimicrobial activity even against multidrug-resistant bacteria and pathogenic fungi. For example, bacteria from Proteobacteria and Actinomycetes families (from olive and walnut-associated microbiomes) were used to synthesize AgNPs that inhibited *Staphylococcus*, *Escherichia*, *Acinetobacter*, *Candida*, and multiple plant pathogenic fungi, demonstrating the broad spectrum of activity possible using microbial mediated nanoparticles [35].

Intracellular and extracellular pathways are the two primary techniques used in microbe-based synthesis. Each has unique benefits and drawbacks. Very pure, frequently well-formed nanoparticles are produced using the intracellular approach. But first the cell walls must be broken, and then the particles must be cleaned. This makes the process more complicated. Extracellular synthesis, on the other hand, makes use of proteins, carbohydrates, or enzymes that the microorganism releases beyond the cell. Particle isolation is greatly facilitated by this. The likelihood of large-scale production may also be increased by using this method. According to research, Pseudomonas indica produces extracellular AgNPs that are effective against the fungi that cause mucormycosis. Additionally, these particles exhibit strong antioxidant qualities. Their potential for medical treatment is highlighted by this [36]. Likewise, synthesis is frequently carried out outside of the cell by marine fungi. Because of this, further processing stages are rather straightforward [34].

For the greatest outcomes, researchers must modify a few crucial elements in microbe-based synthesis. These variables include the growth phase and the type of microbe strain (name, habitat, and metabolism). The culture conditions, including temperature, food source, pH level, and oxygen supply, are also crucial. Additionally important are the incubation period and the silver salt concentration. In order to guarantee particle stability, researchers frequently use the proteins or metabolites that the microorganism secretes. A study that used bacteria from walnut and olive sources, for instance, discovered that different bacterial species produced different numbers of nanoparticles, distinct sizes of particles, and different capacities to kill microorganisms. The bacterial strain identity and source (endophytic vs free living) significantly impacted nanoparticle size, polydispersity, stability (zeta potential), and antimicrobial potency [35]. Similarly, *Myco-Synthesis of Silver Nanoparticles using marine fungi* illustrated that marine vs terrestrial fungal species, and the ionic strength of the medium, influenced NP shape (globular vs more irregular), average size (often in the 10-60 nm range), and stability under saline conditions [34].

Comparatively, microbes often afford better size control and higher monodispersity than many plant extract based methods, in part because microbial cultures can maintain relatively consistent enzymatic secretion profiles. For example, the *Marine fungi* study had mean particle diameters with narrower standard deviation than many plant extract studies of similar silver precursor concentration. However, microbial methods are not devoid of challenges: longer incubation times, risk of contamination, need for strict culture sterility, potential pathogenicity or biosafety issues (especially when working with opportunistic pathogens), and sometimes lower yields unless processes are optimized or genetically engineered.

In terms of applications, microbe-mediated AgNPs have demonstrated strong antibacterial, antifungal, antioxidant, and even antifungal immunomodulatory effects. Even against fungi implicated in mucormycosis, the AgNPs produced from Pseudomonas indica shown exceptional efficacy, suggesting potential for use in therapeutic or medical settings [36]. The approach has cross-disciplinary importance, as evidenced by the testing of other microbially produced AgNPs for their ability to inhibit biofilms, manage plant diseases, and act as antibacterial agents in food safety situations. To sum up, microbe-mediated synthesis is a potent environmentally friendly method for producing AgNPs, with advantages in controllability, scalability, and a variety of functional uses. However, for translation into commercial, medicinal or environmental applications, standardization of strain characterisation, process repeatability, safety and yield is still essential.

3.3 Algal and Cyanobacterial Synthesis

Green synthesis has made algae and cyanobacteria attractive options for producing silver nanoparticles (AgNPs).

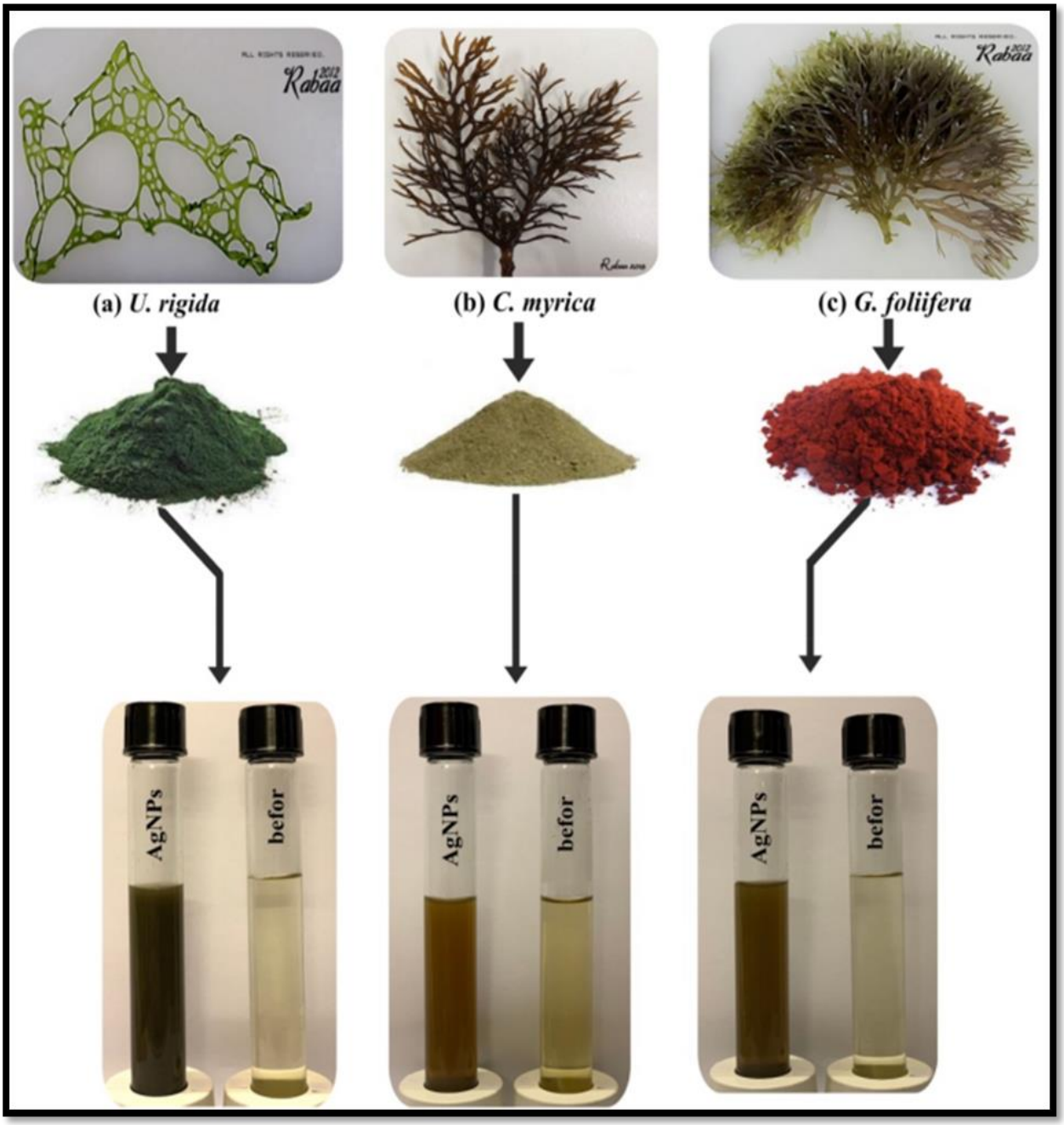

Figure 4. Biosynthesis of AgNPs using different marine algal extracts, **(a)** U. rigida, **(b)** C. myrica, and **(c)** G. foliifera, as reducing and capping agents [37].

These organisms have a wide variety of metabolites, including proteins, lipids, polysaccharides, pigments, and sulfated substances. These substances aid in stabilizing the new nanoparticles and lowering the silver. Algae may also withstand high metal buildup levels. Both freshwater and marine macroalgae, such as seaweeds, and microalgae, such as cyanobacteria, have been employed. AgNPs with favorable physical, chemical, and biological characteristics are produced by them. Because algae are simple to gather and prepare as biomass, using them for this procedure makes sense. Due to the natural chemicals found in the algae, the resultant nanoparticles also exhibit good bioactivity. Additionally, this approach is still eco-friendly.

Algotiml *et al.* (2022) used marine algal extracts to create silver nanoparticles (AgNPs). Three varieties were employed as shown in figure 4: the green alga Ulva rigida, the brown alga Cystoseira myrica, and the red alga Gracilaria foliifera. These extracts served as capping and reducing agents. The spherical AgNPs from U. rigida measured around 12 nm. The spherical particles from C. myrica measured around 17 nm. G. foliifera produced particles that were somewhat larger—about 24 nm. Chemical groups such as hydroxyl (-OH), carbonyl (C=O), and amide groups assisted in binding the particles, according to FTIR studies. These AgNPs demonstrated potent anti-cancer properties in a biological setting. For instance, 92.6% of MCF-7 breast cancer cells were destroyed by AgNPs

capped with U. rigida extract. They also worked well against germs and fungus. At moderate dosages, the study discovered little harm to either normal cells or Artemia salina nauplii [37]. Sargassum coreanum extract, a marine macroalga, was used in another example. AgNPs were created by this extract's reduction of silver nitrate ($AgNO_3$). These particles were employed by researchers to catalyze the degradation of methylene blue dye. The resultant AgNPs dispersed readily in liquid and were spherical, measuring roughly 19 nm in size. Under ideal circumstances, they demonstrated exceptional catalytic efficiency, breaking down more than 99% of the dye [38].

According to mechanistic research, cyanobacteria and algae mostly produce AgNPs via an extracellular process. Numerous chemicals can be found in algae extracts or the water they grow in. These consist of proteins, sulfated polymers, polysaccharides, pigments (such as carotenoids and chlorophylls), and other secondary metabolites. These substances lower the silver ions, which initiates the growth and production of particles. The particles are then frequently capped by the same biomolecules. In addition to preventing clumping and maintaining particle stability in liquid, this coating also modifies the zeta potential, or surface charge. These extract-derived capping agents offer both physical and charge-based stability. Additionally, the quantity and kind of these metabolites are related to the size variation observed among various algae species. Under comparable circumstances, species with higher levels of polysaccharides and phenolic/tannin content typically create smaller, more homogeneous AgNPs [37].

Environmental and procedural parameters also strongly modulate the outcome. Factors such as extract concentration, pH, temperature, light exposure, silver precursor concentration, reaction time, and even agitation influence the kinetics of reduction and subsequent growth stages. For example, in the *Sargassum coreanum* work, heating of the extract and maintaining reaction at ~25 °C with moderate extract:$AgNO_3$ ratios yielded spherical AgNPs of ~19 nm; changes in temperature or extract amount shifted both rate of reduction (visible via UV-Vis SPR peak intensities) and the size distribution [38]. Similarly, *Sargassum ilicifolium* was used in a sunlight-induced synthesis where water-soluble biomolecules (such as alginate or fucoidan) played significant roles; reaction under sunlight accelerated formation, and average nanoparticle size was ~25.7 nm with stable colloidal behaviour [39].

Comparatively, algal and cyanobacterial syntheses often yield smaller particles than many crude plant extract methods under similar precursor concentrations, and with better-defined morphology due to the uniformity of metabolites in algal extracts. They also tend to have better biocompatibility, perhaps because many algal capping agents are themselves biologically tolerated (e.g. polysaccharides, proteins). On the other hand, challenges include slower reaction times (often hours to days), variability due to seasonal or geographical differences in algal metabolite composition, potential difficulty in quantifying the bioactive reducing/capping agent contributions, and often relatively lower yields unless optimized. A more sophisticated and environmentally friendly method of producing silver nanoparticles (AgNPs) is now provided by algae and cyanobacteria. In this field, scientists have advanced significantly. They now identify the ideal reaction conditions, have a better understanding of the extract's composition, and exhibit interesting applications. These applications include serving as catalysts and combating bacteria and cancer. Future research must concentrate on a few crucial areas. The extract preparation and active metabolite measurement methods must be standardized by researchers. They need to figure out how to increase production. Additionally, they must guarantee that the outcomes can be replicated in any setting or season. Lastly, they need to research how safe and stable these particles are inside the body over the long run. By addressing these issues, the promise will be transferred from the lab to actual industrial or clinical use.

3.4 Other Biological Routes

Researchers are presently investigating more regulated biological processes for producing green silver nanoparticles (AgNPs). These techniques go beyond the use of entire organisms, such as algae, bacteria, and plants. They pay particular attention to tactics that employ biopolymers or enzymes. There are numerous advantages to these new techniques. They enable simple modifications (modularity). The designs can be altered, and the results are repeatable. This gives scientists more control over the characteristics of the particle. Additionally, industrial production possibilities may increase and the chemical aids are reusable.

Isolated enzymes, not entire cells or extracts, are used in enzyme production. The reduction of silver ions is accelerated by the enzymes. They also aid in stabilizing or capping the novel nanoparticles.

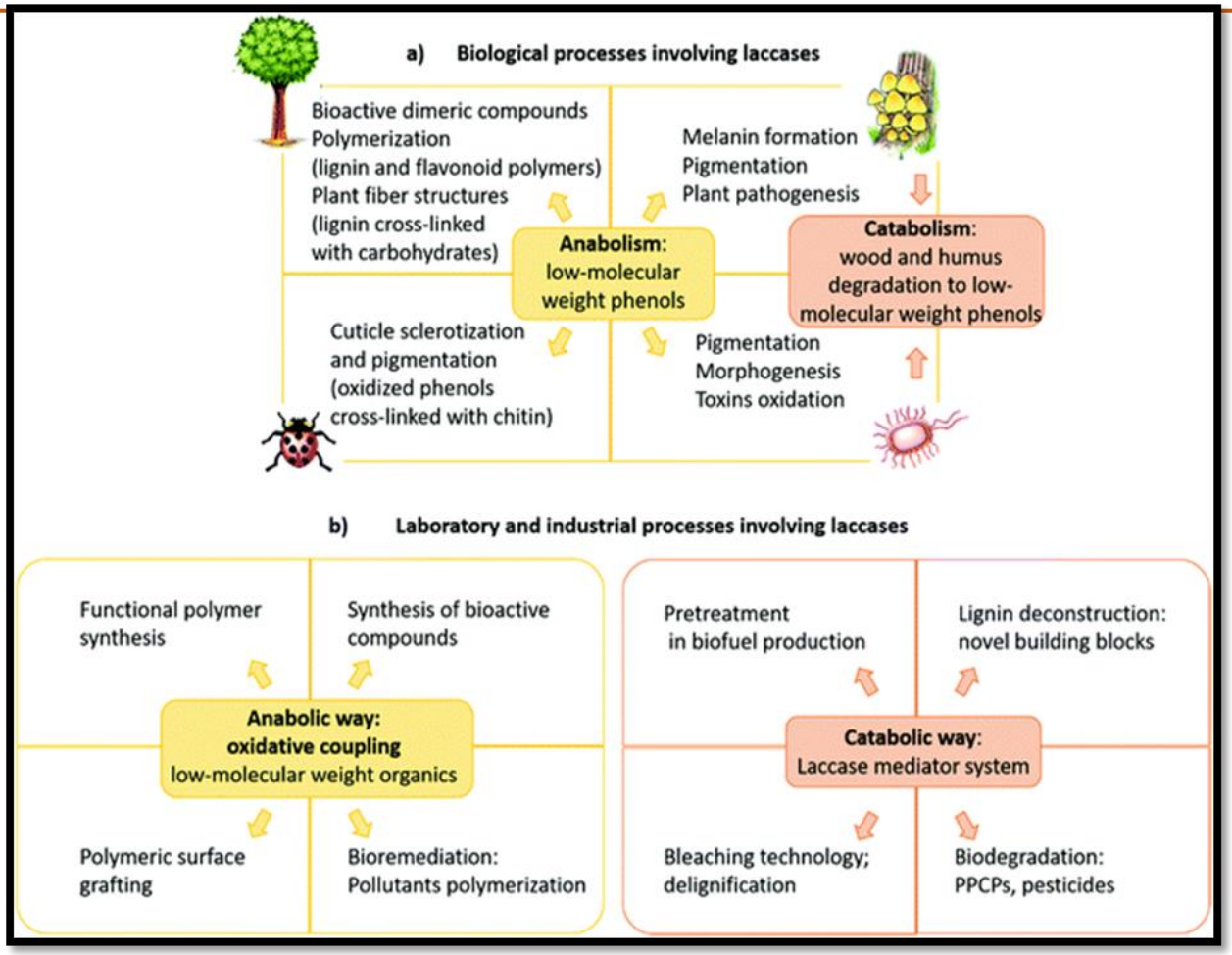

Figure 5. Role of laccases **(a)** Biological **(b)** laboratory and industrial processes involving laccases [27].

Keratinase from Pseudomonas aeruginosa C1M was utilized in a significant recent example. This enzyme stabilized and decreased the silver, among other things. At normal temperature, it converted Ag + ions to Ag 0 metal. Stable AgNPs were produced by the technique. Around 450 nm, these particles displayed UV–Vis absorption. These AgNPs coated with enzymes demonstrated strong antimicrobial activity. They also aided in the breakdown of numerous azo dyes. The particles' dual utility in environmental and health applications is demonstrated by this [40]. The chemical process and the intricate cell environment are separated by enzyme catalysis. This could lessen the variability of the results. Additionally, it facilitates the last cleanup. Scientists can better regulate the underlying mechanism with this strategy.

Another dimension of enzyme-mediated approaches involves oxidative enzymes like laccases, which have been widely used in green synthesis and biocatalysis. While direct reports of laccase-driven AgNP synthesis remain more limited in the recent years, the broader literature recognizes their potential in catalyzing phenolic oxidations and redox coupling, which could serve to reduce silver ions in suitably engineered systems [27].The advantage of leveraging well-characterized enzyme systems is the ability to engineer enzyme variants or immobilize them on supports to control local reaction microenvironments, which could finely regulate nucleation versus growth, particle size, and polydispersity. The role of laccases in Nature varies with the organism and they participate in both anabolic and catabolic processes (Figure 5).

The biopolymer-mediated strategy complements enzyme techniques. This method makes use of natural polymers such as proteins, starch, cellulose derivatives, and chitosan. These substances serve as stabilizing scaffolds, reducing agents, or support structures. Biopolymers offer electrical stability as well as physical bulk (steric hindrance). Additionally, they have local control over the release of chemical lowering agents. Chitosan–AgNP nanocomposites are an excellent example. In this case, chitosan serves as an antibacterial matrix in addition to stabilizing the particles. This concept was applied in a recent study to create antibacterial coatings. Researchers changed the pH level (3–5) and the chitosan concentration (0.5–2%). As a result, they were able to regulate the size fluctuation over 21 days as well as the stability of the nanocomposite in liquid.

Hermosilla *et al.* (2023) produced chitosan fungal beads in another study. A system of fungal extract was present in these beads. The procedure produced reusable silver-chitosan nanoparticles. Even after numerous uses, these particles maintained their antimicrobial properties, enhancing sustainability [41]. Fungal metabolites decreased the silver ions in that investigation. The particles were then retained and stabilized by the chitosan framework. In addition to making reuse simpler, this architecture stopped the nanoparticles from simply dispersing into the solution.

Particle formation is significantly influenced by the biopolymer matrix's composition. The degree of deacetylation (for chitosan), temperature, ionic strength, pH, and the molecular weight of the polymer all affect how nanoparticles nucleate and develop. Many functional groups, such as -NH_2, -OH, and -COOH, are frequently found in biopolymers. These clusters can serve as growth initiators and draw in silver ions. The particle growth may be slowed down or localized as a result. The resulting nanoparticles' homogeneity and size consistency are also determined by how effectively the silver ions migrate within the polymer and how mobile the reducing chemicals are. Over time, biopolymer scaffolds physically prevent particles from clumping together. Their stability in liquid is enhanced as a result. According to reports, compared to simple AgNPs formed only of plant or microbial extracts, chitosan–AgNP systems frequently have smaller average sizes and more uniform sizes. It is still challenging to get incredibly uniform sizes (less than 5% size variance).

There are several trade-offs when comparing enzyme and biopolymer techniques to extracts from whole organisms. Cleaner systems with fewer interfering molecules are produced by the enzymatic method. It provides greater consistency and reusability. Enzymes, however, can be expensive. When the concentration of silver is high, they may also exhibit limited activity. Biopolymer systems are frequently affordable and provide excellent stability and support. However, they might restrict material flow (diffusion restrictions). Additionally, they vary according to the source of the polymer. In crude extracts, the reduction reaction can occasionally be slower than that of highly reactive compounds. Enzyme and biopolymer systems must be carefully scaled up for industrial usage. Enzyme stability, polymer source, effective material movement, and system design are all factors that researchers must take into account.

These controlled biological techniques are promising for applications requiring stable, reusable nanoparticle platforms. Continuous flow reactors, sensor layers, catalysis, and antimicrobial coatings are a few examples of applications. The potential applications of the keratinase-based system extend beyond its ability to combat microorganisms, as evidenced by its capacity to degrade dye [40]. Reusable chitosan–silver composites indicate potential for use in goods that require repeated use, such as filtration membranes or wound dressings [41]. Finally, alternative biological pathways are an intriguing topic for green AgNP synthesis, particularly enzyme and biopolymer approaches. Better control, reusability, scaffold stabilization, and integration into surfaces or devices are all made possible by them. Enhancing the reaction time is the next phase. Additionally, they must strengthen the polymer or enzyme during synthesis conditions. The largest obstacle to overcome is scaling up these systems without sacrificing control over particle quality or performance.

4. Mechanistic Insights into Green Synthesis

It is critical to comprehend how the green production of silver nanoparticles (AgNPs) operates. Scientists can better regulate the particles' physicochemical characteristics thanks to this knowledge. These characteristics include surface chemistry, crystallinity, size, and form. A thorough comprehension also contributes to more reproducible (constant) outcomes. This is essential for increasing production volume and customizing the particles for certain applications. Recent literature has significantly advanced our knowledge in four interconnected areas: molecular interactions in reduction and stabilization; functional group roles (–OH, –COOH, –NH_2, –SH); electron transfer pathways and nucleation-growth kinetics; and in-situ spectroscopic/theoretical modeling to elucidate mechanistic pathways.

At the molecular level, a large body of work has clarified how phytochemicals and other biological molecules act as reducing and capping agents. Phenolic compounds, flavonoids, terpenoids, alkaloids, proteins, and polysaccharides commonly donate electrons (or hydrogen atoms) to reduce Ag^+ ions to Ag^0, while simultaneously oxidizing themselves (often to quinones or analogous oxidized forms) which then participate in capping or stabilizing.

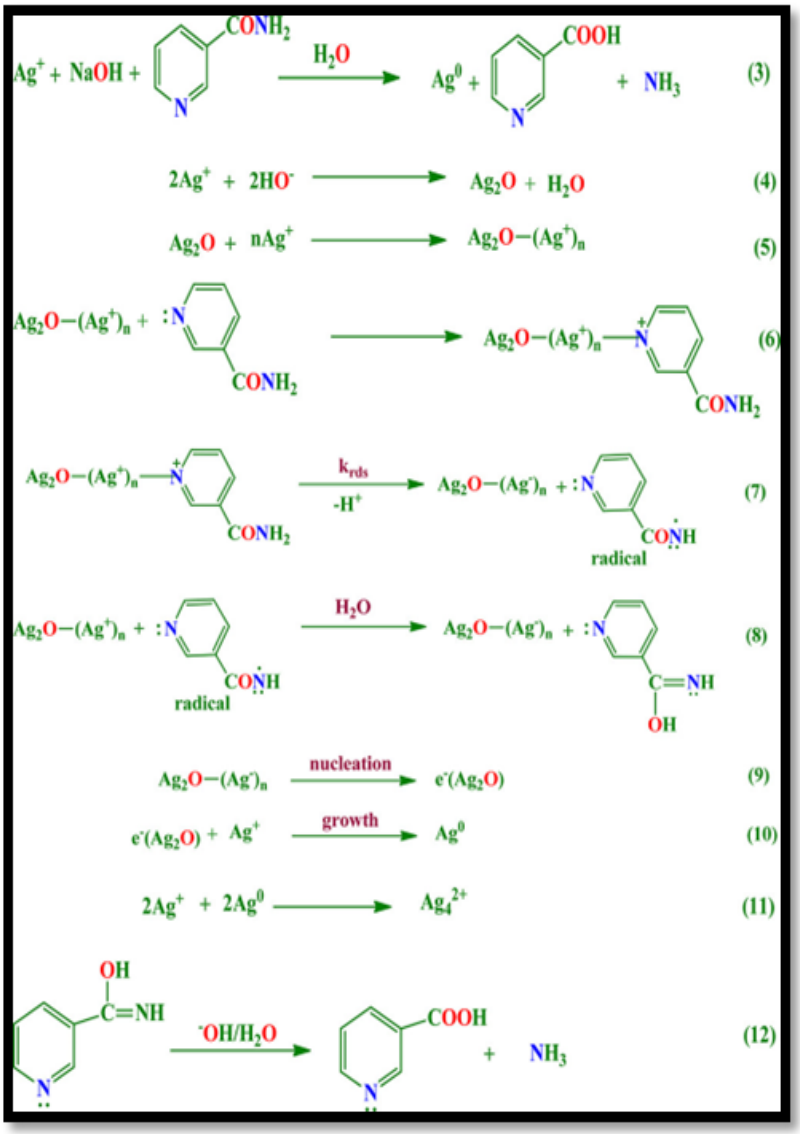

Figure 6. Most Plausible Mechanism Proposed for the Synthesis of Silver Nanoparticles [45]

Ritu *et al.* demonstrated that in phytochemical-based AgNP synthesis, functional groups such as phenolic –OH and carbonyl (C=O), as well as amino groups, are principally responsible both for reduction and stabilization, with characteristic shifts in FTIR bands (e.g. –OH stretching, C=O bending) as AgNP formation proceeds [42]. In studies using ginger extract, FTIR analysis revealed peaks shifting and broadening in the amide I (protein) and phenolic regions, indicating involvement of free amine groups (e.g., from proteins or amino acids) and phenolic hydroxyl in Ag^+ reduction and in capping the resulting Ag^0 surface against aggregation [43].

Finding functional groups is complemented by kinetic and mechanistic research. These investigations aid in the explanation of how the process is impacted by modifications to the reaction conditions. For example, the rate of reduction, particle formation, growth, and final shape are all affected by changes in pH, temperature, reducing agent concentration or silver precursor. The study that used kiwifruit peel extract (Actinidia deliciosa) is a nice example. The UV-Vis SPR (surface plasmon resonance) band grew stronger and moved toward blue as the reaction temperature increased. These modifications indicate that the initial particle creation (nucleation) occurred more quickly and that the resulting silver nanoparticles were smaller. In that system, the mechanistic scheme proposed involves first complexation of silver ions with phenolic (phenoxide) groups, then electron donation resulting in Ag^0 seeds (nucleation), followed by growth where phenolic hydroxyl groups oxidize to quinone-like forms that then serve both as capping/stabilizing moieties [44]. Another example is the detailed mechanistic and kinetic study using nicotinamide as a reducing agent and CTAB as a stabilizer: here, the reaction's dependence on silver ion concentration, base concentration (NaOH), and stabilizer concentration was quantitatively explored, and pseudo-first-order kinetics was observed for many conditions; the mechanistic

proposal involved intermediate silver oxide or hydroxo-silver species that convert to Ag^0, and reduction steps mediated by nicotinamide oxidation, with CTAB assisting stabilization and dispersion [45]. The overall reaction that actually takes place during the SNPs colloid formation is shown in Figure 6**.** According to this mechanism, small-sized silver nanoparticles are formed through the reaction onto the surface of Ag_2O.

Electron-transfer pathways have been more precisely probed via spectroscopic and computational techniques. Plots of absorbance vs time (UV-Vis) coupled with FTIR reveal changes in bond strengths and shifts that correlate temporally with nucleation phase onset and subsequent growth. For instance, in studies with Azadirachta indica leaf extract, monitoring of SPR peak emergence was accompanied by FTIR shifts in protein/amine and phenolic bands, supporting a model in which electron donation occurs from phenolic and amino groups, with capping by proteins to stabilize Ag^0 cores [46]. While full density functional theory (DFT) or molecular docking studies specifically on green-mediated AgNP synthesis are comparatively sparse to date, some "in silico" annotation alongside empirical work (e.g. in the Azadirachta indica study) has been used to model interactions between silver ion surfaces and donating ligands (phenolic, flavonoid), showing favourable binding energies and plausible geometries for stable capping.

The nucleation-growth kinetics of green AgNP formation often follow classical models but with modifications due to biological complexity. In many reports, initial rapid nucleation is followed by slower growth; reaction progress monitored by UV-Vis shows that SPR band intensity increases rapidly then stabilizes. In the kiwifruit peel system, higher temperature and greater reducing agent concentration produced many small nuclei, leading to smaller mean particle size; lower temperature or lower extract concentration resulted in fewer nuclei but larger growth. Such observations, together with the fact that particle size often plateaus after a certain reaction time irrespective of further increases in precursor or reducing agent, suggest that capping molecule saturation, depletion of reducing power, or diffusion limitations intervene as growth proceeds.

Some works have also addressed temporally resolved in-situ spectroscopic insights. For example, FTIR analysis before, during, and after nanoparticle formation shows shifting and attenuation of specific functional group peaks (e.g. –OH, –NH, C=O) as they bind or oxidize; UV-Vis tracking of SPR peak position over time gives insight into nucleation and growth phases; dynamic light scattering (DLS) gives evolving size distributions. A study on ginger-derived AgNPs tracked such changes, showing that functional groups' spectral features shift (due to bonding / adsorption) concomitantly with the emergence of a clear SPR peak, allowing mapping of onset of nucleation [43].

Collectively, these mechanistic studies enable formulation of comparative insights: plant extracts rich in phenolic hydroxyls and low molecular weight proteins tend to produce faster nucleation and smaller, more uniform nanoparticles; higher temperature, alkaline pH, and high reducing agent concentration enhance reduction kinetics but risk uncontrolled growth unless effective capping is present. Systems with stronger binding ligands (e.g. multiple –OH / –NH groups) tend to achieve better colloidal stability (higher absolute zeta potentials) and longer shelf-life, sometimes at cost of slower growth. In summary, mechanistic understanding of green AgNP synthesis has advanced substantially, and current studies coherently show that the interplay among reducing power of biological molecules, their functional group availability, reaction environment (temperature, pH, concentration), and stabilization capacity of capping molecules collectively governs nanoparticle formation. However, there are still some important knowledge gaps. To completely comprehend how the capping molecules interact with the silver surface, researchers must carry out further in-depth computational modeling (such as DFT or molecular dynamics). Additionally, they must employ time-resolved in-situ spectroscopies (e.g., real-time FTIR/Raman or synchrotron X-ray absorption). This will enable them to watch the reaction in real-world synthesis settings. Lastly, they must precisely measure kinetics in a wide range of biological systems. In order to develop predictive design procedures for synthesis, this phase is essential.

5. Characterization of Green-Synthesized AgNPs

Accurate and comprehensive characterisation of green-synthesized silver nanoparticles (AgNPs) is crucial. This stage validates that the synthesis was successful. It helps scientists comprehend the relationship between the particle's structure and its properties. Additionally, it guarantees that others can replicate the outcomes. Complex combinations of biological materials, such as extracts, enzymes or biopolymers, are frequently used in green synthesis.

Therefore, in order to thoroughly analyze the particles, scientists need to employ a variety of methodologies. This makes it easier to distinguish between the stabilizing agents' effects and the fundamental characteristics of the nanoparticle. Researchers have been using a lot of supplementary tools lately. These consist of surface chemical studies, scattering techniques, spectroscopy and microscopy. A comprehensive physical and chemical picture of the biological AgNPs is given by these instruments.

First and foremost, morphological and structural analysis via transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), and X-ray diffraction (XRD) remains foundational. TEM offers direct visualization of nanoparticle size, shape, lattice fringes, crystallinity, and dispersion at high resolution; for example, green-synthesized AgNPs using *Lythrum salicaria* extract exhibited spherical particles ~40 nm in diameter in TEM images, corroborating SPR spectral features [47]. In some works, high-resolution TEM (HRTEM) revealed lattice spacings corresponding to the (111) plane of face-centered cubic silver, confirming crystallinity. SEM complements this with surface morphology and agglomeration patterns over larger fields; when combined with energy dispersive X-ray spectroscopy (EDX), elemental silver peaks (~3 keV) can confirm elemental composition. XRD analysis further establishes crystalline phases: characteristic diffraction peaks corresponding to Ag (111), (200), (220), and (311) planes validate the metallic nature and often allow estimation of average crystallite sizes using the Scherrer equation. In the broader review of green AgNP routes, Asif et al. describe how such structural and morphological techniques are standard in confirming nanoparticle formation, phase purity, and faceting [48]. AFM, though less widely used, complements TEM/SEM by mapping surface topography at nanoscale resolution, helpful particularly when nanoparticles are deposited on substrates in sensor or surface applications.

Optical properties provide rapid, non-destructive evidence of nanoparticle formation and size/shape distribution through the phenomenon of surface plasmon resonance (SPR). UV–Visible absorption spectroscopy is a ubiquitous method: biogenic AgNP colloids typically present a distinct SPR band in the 380–450 nm range, and both intensity and peak position shift depending on nanoparticle size, shape, dielectric surroundings, and aggregation state. In the *Lythrum salicaria* example, the appearance of a pronounced absorption peak near 410 nm offered early confirmation of spherical AgNP formation [47]. Broadening or red-shifting of the SPR peak often correlates with increased polydispersity or aggregation; conversely, narrower peaks imply more monodisperse populations. Some studies also explore photoluminescence (PL) from biogenic AgNPs, particularly when trace biomolecules or defects act as luminescent centers, though such signals are weaker and less frequently reported in green synthesis literature.

Chemical and surface analysis is critical because green synthesis inherently involves biomolecular capping and surface modification. Fourier transform infrared spectroscopy (FTIR) is a standard tool to identify functional groups participating in reduction and capping of AgNPs: shifts in absorption bands corresponding to –OH, –COOH, –NH_2, C=O, C–O–C, and amide linkages between the extract (or capping molecule) and nanoparticle surfaces are interpreted as evidence of binding or coordination. The recent general review on FTIR in nanoparticle studies underscores its power—and limitations—in distinguishing contributions from adsorbates on metallic cores [49]. Following contact with AgNPs, scientists frequently report changes in hydroxyl stretching frequencies or the elimination of peaks in the biomolecule spectra. This validates that the capping was successful. In addition to FTIR, X-ray photoelectron spectroscopy (XPS) provides additional information. The elemental makeup and oxidation states at the surface of the nanoparticle are verified by XPS. The binding energies of metallic silver (Ag0) are often matched by the peaks for Ag 3d5/2 and 3d3/2. This attests to the silver's excellent reduction. Additionally, it aids in the detection of any residual silver oxide or capping agent interaction. The molecules affixed to the surface can be further investigated using Raman spectroscopy or surface-enhanced Raman scattering (SERS). When using biologically produced AgNPs for sensing, this is crucial. To stop the biomolecules from fluorescing or decomposing due to photodegradation, scientists must exercise caution.

Measurements of zeta potential and dynamic light scattering (DLS) offer general information on the behaviour of the nanoparticles in a liquid solution. The polydispersity index (PDI) and hydrodynamic diameter are provided by DLS. The particles have little clumping or a thin layer of solvent surrounding them if the DLS diameter is near (approximately 1.2 times) to the TEM size measurement.

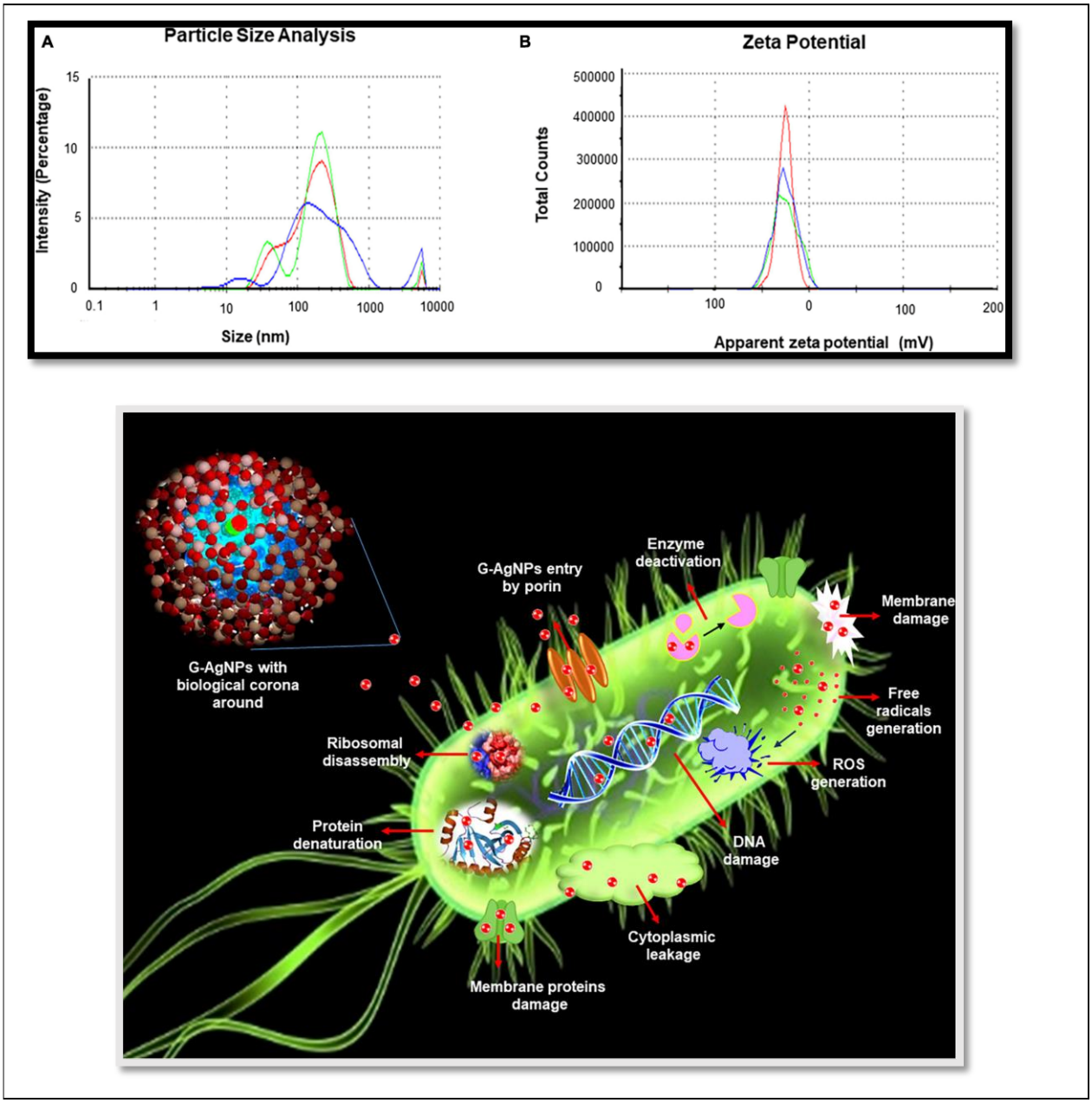

Figure 7. (a) nanoparticles distribution concerning size and intensity and **(b)** zeta potential of G-AgNPs representing highly negative surface charge. **(c)** Schematic representation of silver nanoparticles (AgNPs) antimicrobial mechanisms in Escherichia coli [50].

Significant aggregation or a thick hydration shell is suggested by a significantly greater DLS diameter. The particle's surface charge is indicated by the zeta potential. In general, higher absolute values ($|\zeta| \geq 20–30$ mV) indicate improved electrical stability. This lessens the chance of aggregation. For example, the study Strong Antimicrobial Activity of Silver Nanoparticles (figure 7c) discovered that the size of their green AgNPs, as determined by DLS, matched the shape data. Additionally, they have steady zeta potentials as shown in figure 7 [50]. Previous reviews suggest that DLS and zeta analysis are required to verify colloidal stability before starting any functional testing [51].

Because each single technique yields only partial insight, correlative characterization, combining multiple methods, is increasingly regarded as the gold standard. A robust approach might begin with UV–Vis to verify nanoparticle formation, TEM/SEM + XRD to elucidate size, shape, and crystallinity, FTIR/XPS to assign capping interactions, and DLS/zeta to assess colloidal health. Inclusion of EDX (elemental analysis) or inductively coupled plasma mass spectrometry (ICP-MS) further strengthens quantitative elemental confirmation (silver content, impurities). Over the recent literature, most successful reports use at least four or five complementary techniques to fully support claims of nanoparticle identity, purity, and stability.

In order to properly describe green-synthesized AgNPs, researchers must employ a multi-technique approach. The bar for proof has been raised by recent developments in the combination of scattering techniques, spectroscopic surface analysis and high-resolution imaging. But there are still difficulties. Separating the signal from the nanoparticle core from the capping biomolecules is still challenging. Accurately measuring trace levels of metal oxides or contaminants is similarly challenging. Researchers also need to standardize the stability and size variation measures for various biologically generated systems. Future research in this field will be of much higher quality if complementary methodologies are adequately integrated, all characterisation protocols (such as sample preparation and dilution) are fully reported, and cross-study comparison tables are used.

6. Functional Applications of Green-Synthesized AgNPs

6.1 Biomedical Applications

Silver nanoparticles (AgNPs) produced using green synthesis have emerged as potent medicinal instruments. Their unique physical and chemical characteristics are the cause of this. They naturally combat bacteria, have a changeable surface chemistry, and a high surface-to-volume ratio. Crucially, they frequently exhibit superior biocompatibility in contrast to AgNPs produced using harsh chemicals. The use of green AgNPs in medicine has expanded significantly over the past five years. They now combat viruses, fungus, and bacteria. They are employed in theranostics, which combines diagnosis and treatment, and anti-cancer therapies. They are also used by scientists to help heal wounds and regenerate tissue, as well as to administer drugs. Numerous studies attempt to strike the ideal balance between the particles' effectiveness and safety for the body in all of these applications.

One of the most intensively explored applications is antibacterial, antifungal, and antiviral therapy. Green AgNPs disrupt microbial viability via multiple pathways: generation of reactive oxygen species (ROS), disruption of cell membranes, interference with DNA replication, and protein inactivation. Because of this multimodal attack, they are effective against antibiotic-resistant bacteria and biofilms. A recent comprehensive review notes that green-synthesized AgNPs consistently outperform or synergize with conventional antibiotics or antifungals in in vitro assays, often reducing minimum inhibitory concentrations (MICs) of co-administered drugs [6]. For example, AgNPs synthesized via plant extracts have shown robust inhibition against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*Escherichia coli*) species, with low cytotoxicity in mammalian cell lines. At the same time, the proteomic mechanisms of action (e.g., membrane proteins, metabolic enzymes) are being increasingly elucidated, lending rational targets for nanoparticle design [52, 6].

In the anticancer and theranostic domain, green AgNPs are being engineered to selectively target tumor cells while minimizing normal cell damage through surface functionalization, controlled silver ion release, or synergistic drug conjugation. A recent study using Anzaroot plant extract–derived AgNPs demonstrated cytotoxic effects against MCF-7 breast cancer cells at low micromolar concentrations with negligible effects on healthy cells, attributing selective uptake and ROS generation as underlying mechanisms [53]. Another green synthesis of metal nanoparticles underscores their dual roles as therapeutic and contrast agents (e.g., photoacoustic, Raman labels) in integrated nanomedicine platforms [54]. However, translation to in vivo models remains limited: few studies report biodistribution, clearance, or long-term toxicity in animal systems, emphasizing a major future gap.

Drug delivery and nanoparticle-based carriers represent another exciting frontier. Green AgNPs, often capped with biological ligands (proteins, polysaccharides), can be further functionalized with targeting ligands (antibodies, peptides) for site-specific delivery. Because the green capping layers are often biocompatible and biodegradable, they can mitigate premature immunogenicity or cytotoxicity [6, 54]. In some instances, AgNPs are integrated into polymeric scaffolds, micelles, or hydrogels to control release kinetics and local retention, particularly in implantation or localized therapy contexts.

The P/CH/AgNPs composite film was successfully synthesized, as detailed in Figure 8a, and subsequently characterized for its biological efficacy. The film demonstrated broad-spectrum antibacterial activity (Figure 8b), effectively inhibiting the growth of both Gram-positive (S. aureus and B. cereus) and Gram-negative (P. aeruginosa and K. pneumoniae) pathogens. Furthermore, the in vivo studies confirmed its potential for wound healing.

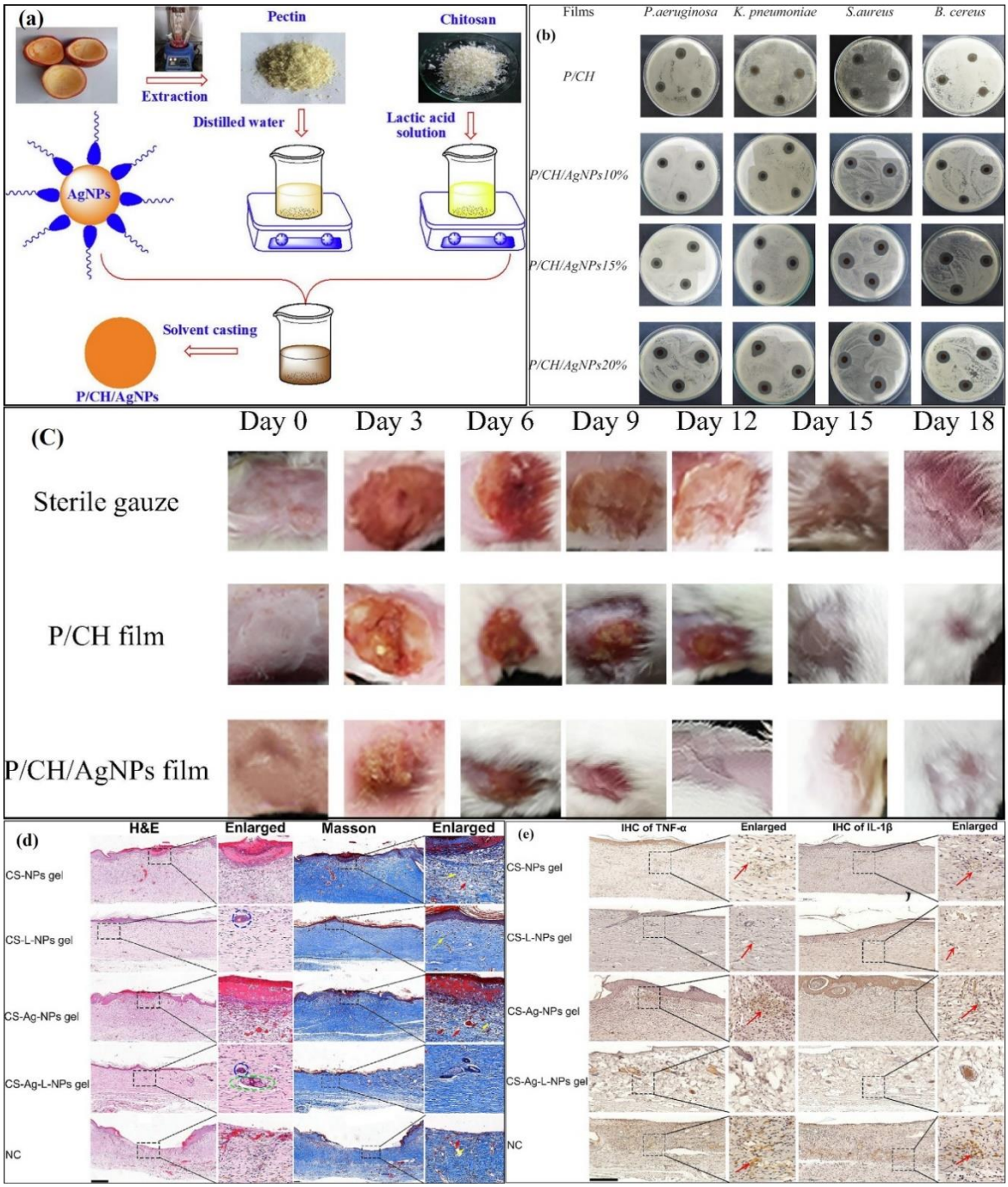

Figure 8 (a) A preparation process of P/CH/AgNPs; **(b)** Antibacterial activities of S. aureus, B. cereus, P. aeruginosa and K. pneumoniae of P/CH/AgNPs; **(c)** Photographic images of wounds treated with strerile gauze, P/CH and P/CH/AgNPs composite film [55]; **(d)** Histological analysis of wound tissues of different groups with H&E and Masson stain Bar 400 μm ; **(e)** Immunohistochemistry of TNF-α and IL-1β in wound tissues of different groups Bar 200 μm [56].

Photographic images (Figure 8c) clearly illustrate the enhanced rate of wound closure in groups treated with the P/CH/AgNPs composite compared to both the sterile gauze and P/CH controls [55]. This accelerated healing was supported by histological analysis (Figure 8d), where H&E and Masson staining revealed improved tissue regeneration and greater collagen deposition in the P/CH/AgNPs group. Crucially, the material also modulated the inflammatory response, as shown by immunohistochemistry (Figure 8e), which indicated a decreased expression of the pro-inflammatory cytokines TNF-α and IL-1β in the treated wound tissues [56].

Among the most immediate translational applications is wound healing and tissue regeneration. Here, green-synthesized AgNPs support healing by simultaneously suppressing infection, modulating inflammation, and promoting cell proliferation and angiogenesis. A study formulated a hydrogel incorporating Cyperus rotundus–derived AgNPs,

demonstrating rapid wound contraction, improved re-epithelialization, and bacterial suppression in *in vivo* models [57]. Another recent work of Nandhini showed that AgNPs accelerate wound closure while regulating inflammatory cytokines and collagen deposition [58]. Additionally, grafted chitosan–AgNP hydrogels have shown excellent healing in chronic wound models, maintaining antibacterial efficacy yet controlled silver release and minimal cytotoxicity [59].

The efficacy of AgNPs is evident from modest animal experiments and laboratory research. Some issues, meanwhile, limit its application in actual medicine. The primary issues continue to be toxicity and biocompatibility. The accumulation of particles in organs (such as the liver and kidney), the release of silver ions, and any oxidative stress must all be closely monitored by researchers. The optimal dosage, the rate at which the body eliminates the particles, and long-term safety are still not well understood in many investigations on green AgNPs. It can be challenging to compare findings from several studies. This occurs because researchers might not employ consistent animal methods or uniform cytotoxicity testing. Additionally, for medical applications, the capacity to scale up and guarantee reproducibility across all production batches is essential. This guarantees that every particle has the same size, shape, and capping. However, a lot of reports on green synthesis only go as far as the lab. The use of green-synthesised AgNPs in medicine is becoming more and more established. Their applications include medication delivery, regenerative medicine, antibacterial therapy, and anticancer treatment/theranostics. They hold great promise for novel medicinal therapies due to their scalable design, numerous functionalities, and biocompatible capping. Thorough standardization, long-term safety testing, and mechanistic animal investigations are vital to bringing a basic concept to actual clinical applications.

6.2 Environmental Applications

Silver nanoparticles (AgNPs) produced using green synthesis have a lot of potential to address environmental issues. They are particularly helpful in environmental sensing, heavy metal removal, dye degradation, and water purification. They have a lot of surface area, strong catalytic power, a surface chemistry that can be adjusted due to biological capping, and inherent antibacterial qualities. They can handle complex pollutant mixes in water thanks to these features. Green AgNP integration into hybrid systems, such as membranes, composites, and biofilters, has been the focus of research in recent years. Additionally, they verify their stability and performance in real-world conditions.

An important focus is placed on the removal of colours and organic contaminants, particularly from textile wastewater. AgNPs produced by biology frequently serve as an electron aid or catalyst in complex oxidation reactions. They are effective with lowering agents as well. AgNPs produced from Cestrum nocturnum leaf extract, for example, produced excellent results. They eliminated more than 90% of the Congo Red dye in 15 minutes when used with NaBH4 as a reducing agent. In 18 minutes, they likewise broke down roughly 78–79% of the methylene blue. This demonstrates their potent catalytic activity and the benefits of working in tandem with reductants [60]. In another study, green-synthesized AgNPs anchored onto reduced graphene oxide, prepared via orange peel extract-mediated methods, showed visible light photodegradation of organic dyes, reflecting how hybridization enhances photocatalytic performance [61]. The nanoparticle, as catalyst, was utilized for reductive degradation of three azo dyes such as methyl orange, methyl red and congo red in aqueous medium at room temperature. Reductive degradation pathway of the said dyes using NaBH4 and silver nanoparticle catalyst was followed under visible light. More than 90% degradation of the dyes was observed within 10 min for methyl orange, and 20 min for both methyl red and congo red. The mechanism of the degradation process and the kinetic parameters were evaluated. The degradation pathway was found to follow a pseudo first-order kinetic model and the activation energy was evaluated to be 244, 204 and 104 kJ mol−1 for methyl orange, methyl red and congo red respectively. The role of common ions on the degradation efficiency was determined and the process was found effective in the river water system [62]. These examples illustrate that green AgNPs can serve as effective catalysts or catalysts support in dye pollutant breakdown.

Beyond dyes, green AgNPs have been exploited for heavy metal remediation. There are two advantages to using agricultural waste for the creation of nanoparticles. It promotes the ideas of green chemistry and aids in waste reduction. The peel of the Murcott Mandarin fruit was used in this particular procedure. Bioactive substances were isolated from the peel by researchers. These substances then served as the stabilizing and reducing agents throughout the creation of the nanoparticles. The resultant silver

nanoparticles shown exceptional capacity to eliminate heavy metals, particularly lead ions, from industrial effluent. Their potent adsorption properties were validated by batch experiments. The nanoparticles absorbed lead ions at a rate of 42.7 mg/g when exposed to optimal circumstances, which included pH 5.5 and 60 minutes of stirring. Furthermore, after filtering the Ag-NP synthesis mixture, the solid residue from the Murcott Mandarin MM-SR was obtained, and it likewise performed well. Under the same ideal circumstances, this residue eliminated lead ions at a rate of 6.6 mg/g. The possibility of employing farm waste to produce materials for wastewater treatment is highlighted by this environmentally benign synthesis approach and the efficient heavy metal removal of the silver nanoparticles. This method produces valuable, practical resources for environmental management while resolving waste disposal issues.

Overall, the study emphasizes the importance of exploring innovative and sustainable pathways for nanoparticle synthesis, highlighting the promising role of silver nanoparticles synthesized from *Murcott Mandarin* peel waste in cost-effective and environmentally benign strategies for heavy metal removal in metal industrial wastewater treatment [63]. The versatile synthesis and applications of AgNPs in the form of photocatalyst, electrochemical sensor, fluorogenic sensor *etc*. towards the detection of hazardous pollutants in the air, water, and soil medium. All the synthesized nanomaterials possess low LOD value with high sensitivity and selectivity towards the detection of pollutants. Due to their biogenic nature, it can also be further recycled and reused [64].

A rapidly growing direction is integration into filtration and membrane systems. For example, Bashir et al. fabricated mixed matrix membranes (MMMs) by embedding green AgNPs (synthesized using *Hibiscus rosa sinensis* extract) within a polyethersulfone matrix for nanofiltration. The resulting membranes exhibited enhanced dye rejection, improved antifouling behavior, and superior water permeability relative to pristine membranes, indicating practical applicability in water purification systems [65]. Similarly, pistachio husk–supported AgNPs were used as a heterogeneous photocatalyst for the degradation of eosin Y under visible light. Also, the reduction of 4-nitrophenol to 4-aminophenol by AgNPs was investigated and after 15 min this reaction was completed. These results demonstrate a very efficient, cost-effective, and benign method in the field of synthesis of nanoparticles and catalysis technology development [66]. In environmental sensing, green AgNPs are often used in colorimetric, fluorescence, or plasmonic detection of environmental toxins. The *Acacia raddiana*–derived AgNPs discussed above acted as colorimetric sensors for multiple heavy metal ions. The presence of specific ions induced shifts in SPR peaks (color change), enabling simple visual detection [67]. Because the biomolecular capping often provides selective binding (e.g. via thiol or carboxyl groups), green AgNP sensors can attain good sensitivity and selectivity while maintaining low toxicity.

Despite promising demonstrations, practical deployment and long-term stability remain key challenges. Reusability, nanoparticle leaching, fouling, and the robustness of green AgNP catalysts or membranes under realistic wastewater conditions (presence of salts, pH fluctuations, competing ions) need systematic evaluation. The toxicological effects of employing AgNPs in open water systems must be carefully considered by scientists. Although the acute toxicity may be reduced by green capping, aquatic life or microbial communities may still be impacted by residual silver ions or nanoparticle residues. Comparative studies provide a promising solution: green AgNPs may typically be locked down (immobilized) by mixing them with support materials like fibers, biochar, or membranes. This decreases the ecological dangers, facilitates recycling, and lessens the quantity of silver that leaks out (leaches) [68, 41]. In conclusion, AgNPs that are green-synthesised hold a strong position in environmental applications. They are particularly helpful in sensing, membrane integration, heavy metal removal and dye degradation. Their benefits like biocompatible capping, catalytic activity and adaptability for complicated composite structures, support advancing them transcend basic lab notions. Assuring long-term stability in actual conditions should be the main goal of future research. Researchers need to develop scalable composite materials, reduce leaching of nanoparticles, and carry out thorough ecological effect analyses. This will advance the practical application of these green nanomaterials.

6.3 Energy and Catalysis

Green-synthesized silver nanoparticles (AgNPs) integrate electrical, surface chemical, and plasmonic properties for sustainable technology. This gives them a unique opportunity to be used in catalytic and energy conversion processes. The usage of biologically produced AgNPs will be examined in detail in this section. Plants, microorganisms, or enzyme pathways are examples of synthesis techniques. Photocatalytic

degradation, electrocatalysis and other green energy conversion or storage applications are among the applications addressed here. The underlying mechanisms, performance indicators attained, and areas for further research are highlighted in this section.

6.3.1 Photocatalytic Applications

Green-synthesized AgNPs have emerged as versatile photocatalysts or co-catalysts owing to their localized surface plasmon resonance (LSPR), which amplifies light absorption, enhances charge separation, and fosters reactive radical generation.

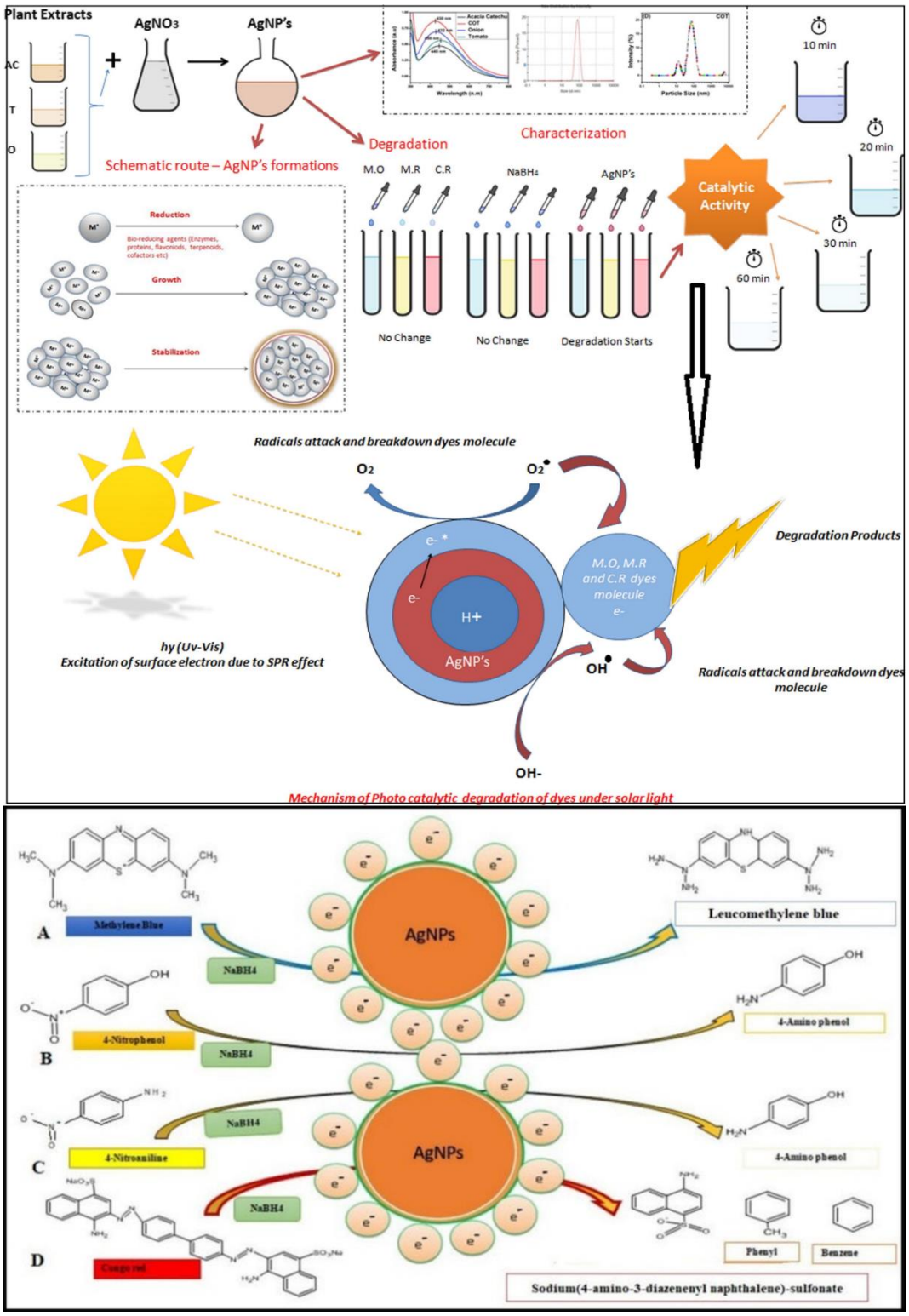

Figure 9. Schematic representation of the mechanism of nanoparticles and degradation [71]. Degradation mechanism of toxic dyes and their catalytic degradation products through the involvement of the AgNPs. **(A)** Methylene blue, **(B)** 4-nitrophenol, **(C)** 4-nitroanaline, and **(D)** congo red [60].

Table 3. A comparative analysis of representative green-AgNP photocatalytic systems

Biological source	Pollutant target	Light source	Efficiency	Advantages	Ref
Isoimperatorin-mediated AgNPs	Model dye degradation	Visible light	~90 % in ~3 h	Good stability, dual electrochemical activity	[69]
Green tea–derived AgNPs	Methylene blue	Visible	~85 % in ~2 h	Simple plant extract route	[70]
Microwave-assisted (plant-based) AgNPs	Methylene blue	Visible	~95 % in 60 min	Rapid route, enhanced catalyst dispersion	[74]
AgNP / TiO_2 hybrid	Phenolic pollutant	Visible / UV	92 % in 2 h	Enhanced charge separation by heterojunction	[75]

In pollutant degradation under visible light, multiple studies have reported that biosynthesized AgNPs can effectively degrade dyes (e.g., methylene blue, rhodamine B) or organic pollutants. For instance, isoimperatorin-mediated AgNPs achieved high photocatalytic degradation efficiency and showed good electrochemical activity under visible light [69]. In other work, green tea–derived AgNPs acted as visible-light driven catalysts for dye degradation with stable performance over multiple cycles [70].

Figure 9 schematically details the catalytic degradation mechanism mediated by AgNPs for various toxic dyes and pollutants [71]. Acting as an electron relay (often with $NaBH_4$), the nanoparticles significantly enhance reaction kinetics. This effect is demonstrated through the reduction and breakdown of Methylene blue, 4-nitrophenol, 4-nitroaniline, and Congo red. Specifically, AgNPs facilitate the cleavage of chromophoric groups in the dyes and catalyze the conversion of the highly toxic nitro group (NO_2) in compounds like 4-nitrophenol to the less harmful amino group (NH_2) derivative, thus lowering the activation energy for environmental remediation. The mechanistic underpinnings often involve plasmonic excitation of AgNPs, injection of "hot" electrons into adjacent semiconductor supports or into dissolved oxygen, generation of reactive oxygen species (e.g. $\cdot O_2^-$, $\cdot OH$), and oxidative attack on pollutants. The presence of AgNPs can also suppress recombination of photo-generated electron–hole pairs by acting as electron sinks or charge mediators. Recent works also explore heterojunctions or hybrid composites (e.g. AgNPs deposited on TiO_2, ZnO, or g-C_3N_4 matrices) to improve photonic absorption and carrier mobility. In a broader review of AgNP-catalyzed reactions, recent advances outline how size, shape, and surface capping agents crucially tune catalytic performance, especially by modulating interfacial charge transfer kinetics [72]. Some of green-AgNP photocatalytic systems are compared in table 3.

6.3.2 Electrocatalysis and Green Energy Conversion

Beyond photocatalysis, green-synthesized AgNPs have found emerging application in electrocatalytic and energy conversion sectors, including fuel cells, oxygen reduction reaction (ORR), hydrogen evolution reaction (HER), and in advanced battery/capacitor electrodes. In particular, the surface chemistry of biogenic AgNPs—with biomolecule capping ligands—can confer better dispersion, accessible active sites, and favorable electron transfer kinetics.

One domain is in alkaline media ORR, where AgNPs have been recognized as lower-cost alternatives to platinum. Biogenic AgNPs may be immobilized on conductive carbon supports or carbon nanostructures, where the interface between Ag and carbon facilitates electron transport. Silver, gold, and platinum nanoparticles produced by biology have shown promise for use in fuel cells, water splitting, and supercapacitors. One important issue is emphasized in this article: it is critical to carefully regulate the particle's morphology (form) and interface engineering (how it attaches to the surrounding material). Catalytic turnover, a high rate of chemical reaction, requires this meticulous management [76]. Although most works focus on other metal systems, the design principles translate to AgNPs when stabilized in a green matrix.

In the realm of hydrogen evolution (HER) and water splitting, Ag has lower intrinsic HER activity than noble metals like Pt, but when combined with suitable co-catalysts (e.g. MoS_2, Ni, or graphitic carbon), AgNPs can act as electron conduits or plasmonic boosters under light-assisted electrolysis. For instance,

plasmonic excitation in Ag can generate localized heating or hot carriers that reduce overpotentials for HER or oxygen evolution reaction (OER). Some recent "green energy breakthroughs" highlight how nano-catalysts (including plasmonic metals) are being integrated into bioenergy systems and photothermal catalysts to drive solar fuel generation or improve electrocatalysis efficiency [77]. Similarly, green-synthesized AgNPs have been explored in sensor–electrocatalyst hybrids for energy monitoring and catalysis coupling. For example, hybrid electrodes formed by green AgNPs on carbon cloth have been used to detect and catalyze degradation of pollutants in simultaneous electrochemical setups [78].

6.3.3 Challenges, Perspectives, and Design Recommendations

While promising, the use of green-synthesized AgNPs in energy and catalysis is still nascent and faces several challenges. First, capping biomolecules (e.g. phenolics, proteins, flavonoids) that confer stability in aqueous media can hinder accessibility of catalytic active sites or impede electron transfer; thus, post-synthesis ligand exchange or controlled partial removal may be necessary. Second, reproducibility in nanoparticle size, crystallinity, and plasmonic features is crucial—variations in biological extract composition over seasons or batches can hamper consistency. Third, long-term stability under operational conditions (illumination, redox cycling, pH extremes) must be demonstrated, as agglomeration and leaching might compromise performance.

Future research should concentrate on four key areas to address these problems. Researchers ought to start by developing hybrid composite architectures. These designs combine robust support materials with green AgNPs. Metal oxides, 2D materials, and conductive carbon are a few examples. This guarantees that the structure remains sturdy and that charge transfer occurs with ease. Second, plasmonic coupling designs need to be the main focus. To get the most near-field improvement, this entails modifying the particle orientation and spacing. Third, researchers ought to employ design-of-experiments (DoE) or machine learning techniques. They can use this to determine the ideal synthesis parameters for excellent catalytic performance. Fourthly, environmentally friendly AgNP catalysts require thorough benchmarking. This entails contrasting them with their conventional, chemically produced counterparts. The exact identical reaction conditions must be used for the comparison. Researchers are required to provide stability data, durability testing, and turnover rates. In conclusion, green-synthesised AgNPs have great potential for use in catalysis and energy conversion. They work especially well as plasmonic boosters in hybrid electrocatalytic systems or as visible-light photocatalysts. Moving past straightforward laboratory experiments to developing stable catalysts with high reaction rates should be the main goal of the upcoming years. This objective needs to be directed by a methodical comprehension of the mechanism and accepted benchmarking procedures.

6.4 Sensing and Detection

In the field of sensing and detection, green-synthesised silver nanoparticles (AgNPs) quickly became well-known. They are particularly helpful in environmental monitoring, chemical sensing, and biosensing. This is due to their huge surface area, strong plasmonic response, simple attachment of functional molecules to their surface, and tunable electronic characteristics. The most recent advancements in green AgNPs-based biosensors and nanosensors will be thoroughly examined in this section. It will dissect the basic detection methods, evaluate the performance of various systems, and pinpoint the key prospects and difficulties for advancing this field.

Green AgNPs have been employed in a variety of detection systems in the biosensing field. These consist of hybrid, photonic, and electrochemical systems. They aid in the highly sensitive and selective detection of biomolecules, pathogens, or trace amounts of chemicals. Their versatility arises from multiple signalling strategies: (i) localized surface plasmon resonance (LSPR) shifts or aggregation-induced changes, (ii) surface-enhanced Raman scattering (SERS), (iii) metal-enhanced fluorescence (MEF) or fluorescence quenching, and (iv) intrinsic Ag nanocluster fluorescence or electron-transfer mediated electrochemical readouts. The signaling mechanisms of AgNPs in optical and electrochemical biosensors were examined in a recent open-access review. The article highlights that AgNPs have a customizable form and a high surface-to-volume ratio. Very low detection limits and fast reaction times are made possible by these qualities. This is particularly beneficial for point-of-care testing, which requires immediate results [79].

Green-synthesized AgNPs optical biosensors typically function in two ways. They make use of plasmonic band shifts or color changes.

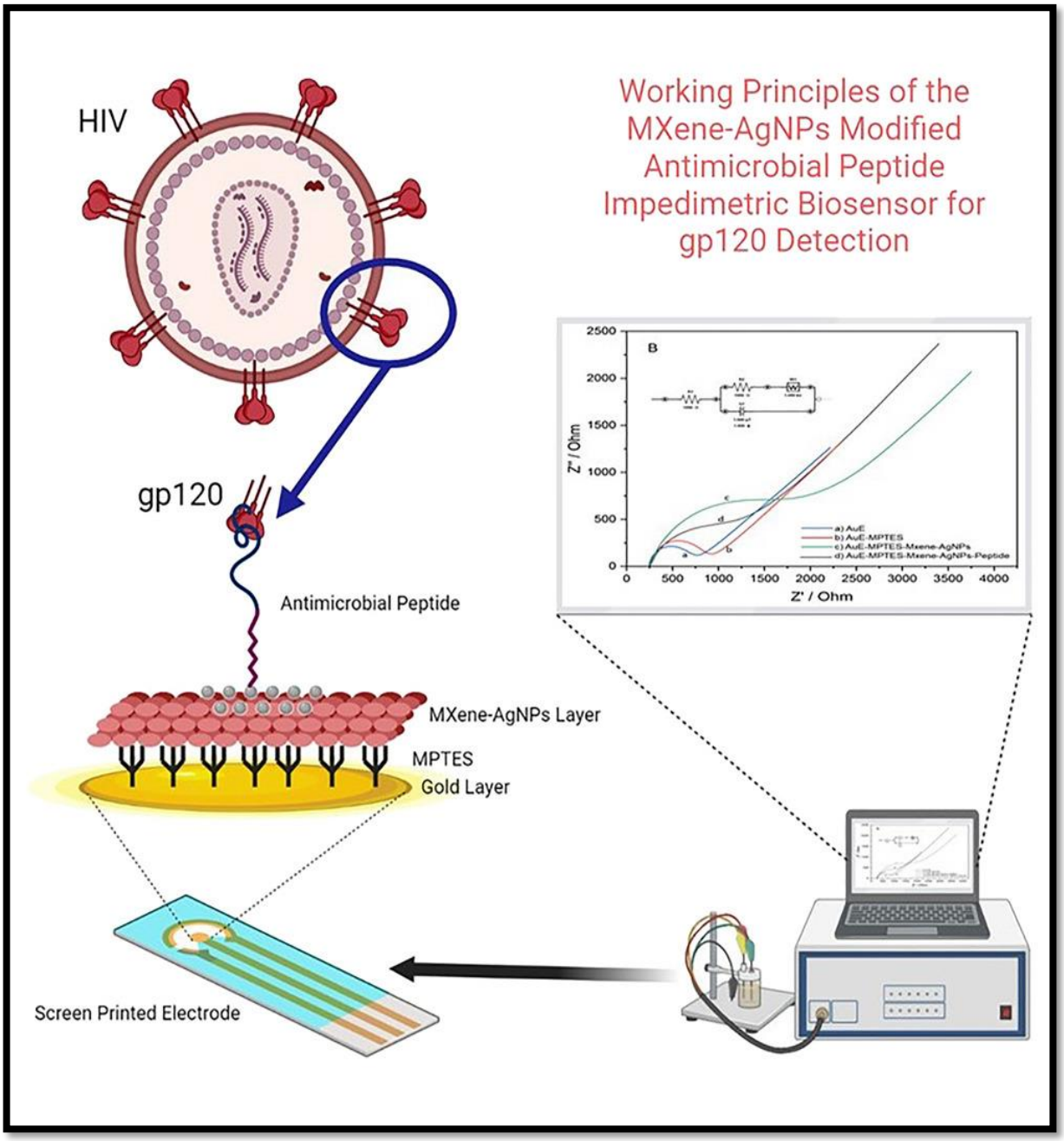

Figure 10. An antimicrobial peptide modified biosensor was developed on a MXene-AgNP [82]

These alterations occur when the nanoparticles begin to cluster together or when the target material (analyte) attaches to the AgNPs. For example, in a study by Srikhao *et al.,* AgNPs synthesized using spent coffee ground extract were embedded into a paper substrate to create a smartphone-compatible colorimetric sensor of hydrogen peroxide. The binding or oxidation led to a discernible change in color and spectral shift, with a detection limit comparable to commercial strips and excellent stability across 100 days [80]. In another example, Rashidi et al. synthesized AgNPs using Smyrnium leaf extract and deployed them in a colorimetric ammonia sensor: gradual shift in λ_max from ~580 nm to 490 nm was used to detect ammonia in the 0.5–200 ppm range, with a limit of detection as low as 0.028 ppm [81]. These results demonstrate the high sensitivity and dynamic range achievable with green AgNP-based optical sensors.

In the electrochemical sensing arena, green AgNPs have been deployed as electrode modifiers to enhance electron transfer, increase the active surface area, and catalyze redox transformations of target analytes. A study by Pektaş *et al.* reported an amperometric glucose biosensor using a carbon paste electrode modified with green-synthesized AgNPs derived from waste tea; the green-AgNP modification resulted in lower overpotentials and higher current responses for glucose oxidation, with favorable stability [83]. Similarly, Uygun & Tasoglu (2024) described

employing a label-free impedimetric biosensor in the development of sensors for bacterial detection. This sensor made of AgNPs that were functionalized on interdigitated electrodes via a green pathway (Figure 10). By altering the impedance (electrical resistance), the device was able to detect bacterial cells with high sensitivity [82]. In another application, Thongwattana et al. modified screen printed carbon electrodes (SPCE) using AgNPs derived from pineapple peel. This aided in the identification of human serum albumin in blood. Over a range of 10–400 µg/mL, the drug could be reliably detected by the AgNP SPCE platform. Additionally, it outperformed basic, bare electrodes in terms of electrochemical performance [84].

Several important characteristics are evident when comparing the performance of various green-AgNP sensor systems. These consist of the linear dynamic range, the limit of detection (LOD), the reaction time, the stability across several applications, the selectivity against interfering compounds and the reproducibility. However, there are still a number of difficulties and limitations in spite of these encouraging actions. First, the biomolecule capping agents that stabilize green AgNPs can either slow down the electron transport or physically block the target chemical from reaching the sensor. In order to balance stability and sensor activity, scientists may need to thin the layer or regulate the partial elimination of these compounds. Second, variations in the size, shape, and surface chemistry of nanoparticles may result from batch-to-batch variations in biological extracts. This has a direct impact on the reliable operation of the sensor. Third, extensive testing is required to ensure long-term stability under actual circumstances, such as humidity, temperature fluctuations, or exposure to other compounds. This entails making sure the baseline performance endures across numerous applications and looking for interference from ions or organic compounds. Fourth, the majority of green AgNP sensors that have been published are still only laboratory proof-of-concept concepts. They must be combined with microfluidics, reduced in size, the sensor must be properly packaged, and appropriate calibration procedures must be developed in order to be scaled up into reliable, field-deployable systems.

Future studies should concentrate on a few key topics in order to advance this discipline. Graphene, MXenes or carbon nanotubes are examples of strong conductive scaffolds that could be used with green AgNPs in hybrid sensor platforms. Sensitivity, stability and electron transport could all be significantly enhanced by this combination. Two, AgNPs functionalized with various recognition molecules could be used in multiplexed sensor arrays. This would make it possible to identify several target compounds at once. Three, their field application potential would be enhanced by integration with on-chip electronics, low-cost readout mechanisms (such as smartphone-based devices) and real-time data analytics. Four, the optimization and standardization process could be accelerated by using machine learning techniques to connect AgNP synthesis parameters, sensor shape, and performance indicators. Lastly, under the same circumstances, researchers should compare green-AgNP sensors directly to traditional sensors (manufactured from harsh chemicals or employing gold nanoparticles). This comparison must employ the same target chemical and environment to clearly highlight the benefits and drawbacks of the green method.

Finally, our study demonstrates that green-synthesized AgNPs offer a promising and adaptable platform for sensing and detection. They may be tailored for various target substances in the chemical, medicinal, and environmental domains thanks to their special blend of plasmonic, electrochemical, and surface functionalization capabilities. Researchers must address the impacts of the capping agents, the extract's variability, long-term stability and full integration into functional sensing systems in order to translate their findings into stable, reproducible, and real-world devices.

7. Comparative Analysis: Green vs. Conventional AgNP Synthesis

7.1 Energy Efficiency, Cost-Effectiveness, and Scalability

Comparing the Costs of Conventional and Green Synthesis Conventional chemical processes often use potent reducing agents (such as sodium borohydride or hydrazine) to create AgNPs. They also frequently call for high temperatures or specialized tools, such as UV devices or microwaves. All of these variables contribute to higher energy consumption and prices for inputs. Green synthesis, on the other hand, makes use of microorganisms, biopolymers, or plant extracts. Usually, this mechanism operates in milder environments. Much less energy is needed because it employs an aqueous medium and ambient or about room temperature. This process uses abundant natural agents that both decrease and cap the silver in place of costly or hazardous chemicals. For instance, according to a recent assessment, the synthesis of green nanoparticles (for a variety of metals, including silver)

can save about 30% on energy usage. Compared to traditional methods, it can reduce expenses by as much as 40% and boost production output by almost 50% [12]. Similarly, a recent review work underscores that plant extract-based green AgNP methods reduce chemical reagent costs and hazardous waste disposal burdens, albeit at somewhat larger reaction times or requiring optimization of extract concentration, pH, etc., to achieve high yields and narrow size distributions [11].

However, challenges to scalability remain. Conventional methods are more mature in terms of well-controlled large-scale manufacturing, with established protocols for chemical reduction in industrial reactors. Green methods, though promising, often suffer from batch variability (seasonal/geographic variation in biomass composition), potential issues in reproducibility, and biomass upstream processing (collection, extract preparation) adding hidden costs. The review highlights that while biomass-mediated routes lower raw material and environmental costs and improve eco-sustainability, their yields/outputs are less consistent than chemical methods, and that achieving standardization and scale-up will require more work [3].

To illustrate quantitatively, a comparative cost analysis in a study using plant by-products showed that biosynthesized AgNPs could be produced at ~€8-9 per gram, including all extract preparation, under laboratory conditions; although this is competitive, conventional routes in large scale may undercut this when optimized reactants and economies of scale are leveraged (chemical reagents purchased in bulk, continuous reactors, shorter reaction times) [85].

7.2 Toxicological Profile and Biocompatibility

One of the strongest arguments for green synthesis is that the capping and stabilizing agents from biological sources (plant phenolics, flavonoids, proteins, polysaccharides) tend to confer superior biocompatibility, reduced leaching of toxic ions, and lower acute toxicity versus chemically synthesized AgNPs whose surfaces may include residual toxic reagents or harsh surfactants. The review compiles multiple in vitro/in vivo studies showing that green AgNPs typically elicit less cytotoxicity at equivalent dosages (on mammalian cell lines) and reduced oxidative stress, especially when properly purified of excess bioextract [1].

Comparative experimental studies lend empirical weight: a study comparing chemically synthesized AgNPs vs. those synthesized using *Paeonia lactiflora* extract (green route) found that the green-synthesized ones exhibited significantly better antibacterial, antibiofilm, antioxidant activity, while having notably lower cytotoxicity toward normal fibroblast cell lines at lower concentrations [86]. Similarly, in *Biogenic silver nanoparticles' antibacterial activity and cytotoxicity on human hepatocarcinoma (Huh-7) cells*, differing green syntheses yielded MIC values much lower than similarly sized chemically synthesized AgNPs, but also correspondingly different cytotoxicities, highlighting that even among green routes variation exists depending on capping agents and particle size/shape [87].

That said, green synthesis does not universally eliminate toxicity; certain studies have reported that green AgNPs still induce dose-dependent oxidative stress, mitochondrial dysfunction, or organ histopathology (e.g. neural tissue changes in rodent brain) at high exposures, though often less severe or requiring higher dose compared to conventional AgNPs. These results highlight the fact that physicochemical characteristics (size, shape, surface charge and extract purity) are crucial; biocompatibility is enhanced but not assured.

7.3 Regulatory and Environmental Impacts

Green AgNP synthesis aligns well with sustainable development and green chemistry principles from the perspective of regulators and the environment. Conventional techniques employ organic solvents, surfactants, or hazardous compounds. These compounds frequently require costly hazardous waste treatment and solvent recovery. The environment is heavily burdened by this. Stricter environmental risk measurements are being demanded by regulatory agencies more and more. The amount of ionic silver that leaks out, the number of nanoparticles that enter ecosystems, and the impact they have on organisms that are not the intended targets are some examples of these measures. Additionally, they require comparable criteria for particle characterization (such as size, shape, and surface chemical) and lifecycle analysis.

Generally speaking, green AgNPs have less regulatory issues. They produce fewer toxic byproducts and employ less hazardous materials. This could result in simpler regulations for disposing of rubbish. Additionally, they are more in line with sustainable product schemes and eco-labels. However, regulatory

challenges persist: first, standardization is lacking—two green syntheses may produce AgNPs with very different behaviours due to slight differences in extract composition. Second, long-term fate in environments (soil, aquatic) still underexplored for green AgNPs: do they aggregate, do capping biomolecules degrade, does silver ion release occur under environmental conditions? Recent environmental toxicology studies such as *Acute Dermatotoxicity of Green-Synthesized Silver Nanoparticles (AgNPs) in Zebrafish Epidermis* demonstrate that green-AgNPs are not free from ecological risks; even green route nanoparticles exhibited histopathological changes under certain exposure regimes [88].

7.4 Synthesis Yield, Size/Morphology Control, and Reproducibility

While conventional chemical methods are generally more straightforward to adjust to produce narrow size distributions, controlled shapes (e.g. rods, cubes, plates), and high crystallinity (due to high temperatures or templating), green methods have made considerable advances, but with greater complexity. Biological reducing agents are complex mixtures: their concentrations (of flavonoids, phenolics etc.), redox potential, pH, and other reaction parameters vary with plant species, harvest time, geography, and extraction method. This leads to wider size distributions, sometimes lower crystallinity, and variable shapes. Such variability can weaken reproducibility and comparability between studies.

Nevertheless, green methods are increasingly incorporating methods to better control size and morphology: such as optimization of extract concentration, reaction time, temperature, use of hybrid methods (e.g. combining green reducing agents with mild chemical reducing agents), templating agents or scaffolds, bioreactors for microbial methods [89]. Also, the plant-by-product study (Eucalyptus, Camellia etc.) showed that controlling extract concentration and using phenolic content as an indicator could yield more uniform particles (zeta potential −31 - −36 mV; size 40-86 nm) with consistent performance [85]. Table 4 summarises the comparison of conventional and green synthesis methods.

7.6 Implications for Choice and Best Practices

Given the above, green-synthesized AgNPs are not universally superior but often offer a better balance of sustainability, safety, and acceptability when properly optimized. For applications where biocompatibility and minimal environmental impact are critical (e.g. biomedical devices, wound dressings, environmental sensors), green routes are increasingly preferred. For other applications (e.g. industrial catalysis under harsh chemical/elevated temperature conditions), conventional methods may still offer advantages in performance if proper safety and waste treatment measures are in place.

Best practice recommendations include: rigorous characterization of green AgNPs (size, morphology, surface chemistry, ion release profile), using standard toxicity assays (including comparing green vs. chemically synthesized products under identical conditions), optimizing biomass/plant extract preparation (standardizing harvest, extraction, storage), improving purification to remove residual biomass components, process design to minimize energy and waste, and life cycle assessment (LCA) to evaluate net environmental benefit. In conclusion, there is substantial evidence to support the notion that green synthesis has major advantages over conventional techniques. These benefits are related to cost, environmental effect, and safety. However, traditional chemical or physical processes continue to perform better for applications that require high catalytic performance, large-scale industrial use or extremely high precision. To fully seize the lead in these challenging fields, green procedures must first undergo additional optimization to improve repeatability, yield, and regulatory compliance.

8. Challenges and Limitations

In the last ten years, there has been great development in the green synthesis of AgNPs. There are, however, some key hindrances and limitations that hinder their application in practice. In the following, four major concerns are discussed: safety and toxicity risks, stability and shelf-life, scalability, and reproducibility and yield. It discusses how these concerns are currently being addressed and what remains to be discovered. One of the greatest challenges lies with the problem of yield optimization and reproducibility. Green synthesis involves the use of complex mixtures like microbial products or plant extracts. The exact composition of these extracts (phenolics, proteins, and carbohydrates) is different. What plant is used, which part of the year it is being harvested, where it is growing, how old the plant is, and the procedure that researchers employ to produce

or store the extract influence these changes. Particle formation, growth, and the rate of decrease in silver are all influenced differently by this variation. Consequently, the final AgNP size, shape, size range and surface charge often differs batch by batch.

Green procedures are more costly than traditional processes, yet they yield lesser yields and are not reproducible according to an evaluation. This must be rectified by stringent standards [11]. Researchers attempt various strategies to address issue. First, the active reducing agents—such as total phenolic content—are quantitated. Rather than working with crude extracts, purified macromolecules are utilized by them. They strictly control temperature, pH, and stirring rate, among other reaction conditions. Design-of-Experiment (DoE) and other optimization methods are also employed by them. Yet, few papers provide sound statistical proof from a large number of tests. Reproducibility remains an open problem.

Another issue that bears a close resemblance is scalability and large-scale manufacturing. Many small-volume laboratory experiments have worked well. But there are many engineering hurdles when scaling up green AgNP synthesis. Controlling the biomass up front (harvesting, drying, and milling) is expensive and adds complicated logistics. It is hard to ensure consistent raw material quality and availability. Cleaning the final product at scale—that is, removing excess biomolecules and silver ions—becomes much harder. Repeated operations such as membrane filtration or centrifugation must be done for this. These processes add expense and decrease production speed. Continuous reactors and solvent recovery systems, which are easier to control, are often employed in traditional chemical synthesis. Green technologies require high production rates, states a paper on issues associated with scaling. They must also inhibit clumping of particles during scale-up. In order to compete with chemical methods, they need to integrate purification steps directly within the process (e.g., continuous flow filtration) [3]. Another major hinderance remains the lack of standardized, scalable reactor designs for biological agent-based synthesis.

Following synthesis, stability, toughness and shelf life become other major limiting factors. Even when scientists produce good green AgNPs initially, it is challenging to preserve their stability in liquid, form, and functionality. This is true whether it's used, stored, or moved. The biomolecules in the may come apart after some time. The particles can oxidize, grow asymmerically (Ostwald ripening), or aggregate. This often happens when there is pH change, salt change, temperature change, or exposure to light. Green only maintain most of their activity for a few cycles according to a number of studies. Long periods of stress in practical use lead them to fail. As per a recent study, such instability often leads to the performance shifting or failing of apps. For example, catalysts fail or sensors lose their baseline measurement [87]. To address this, post-synthesis surface engineering or partial ligand exchange (with stronger binders or polymers), incorporation of protective shell layers (e.g. silica, polymer shells), or embedding within stabilizing matrices (e.g. hydrogels, carbon supports) have been explored. Yet, care must be taken to avoid diminishing active surface accessibility. More systematic studies on long-term aging, accelerated stability testing, and correlation between nanoparticle physicochemical drift and application performance are necessary.

Finally, safety, toxicity, and ecotoxicological risks remain paramount concerns. While one of the prime motivations for green synthesis is to reduce toxicity, green-synthesized AgNPs are not inherently benign. Their biological interactions, silver ion release kinetics, generation of reactive oxygen species (ROS), and accumulation in biological systems must be rigorously assessed. Several recent works emphasize that AgNP toxicity is strongly size-, shape-, surface-charge-, and capping-agent-dependent, and that residual biomolecules or impurities from extracts may themselves introduce toxicity or interfere with assays. The review *Synthesis, applications, toxicity and toxicity mechanisms of AgNPs* underscores that the mechanisms of cytotoxicity—e.g. oxidative stress, membrane damage, DNA damage, mitochondrial disruption—remain only partially deciphered, especially in complex biological matrices [90]. Comparative studies have shown that green AgNPs often exhibit lower cytotoxicity and ion leaching than chemically synthesized counterparts, but at high concentrations or prolonged exposures, hepatotoxicity, neurotoxicity, or organ histopathology may still occur. A 2025 review on tailored silver nanoparticles mentions low yields and potential toxicity as ongoing challenges in biomedical translation [91]. In environmental contexts, release of AgNPs or ionic silver into aquatic or terrestrial systems may disrupt microbial communities, plant root function, or invertebrate health; even green-capped AgNPs sometimes show measurable ecotoxicity in zebrafish or crustacean assays [92].

Table 4. List of issues and mitigation strategies

Research gap	Strategy	Key metric(s) & outcome	Reference
Reusability / photocatalyst activity loss across cycles (practical stability for environmental catalysis)	Use of bacterially produced AgNPs (endophytic *Bacillus amyloliquefaciens*) with characterization and reuse tests under natural sunlight/UV to evaluate dye degradation and recyclability	Photocatalytic MB degradation: 73.9% (sunlight) / 87.4% (UV) after 210 min; reusability: only 11.6% decrease in activity after 5 cycles (demonstrates robust short-term recyclability).	[93]
Cytotoxicity and genotoxicity concerns for green AgNPs; need for systematic in vitro assessment	Systematic in vitro cytotoxic and genotoxic assays across cell types; discussion of dose-dependent responses and mechanisms (ROS, DNA damage)	Reported wide range of IC_{50} values depending on source and coating; emphasized need for standard panels and standardized dosing metrics for comparability. (Paper compiles and compares studies.)	[94]
Batch-to-batch variability and optimization of reaction parameters to reduce polydispersity and hemolytic/cytotoxic effects	Controlled optimization of extract:Ag^+ ratio, pH, temperature and time; hemolysis assay used to quantify cytotoxicity and select optimal conditions	Optimized conditions produced narrowly distributed particles and negligible hemolysis at application-relevant concentrations; provides an experimental template for reproducibility.	[28]
Scalability and translation bottlenecks: low and variable yields, process standardization for industrialization	Comprehensive review of biomass-mediated routes; synthesis parameter harmonization and recommendations for scale-up (process flow, QA/QC)	Critical analysis: green routes often show lower throughput vs. chemical routes; identifies parameters limiting yield and proposes pilot-scale benchmarks for future work.	[3]
Stability and controlled release for biomedical uses (preventing aggregation, improving shelf life)	Entrapment of biogenic AgNPs into polymeric/hydrogel matrices (alginate/gelatin/alginate-chitosan composites) to improve colloidal stability and controlled release	Hydrogel-embedded AgNPs retained antimicrobial and anticancer efficacy with improved storage stability vs. free colloids; demonstrated improved in-vitro retention and sustained release profiles.	[95]
Phytochemical variability causing composition and performance variability (plant extract source, seasonality, geography)	Analytical profiling of extracts and linking dominant phytochemicals to reduction/capping roles; recommendation for extract characterization standards (e.g., polyphenol content)	Demonstrated that extract composition drives NP size, zeta potential and biological response; recommends minimal reporting standards to improve cross-study comparability.	[96]
Yield and functional-property tradeoffs (higher yield often worsens polydispersity / stability)	Systematic process optimization and reporting of optimization curves (Ag^+ conc., extract volume, contact time) to reach acceptable tradeoffs between yield and	Example studies report yield improvements (up to ~60–80% in optimized lab protocols) but note that increased yield sometimes increases polydispersity unless capping is	[97]

	monodispersity	strengthened.	
Purification, surface contamination and impact on downstream applications (need for standard purification & characterization pipeline)	Comprehensive reviews and recommendations for purification workflows (centrifugation/washing, dialysis, chromatographic steps) and a minimal characterization panel (DLS, zeta, TEM, XPS, FTIR, residual organics quantification)	Meta-analysis shows that insufficient purification correlates with false positives in bioactivity assays; authors propose a standardized QC checklist to reduce false variability across labs.	[98]

Future studies must incorporate life cycle risk assessment, controlled ion-release kinetics investigations, long-term in vivo/in situ ecotoxicological testing, and the creation of degradable or "self-deactivating" capping systems that gradually release less free silver in order to solve this. Table 4 lists the research gap and strategies to address these gaps. One helpful tactic in light of these interconnected difficulties is to apply an integrated evaluation approach, which combines process economics, stability testing, toxicity screening, and synthesis optimization in concurrently rather than sequentially. Progress might be accelerated by using machine learning or multivariate optimization to balance yield, stability, and biocompatibility concurrently.

In conclusion, significant issues with reproducibility, scalability, long-term stability and safety limit the promise of green-synthesized AgNPs. It will need intense multidisciplinary effort to address issues, integrating toxicology, chemistry, process engineering and materials science to create platforms that are reliable, secure and commercially viable.

9. Future Perspectives

The green synthesis of silver nanoparticles (AgNPs) has developed rapidly. Still, there are principal issues that hinder their large-scale, real-world application. Future advancements require joint endeavour from numerous diverse disciplines. Some new trends hold tremendous potential. These are computational optimization, hybrid complex synthesis, medical applications, and compliance with circular economy principles and policy. By following these avenues, the AgNP technology shall become reliable, safe, scalable, and socially accepted. Reusing artificial intelligence (AI) and machine learning (ML) is one intriguing future direction. Elementary investigations now use hybrid models. For example, they use Response Surface Methodology (RSM) and Artificial Neural Networks (ANN). This can predict particle size or size distribution based on synthesis factors. A recent publication utilized RSM-ANN-LM (Levenberg-Marquardt) modeling. This model accurately predicted AgNP size (R^2 approx 0.985). It was based on four input factors—temperature, precursor-to-extract ratio, agitation, and pH. The aim was to obtain small and uniform particles [99]. Research also utilized design of experiments to statistically enhance AgNPs produced by fungal culture water in order to enhance their function [100]. Further research can extend this method. It can construct robust databases of synthesis results. It can employ ML methods such as Bayesian optimization to analyze numerous parameters. It must also link ML to reaction speed models and thermodynamics. This will enable scientists to know why things happen instead of simply predicting that they do happen.

Synergistic and hybrid green synthesis is yet another promising field. Here, various biological sources are mixed together, or biological agents are combined with gentle chemical or physical means. This way, researchers can employ the most desirable aspects of each. For example, scientists employed extracellular polymeric substances (EPS) from the microalga Graesiella emersonii as a reducer and stabilizer. They included auxiliary stabilizing agents (such as tetracycline). This blend rendered AgNPs under mild light. The method enabled tunable shape and improved antibacterial/antioxidant activity [101]. Biomass-derived byproducts such as agricultural waste (rice husks, straw, spent coffee grounds) are increasingly used as reducing and capping agents, offering inexpensive and sustainable feedstocks, though precise control over extract composition remains a challenge [102]. Future work could explore modular and layered synthesis protocols: e.g. using one biological source for reduction, another for stabilization or functionalization; sequential or dual-stage synthesis; co-reduction or templating; or "bio-

inspired" hybrid frameworks that embed AgNPs in biodegradable polymeric scaffolds.

Translational biomedical research and clinical evaluation stand out as a critical need. Despite numerous in vitro studies demonstrating antimicrobial, anticancer, antioxidant, and wound-healing potentials of green AgNPs, clinical or preclinical in vivo trials remain few. Notably, the biosynthesized AgNPs using *Trillium govanianum* rhizome extract demonstrated in vitro anticancer and anti-inflammatory potentials plus good hemocompatibility [103], but in vivo toxicokinetics, pharmacodynamics, long term safety, clearance, immunogenicity, and dosage optimization require much deeper study. Bridging this gap will require standardized toxicity assays, regulatory-grade animal studies, and early phase human studies, ideally with well-characterized, reproducible AgNP preparations. Importantly, engineered stability, biocompatible surfaces, and reduced silver ion leaching will be important design metrics for translation.

Another future direction is embedding green-AgNP technologies within circular economy and waste-to-nanomaterials concepts. Use of agricultural, agro-industrial, or food processing waste as reducing/stabilizing agents not only lowers cost and environmental footprint but also valorizes waste streams. The trend of using cereal waste (straw, husk, bran), plant by-products, and lignin as sustainable reducing agents has been well introduced [102], but more work is needed for life cycle assessment (LCA), environmental cost accounting, and scale to large biomass supply chains. Additionally, the development of biodegradable or degradable capping agents or supports that themselves contribute to environmental sustainability (e.g., biopolymers, waste-derived polymers) will help ensure end-of-life safety.

Finally, global policy, regulatory harmonization, and open-science frameworks will be essential to support safe, equitable, and widespread adoption. Without standardization of definitions (what counts as "green"-synthesized), comparability of characterization (size, shape, surface chemistry, capping, ion-release), and shared data on toxicity, scaling, and performance, adoption into industry, healthcare, agriculture, or environmental remediation will be hindered. International-level guidelines for nanoparticle safety assessment, eco-labels for green nanomaterials, and regulatory pathways for nanomaterials in different regions (Asia, EU, Americas) need to integrate green synthesis considerations. Open data initiatives, repositories of well-characterized green AgNPs, and cross-laboratory validation studies would contribute to scientific rigor and trust. As an example, comprehensive reviews such as *Toxicological Aspects, Safety Assessment, and Green Toxicology of AgNPs* already emphasize the necessity of better in vivo/in vitro work and clearer reporting standards [104]. In conclusion, if adequate mechanistic knowledge, standardized and repeatable procedures, AI/ML tool integration, hybrid synthesis and waste valorisation, translation to clinical/regulatory pipelines, and alignment with sustainability and policy frameworks are all in place, the future of green synthesis of AgNPs is bright. Pursuit of these directions promises to bring green-AgNP technologies into real-world applications with high safety, performance, and societal benefit.

10. Conclusion

This paper verifies that green-synthesized AgNPs are an effective and multi-functional material. Green synthesis has a significant advantage over conventional chemical routes in terms of cost, safety and environmental concern. The AgNPs synthesized in green method hold high promise in biomedical, environmental and energy applications. This achievement is a result of their biocompatibility as capping agents and strong catalytic activity. Synthesis using agricultural waste and algal extracts, further, enhances the alignment of AgNPs with circular economy strategies. To become fully operational, the field must overcome a variety of challenges. Standardization of extract characterization and synthesis protocols should be prioritized by researchers for obtaining repeatable results. They also need to address process engineering problems to enable industrial production and economically viable purification. Mechanistic animal research and comprehensive ecological impact surveys are crucial for establishing long-term stability and biosafety. Machine learning methodologies and the use of hybrid synthesis methods provide powerful paths for reliable control and optimization of AgNPs. Systematic study of these important steps will make sure that green AgNP technology is an effective instrument for future sustainable applications.

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