

## Review Article

# Biosynthesis of Silver Nanoparticles and Its Applications

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Silver nanoparticles possess unique properties which find myriad applications such as antimicrobial, anticancer, larvicidal, catalytic, and wound healing activities. Biogenic syntheses of silver nanoparticles using plants and their pharmacological and other potential applications are gaining momentum owing to its assured rewards. This critical review is aimed at providing an insight into the phytomediated synthesis of silver nanoparticles, its significant applications in various fields, and characterization techniques involved.

## 1. Silver Nanoparticles

Silver is a soft, white, lustrous transition metal possessing high electrical and thermal conductivity. It has been known longer than the recorded history due to its medical and therapeutic benefits before the realization that microbes are agents for infections. It is used in many forms as coins, vessels, solutions, foils, sutures, and colloids as lotions, ointments, and so forth. It is the foremost therapeutic agent in medicine for infectious diseases and surgical infections. The benefits of silver are more than the risk factors [1].

Nanoscience is a new interdisciplinary subject that depends on the fundamental properties of nanosize objects [2, 3]. Nanoparticles possess wondrous optical, electronic, magnetic, and catalytic properties than the bulk material owing to their high surface area to volume ratio [4, 5]. Metal nanoparticles like silver and gold show different colors due to their Surface Plasmon Resonance (SPR) phenomenon. It is a collective oscillation of free electrons of the metal nanoparticles in resonance with the frequency of the light wave interactions causing the SPR band to appear in the visible and infrared region [6].

Metallic nanoparticles are produced by various methods, the more common ones being chemical and physical methods. The aforesaid methods produce pure and well-defined nanoparticles, but the chemicals used in the synthesis are toxic, energy consuming, expensive, and not suitable for biological applications. The syntheses of metal nanoparticles

is coveted in the past three decades, but research plant extract based nanosynthesis mushroomed only in the last decade [7–13].

Silver nanoparticles have received attention due to their physical, chemical, and biological properties that attributed to the catalytic activity and bactericidal effects and found applications in nanobiotechnological research [14, 15]. They are used as antimicrobial agents in wound dressings [16–18], as topical creams to prevent wound infections [19], and as anticancer agents [20].

## 2. Publication Scenario

A literature search was primarily conducted by coining silver nanoparticles as a keyword in “Scopus” database, which yielded 20,022 articles. In order to illumine the green synthesis of silver nanoparticles, the search was refined by the following keywords: plant extracts, applications, and green synthesis under the search results of silver nanoparticles which generated 990, 847, and 853 articles, respectively. The publication scenario regarding synthesis of silver nanoparticles from standard publishers (1980 to September 2014) is documented in Table 1. The electronic search includes only the research articles and reviews published in English conducted in the month of September in 2014. A consequent focused research on the green synthesis of silver nanoparticles revealed that nearly 1000 articles are pertinent to silver nanoparticles and

TABLE 1: Publication scenario of synthesis of silver nanoparticles under standard publishers (1980–2014).

S. number	Name of the publishers	Total number of journal articles
1	American Chemical Society	3323
2	WILEY	3026
3	Royal Society of Chemistry	2140
4	Elsevier Science	8790
5	Springer	2668
6	Taylor & Francis	299

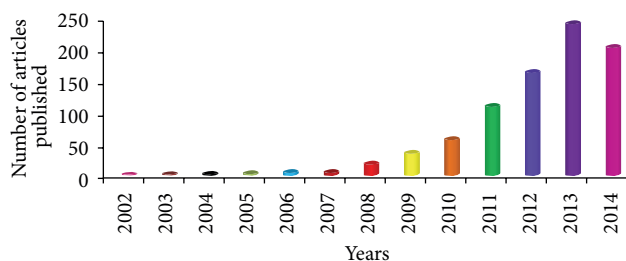


FIGURE 1: Publications trend of phytomediated synthesis of silver nanoparticles in journals under Scopus database.

only 250 articles germane to critical reviewing are used in this paper. The publication trends regarding phytomediated synthesis of silver nanoparticles from the Scopus database (2002 to September 2014) are shown in Figure 1.

There are few review articles based on the green synthesis of silver nanoparticles. The escalation of antimicrobial, catalytic, imaging, and electrochemical applications of metallic nanoparticles like silver, gold, palladium, and so forth are documented [21]. Kulkarni and Muddapur (2014) highlighted the synthesis of metallic nanoparticles, their unique properties, and the rareness of an effective method of synthesis to produce homogeneous dimensions and their applicability [22]. There are several greener routes to synthesis of nanoparticles of zerovalent metals, metal oxides, and salts emphasizing recent developments [23].

Synthesis of metallic nanoparticles using plant extracts is inexpensive, easily scaled-up, and environmentally benign. Different characterization techniques are used in evaluating its potential applications [24].

The production of silver nanoparticles, applications in medical field, health, and environmental concerns due to these nanoparticles, and mechanism of action are deliberated for human betterment in a recent article [25, 26]. Antimicrobial properties of silver nanoparticles and their futuristic view of research [27], environmental perspective of properties of silver nanoparticles in the development of synthetic protocols and applications [28], antibacterial effects of silver nanomaterials, proposed antibacterial mechanisms and possible toxicity to higher organisms [29], current uses of silver nanoparticles in clinical medicine as therapeutics, in diagnosis and imaging, toxicity, and potential applications in future [30] are well-documented.

Short-term exposure to the colloidal AgNPs is nontoxic in oral, ocular, and dermal toxicity tests conducted in mice and guinea pigs. Long-term toxicity studies are necessary for the safe use of the colloidal AgNPs [31]. The development of greener processes for nanomaterial synthesis, preparation of functionalized metal particles, advancement in core synthesis, surface functionalization and shape control, and future challenges to develop greener approaches are illustrated in a recent paper [32]. The scattering, absorption cross section, extinction, and quadrupolar coupling of different silver nanoparticles examined reveal that the optical properties depend on the size of the nanoparticles [33].

This review is an encapsulation of phytomediated synthesis of silver nanoparticles and their varied phases of applications. The yearly publication analysis revealed that there is a steady increase in the research work published on the aforesaid topic and this is expected to be more towards the end of the 21st century.

### 3. Types of Nanosynthesis

The synthesis of metallic nanoparticles involves top-down and bottom-up approaches by chemical, physical, and biological means. Biogenic syntheses of silver nanoparticles are classified under bottom up approach [34]. Several methods have been employed to synthesize silver nanoparticles, including chemical reduction, microwave-assisted synthesis, ultrasonic-assisted reduction, electrochemical reduction, template method, photoinduced or photocatalytic reduction, irradiation reduction, microemulsion method, and biochemical reduction.

**3.1. Chemical Synthesis.** A one-pot method of reduction of  $\text{AgNO}_3$  using  $\text{N}_2\text{H}_4 \cdot \text{H}_2\text{O}$  in the presence of  $\text{CH}_3\text{COONa}$  at room temperature for 2–3 h, with water as a solvent [35], was employed. The size-controlled production of silver nanoparticles (4–8 nm) was obtained by a modified  $\text{AgClO}_4$  reduction method by  $\text{NaBH}_4$  without the addition of any stabilizing agent [36]. The particle size of polyethylene glycol mediated silver nanoparticles using  $\beta$ -D-glucose at  $45^\circ\text{C}$  was found to be dependent on the time of synthesis within 24–48 hours [37]. Gamma rays have been used to synthesize the chitosan stabilized silver nanoparticles [38]. Hydrazine, formalin, and ascorbic acid produced AgNPs (20 nm) by the reduction of  $[\text{Ag}(\text{NH}_3)_2]^+$  complex in sodium dodecyl sulfate (SDS) micellar solution [39].

Silver colloids were prepared by treating silver oxalate with polyvinyl pyrrolidone (PVP) under microwave irradiation and the particle size controlled by altering several factors [40]. Electrochemical fabrication of silver nanoparticles on the glassy carbon electrode by conventional reduction method was accomplished using 1-butyl-3-methylimidazolium tetrafluoroborate [41, 42]. AgNPs of size ranging within 5–10 nm were obtained under microwave irradiation within 30–60 s using an antioxidant, glutathione [43].

Silver nanoparticles (9–30 nm) were obtained by the reaction of hydrazine hydrate, sodium citrate as a reducing agent,

and sodium dodecyl sulphate as stabilizing agent. The highest antibacterial activity was also observed at very low concentrations below  $6.74 \mu\text{g/mL}$  [44]. The reduction of silver ions by the hydrolyzate of Carboxymethyl Cellulose Sodium (CMS) was acquired by microwave irradiation, whereas it is not possible with conventional methods. The concentration of CMS has little effect on the size distribution, while the impact of  $\text{AgNO}_3$  concentration is apparent [45]. Anisotropic silver nanoparticles are obtained rapidly by microwave aided decomposition of silver oxalate in a glycol medium using polyvinyl pyrrolidone (PVP) as the capping agent [46].

Aqueous-gaseous phase reaction of silver nitrate solution and ammonia gas results in rapid formation of AgNPs of size 10 nm [47]. Colloidal dispersions of AgNPs are obtained commonly by chemical reduction methods [48, 49]. The use of sodium dodecyl benzene sulfonate (SDBS) improves the distribution of AgNPs synthesized in an electrochemical method [50]. Reaction of silver citrate and polyvinyl pyrrolidone (PVP) in a pulsed sonoelectrochemical technique yielded silver nanoparticles [51]. The reduction of sodium borohydride in the presence of dodecanethiol produces size dependent silver nanoclusters [52].

Radiolytic reduction of citrate and silver ions produced silver nanocrystallites, the size and shape of which depended on the citrate ions in the solution [53]. Uniform silver nanowires are obtained in polyol process which is a different approach for large scale production [54]. Reduction of silver nitrate with hydroxylamine hydrochloride at alkaline pH produced stable, highly SERS-active silver colloids of particle size between 23 and 67 nm [55]. A Tollen's process used for the preparation of AgNPs resulted in electrodeless deposition of silver of size within 20–50 nm and forms stable dispersion in water or submonolayer coating on microscale colloids [56].

Different studies have been used to increase the particle size from 20–45 nm to 120–170 nm in the synthesis of silver sols using silver nitrate and sodium borohydride [57, 58]. Electroreduction of silver ions in acetonitrile containing tetra-butyl ammonium salts yielded AgNPs of 2–7 nm in size. Different kinds of counter electrodes have been used to study the influence of different electrochemical parameters on the final size of the nanoparticles [59]. The reduction of  $\text{Ag}^+$  by 1-hydroxyalkyl radical generated in the radiolysis of 2-propanol and  $\gamma$ -irradiation of  $1.0 \times 10^{-4} \text{ M}$   $\text{AgClO}_4$  solution produced colloidal silver shoals of long-term stability [60]. AgNPs of 7 nm size with unusual narrow Plasmon absorption band were produced in the presence of polyethyleneimine [61]. Monodispersed silver nanoparticles in liquid phase are synthesized by functionalized AOT reverse micelles and size-selected precipitation method [62].

The reduction of silver ions in ethanol takes place in the absence of light with the nonionic surfactants in the solution [63]. Reverse micelles enable control of the size of silver sulfide nanoparticles [64]. The agglomeration of oligomeric clusters of silver atoms ( $\text{Ag}^0$ ) formed by the reduction of various complexes with silver ions ( $\text{Ag}^+$ ) results in colloidal Ag particles [65]. Stable silver colloids are obtained by spontaneous reduction of silver ions in the presence of nafion and basic, air saturated solution of 2-propanol [66].

## 4. Microbe-Assisted Synthesis of Nanosilver

**4.1. Bacterial-Induced Synthesis.** *Lactobacillus fermentum* suppresses the growth of *P. aeruginosa* and controls the formation of biofilm in the synthesis of biogenic silver nanoparticles [67]. Anisotropic nanoparticles synthesized by *Bacillus flexus* produced spherical (12 nm) and triangular (61 nm) nanoparticles [68]. An incubation period of 3–5 days at room temperature is needed for the production of AgNPs using *Bacillus cereus* [69]. The stability and synthesis of AgNPs depend on cell-free culture supernatants of psychrophilic bacteria (*Pseudomonas antarctica*, *Pseudomonas proteolytica*, *Pseudomonas meridiana*, *Arthrobacter kerguelensis*, and *Arthrobacter gangotriensis*) and mesophilic bacteria (*Bacillus indicus* and *Bacillus cecembensis*) [70]. The spore crystal mixture of *Bacillus thuringiensis* is used to synthesize AgNPs of mixed morphology (cubic and hexagonal) of size 15 nm [71].

Parameters like temperature, pH, and concentration of  $\text{AgNO}_3$  control the size of AgNPs synthesized using *Escherichia coli*, *Klebsiella pneumoniae*, *Escherichia coli*, and *Enterobacter cloacae* which effectively produced silver nanoparticles [72, 73]. The interaction of *Plectonema boryanum* UTEX 485 with aqueous  $\text{AgNO}_3$  for 28 days spawned precipitation of spherical silver nanoparticles [74]. The silver ions get reduced rapidly within 5 minutes with the addition of the cell filtrate of *Enterobacteriaceae* (*Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae*) to silver nitrate solution [75]. The size and shape of the silver nanoparticles synthesized using microbes depend on the interaction of silver ions with bacteria [76, 77]. *Pseudomonas stutzeri* AG259, isolated from silver mine, produced silver nanoparticles of well-defined size and distinct morphology within the periplasmic space of the bacteria [78].

**4.2. Fungal-Derived Synthesis.** Polydispersed spherical AgNPs of size ranged within 17–33 nm was synthesized using cell free filtrate of *Helminthosporium tetramera* and showed significant antibacterial activity [79]. *E. coli* was found to be more susceptible to silver nanoparticles than *S. aureus* [80]. The thermophilic fungus *Humicola* sp. reacted with  $\text{Ag}^+$  ions, reduces the precursor solution, and leads to the formation of extracellular nanoparticles [81]. Ideal conditions such as temperature  $37^\circ\text{C}$ , pH-6.0, and substrate concentration of 2.0 mM silver nitrate are required to synthesize AgNPs from *Aspergillus niger* [82]. Patenting of research work on microbial synthesis of nanoparticles is also on an increase in recent research. One such significant work is the synthesis of AgNPs (5–50 nm) harnessing wet biomass of *Trichoderma reesei* fungus at  $28^\circ\text{C}$  after 120 hours of continuous shaking [83]. The spherical, semipentagonal, and hexahedral structures (10–60 nm) of silver nanoparticles were formed using *Bipolaris nodulosa* [84].

AgNPs produced using *Pleurotus sajor caju* have good antibacterial action against *Pseudomonas aeruginosa* and *Escherichia coli* compared to *Staphylococcus aureus* [85]. Highly stable and crystalline silver nanoparticles (10–60 nm) were produced in solution by treating an aqueous silver nitrate solution with fungus *Fusarium semitectum* [86].

Extracellular mycosynthesis of AgNPs by *Fusarium acuminatum* isolated from infected ginger produced nanoparticles of size in the range of 5–40 nm size within 15–20 min. The reduction of the silver ions occurs possibly by a nitrate-dependent reductase enzyme and showed efficient antibacterial activity against *Staphylococcus aureus*, *Salmonella typhi*, *Staphylococcus epidermidis*, and *Escherichia coli* [87]. Nanocrystalline AgNPs of size 13–18 nm were produced using cell-free aqueous extract of *Trichoderma asperellum* after 5 days of incubation [88].

*Aspergillus flavus* accrued silver nanoparticles on its cell wall in 72 h but was found to be dislodged by ultrasonication [89]. Rapid reduction of  $[\text{Ag}(\text{NH}_3)_2]^+$  to  $\text{Ag}^0$  takes place when a quantity of -OH was introduced into the dried cells of the bacterium *Aeromonas sp.* SH10 [90]. Extracellular synthesis of well-dispersed AgNPs in the size range 5–25 nm was achieved in a few minutes when the silver ions were treated with *Aspergillus fumigates* [91]. *Fusarium oxysporum* aided synthesis of silver nanoparticles resulted in agglomeration [92], whereas a conventional halogen-tungsten lamp method of synthesis produced AgNPs within an hour with less aggregation [93].

The reduction of silver ions takes place by the enzymes present on the surface of *Verticillium* and even after the formation of AgNPs the cells were found to multiply [94]. Thus, the microbially assisted syntheses of silver nanoparticles have developed the biomimetic conduit towards plant species. The enzymes present in the microorganisms are responsible for the reduction of silver ions forming silver nanoparticles [95]. These organisms are susceptible to higher concentrations of silver ions [96]. Hence, the nanosilver synthesized by microorganism's exhibit certain difficulties when used in biomedical applications.

## 5. Conventional Methods of Nanoparticle Synthesis: A Critical View

The exploitation of reducing agents in the nanoparticles synthesis has opened a crucial pathway which threatens the environmental sustainability and also limited the uses of these noble materials towards biological applications. The use of hazardous chemicals and the amount of capital involved in the synthesis process leads to an energy exhaustive process which eliminates the conventional methods from being environmentally friendly [97–102]. Chemical synthesis of silver colloids mostly leads to aggregation as the period of storage increases [2]. The aforesaid challenges have recommended the integration of the green chemistry principles in the synthesis of metallic nanoparticles. Environmentally friendly solvents and reducing and stabilizing agents are the crucial constituents for the green nanosynthetic routes [103].

## 6. Plant-Mediated Synthesis

Nanosilver of different sizes were obtained using extracts of *Myrmecodia pendans* (10–20 nm) [104], *Tectona grandis* (30–40 nm) [105], *Syzygium cumini* (10–15 nm) [106],

*Rhynchoetechum ellipticum* (51–73 nm) [107], *Alternaria alternata* (27–79 nm) [108], *Citrus maxima* (2.5–5.7 nm) [109], *Desmodium gangeticum* (18–39 nm) [110], latex of *Thevetia peruviana* (10–30 nm) [111], *Lycopersicon esculentum* Mill (30–40 nm) [112], *Piper pedicellatum* (2–3 nm) [113], *Centella asiatica* L. (30–50 nm) [114], *Boswellia serrata* [115], Neem leaves (43 nm), Triphala (59 nm) [116], *Ocimum sanctum* leaf [117], Pomegranate seed (30 nm) [118], *Mentha piperita* (90 nm) [119], *Murraya koenigii* (10–25 nm) [120], and so forth as capping agents.

The size of silver nanoparticles synthesized using the antioxidant constituents from blackberry, blueberry, pomegranate, and turmeric extracts was found to be between 20 and 500 nm size, depending on the nature of extracts and method of preparation [121]. *P. maderaspatensis* proved to be a good catalyst for the initiation of the reaction, which rapidly produced AgNPs within a short span of 24 h with a particle size as 59 nm [122]. The zeta potential value (−18 mV) of AgNPs synthesized by *Delonix elata* after 24 h incubation indicates its stability [123]. AgNP thin films of large area were obtained using guava leaves extract through SILAR method [124]. AgNPs of various shapes like truncated octahedron, rhombic-dodecahedron, cubic, octahedron, and octagon structures with particle size ranging from 75.50 nm to 1.22  $\mu\text{m}$  were formed using banana stem extract [125].

Nanotriangles and hexagon shape AgNPs were synthesized using *Potamogeton pectinatus* L. and continuous growth taking place as the concentration of silver nitrate was increased, finally resulting in polydispersity [126]. Polyphenol rich extracts of *Rumex hymenosepalus* aided synthesis yielded a mixture of face-centered cubic and hexagonal structured AgNPs of size 2–40 nm [127]. High pH and temperature are reported to be the optimized conditions for the rapid synthesis of *Cissus quadrangularis* mediated silver nanoparticles [128]. Water-soluble organics present in the plant materials were mainly responsible for the reduction of silver ions to AgNPs [129]. Nearly 50% free radical scavenging activity was observed for the AgNPs synthesized using *Prunus armeniaca* (apricot) fruit extract in DPPH and ABTS assay [130]. Needle shaped AgNPs of size 82.46 nm were obtained with the root extracts of *Coleus forskohlii* [131].

*Malva parviflora* was found to produce monodispersed AgNPs in less time than the *Beta vulgaris*, *Anethum graveolens*, *Allium kurrat*, and *Capsicum frutescens* [132]. Water soluble compounds like saponins present in the leaf extract of *Memecylon edule* are reported to be responsible for the reduction of silver ions under incubation at 150 rpm in a shaker in dark condition and formed mainly square shaped AgNPs in the size range 50–90 nm [133]. Spherical shaped AgNPs (average size of  $18.2 \pm 8.9$  nm) were formed using the leaf methanolic extract of *Vitex negundo* and showed antibacterial activity against both Gram positive and Gram negative bacteria [134].

The alteration in the size distribution and decreased rate of synthesis of AgNPs in protein-depleted fractions confirmed the involvement of cellular proteins of unicellular algae *Chlamydomonas reinhardtii* in the biosynthesis of AgNPs [135]. The extracts from tissue culture-derived callus and leaves of the salt marsh plant, *Sesuvium portulacastrum*

L., were used for the synthesis of AgNPs and stabilized by polyvinyl alcohol [136]. The reduction of AgNO<sub>3</sub> by eugenol present in the clove extract takes place due to the inductive effect of methoxy and allyl groups present at the ortho and para positions of proton releasing -OH group from one molecule of eugenol. This is followed by the formation of resonating structure in the anionic form of eugenol [137].

The polyol and the water soluble heterocyclic components present in the leaf broth of *Cinnamomum camphora* are reported to be responsible for the reduction of silver ions [138]. A bifunctional tripeptide (DDY-OMe) with one Tyr residue and two carboxyl groups in the Asp residues of *Chlorella vulgaris* produce small Ag nanoplates in good yield [139]. Highly stable silver and gold nanoparticles of sizes 10–20 nm and 15–25 nm, respectively, were produced using *Emblica officinalis* extract [140]. Pure silver, gold, and bimetallic nanoparticles were formed by the reduction of metal ions facilitated by the reducing sugars and terpenoids present in the *Azadirachta indica* leaf broth [141].

## 7. Various Methods of Synthesis of Silver Nanoparticles

Several methods are employed to synthesize silver nanoparticles. A thorough search of the literature revealed diverse methods for the production of silver nanoparticles. Research pertaining to different methods is reviewed and presented below.

**7.1. Synthesis at Room Temperature.** *Alternanthera dentata* aided synthesis of AgNPs resulted in nanoparticles within 10 min at room temperature and the nanoparticles exhibited antibacterial activity against *E. coli*, *P. aeruginosa*, *K. pneumonia*, and *E. faecalis* at 50 ppm concentration [142]. The flavonoids and proteins in the *Tephrosia purpurea* leaf extract are the key factors for the formation of AgNPs. The size of AgNPs was found to be 16 nm with good agreement with XRD results [143]. The AgNPs (30 nm) were prepared in 10 min through the reduction of silver ion by aqueous extract of *Alternanthera sessilis* and found that the proteins and ascorbic acid are responsible for the synthesis [144].

AgNPs synthesized using the leaf extracts of *Mangifera indica*, *Eucalyptus tereticornis*, *Carica papaya*, and *Musa paradisiaca* under ambient temperature resulted in different size and shapes, namely, 50–65 nm (ovular), 60–150 nm (oval), 25–40 nm, and 10–50 nm (round and irregular), respectively [145]. The complete reduction of silver ions by aqueous extract of *Padina tetrastromatica* was observed after 72 h at room temperature under shaking condition [146]. Environmentally benign aqueous extract of *A. dubius* was used as an effective capping and reducing agent in the synthesis of silver nanoparticles of size 10–70 nm [147].

Rapid synthesis of silver nanoparticles by a Bryophyte, *Fissidens minutus*, was obtained under room temperature [148]. Metallic AgNPs (10 nm) formed within few minutes at room temperature using aqueous sorghum extract [149]. Cubic (20 nm) and hexagonal (10–50 nm) shape AgNPs were obtained after 4 h under room temperature using *Argemone*

*mexicana* leaf extract and were found highly toxic against pathogenic bacteria and fungi at 30 ppm [150]. Cubic AgNPs of size 50–150 nm were synthesized using *Eucalyptus hybrida* leaf methanolic extract with aqueous solution of silver nitrate (AgNO<sub>3</sub>) at ambient temperature [151]. Polydispersed AgNPs (5–30 nm) were formed by the reduction of silver ions using *Mentha piperita* at room temperature within 15 min [152].

**7.2. Synthesis at Higher Temperature.** The reduction of silver ions to AgNPs was carried out by heating the mixture of root aqueous extract of *Withania somnifera* and aqueous Ag(NO<sub>3</sub>)<sub>2</sub> at 60–80°C for 20 min [153]. Comparative study of various methods in the synthesis of AgNPs using *Amaranthus polygonoides* revealed that higher temperature method results in the rapid synthesis [154]. The visibility of prominent reddish brown color was observed at 60°C within 20 min which indicates the formation of AgNPs using marine seaweed *Gracilaria corticata* [155]. The reductive capability of reducing sugars and flavonoids present in *Cacumen platycladi* was increased at 90°C and lead to the formation of AgNPs (18.4 nm) with narrow size distribution [156].

The *Cycas* leaf extract solution treated with 0.25 M AgNO<sub>3</sub> solution kept on the steam bath for 10 min produced spherical shape AgNPs of diameter in the range of 2–6 nm [157]. Continuous stirring of silver nitrate solution with *Allium cepa* extract at 50–60°C yielded average sized AgNPs (33.6 nm) and showed complete inhibition against *E. coli* and *Salmonella typhimurium* at 50 µg/mL [158]. Nanosilver of 10–20 nm size was produced by heating the mixture of silver nitrate and latex of *Jatropha curcas* for 4 h in an oil bath with constant stirring [159]. The increase in temperature to 95°C fastens the reaction of *Magnolia kobus* leaf broth and silver nitrate to produce AgNPs up to 90% within 11 min [160].

**7.3. Synthesis Using Microwave Irradiation.** Peels of citrus fruits (orange, grapefruit, tangelo, lemon, and lime) used in microwave aided synthesis of AgNPs revealed orange peel extract to give silver nanoparticles (7.36 ± 8.06 nm) in 15 min compared to other extracts [161]. Spherical shaped AgNPs of 15–20 nm size synthesized using *Acacia farnesiana* (sweet acacia) seed extract under microwave irradiation showed better inhibitory action against *E. coli*, *S. aureus*, *B. subtilis*, and *P. aeruginosa* [162].

*Cuminum cyminum* seed powder extract and silver nitrate solution in the ratio 1:10 were irradiated in a domestic microwave for 120 seconds to give AgNPs [163]. Microwave-assisted synthesis of AgNPs was obtained in 8–10 min by the extract of *Cymbopogon citratus* (Lemongrass) at pH of 8.0, when it is irradiated to 90 watts [164]. Microwave irradiation is considered better for the reduction of silver ions to silver nanoparticles. This method yields smaller particles of uniform size distribution [165].

**7.4. Synthesis by Sonication.** Rapid synthesis of silver nanoparticles using *Portulaca oleracea* was observed in sonication method compared to that of room and higher temperature conditions and found to be less than 60 nm [166]. *Pisonia grandis* derived AgNPs were found to be

uniform in sonication due to the acceleration effect in chemical dynamics and rates of the reactions. Ultrasonic energy might have interfered in the chemical route of synthesis by generating free radicals [167].

**7.5. Light Induced Synthesis.** Polydispersed silver nanoparticles of size 8–10 nm were expeditiously synthesized from aqueous silver nitrate through a simple method using *Cynodon dactylon* leaf extract under sunlight [168]. *Solanum trilobatum* Linn extract yielded cubic and hexagonal shape (15–20 nm) AgNPs under sunlight irradiation and when mixed with shampoo was found to enhance the antidandruff effect against fungal pathogens (*Pityrosporum ovale* and *Pityrosporum folliculitis*) [169]. AgNPs reduced in size significantly to 10–50 nm, after ultrashort laser pulses, by the irradiation of silver nitrate and *Euphorbia milii* solution using xenon lamp [170].

## 8. Pharmacological Applications

**8.1. Antimicrobial Activity.** Silver nanoparticles synthesized using *Abutilon indicum* leaf extract exhibited highly potent antibacterial activity on *Staphylococcus aureus* (16.8 mm), *Bacillus subtilis* (18.3 mm), *Salmonella typhi* (14.5 mm), and *Escherichia coli* (17.2 mm) [171]. The impregnation of *Ipomea carnea*-AgNPs with a cellulose acetate membrane to form a structured antimicrobial membrane showed 14 mm zone of inhibition on *Mycobacterium smegmatis* [172]. *Boerhaavia diffusa* mediated AgNPs showed higher sensitivity against *Flavobacterium branchiophilum* than the other two fish bacterial pathogens *Aeromonas hydrophila* and *Pseudomonas fluorescens* [173]. Lingo-berry and cranberry juice assisted AgNPs are found to be more active against *S. aureus*, *B. subtilis*, and *B. cereus* and less active against *C. albicans* and food borne *B. cereus* [174].

The growth of *V. alginolyticus*, *K. pneumoniae*, *P. aeruginosa*, *B. subtilis*, and *P. shigelloides* was highly inhibited by the AgNPs synthesized using the inflorescence of the *Cocos nucifera*. The binding nature of AgNPs to bacterial cell wall is evidenced microscopically [175]. Antidermatophytic activity of AgNPs synthesized using lemon peel extract showed maximum zone of inhibition  $12 \pm 0.3SD$ ,  $11 \pm 0.5SD$  against *T. mentagrophytes* and *C. albicans*, respectively, but no activity against *T. rubrum* [176]. Triangular, hexagonal, and spherical AgNPs of size between 78 nm and 98 nm formed by the leaf extracts of *Caesalpinia coriaria* showed better antibacterial activity against *Escherichia coli* (12 mm) and *Pseudomonas aeruginosa* (18 mm) [177]. Apoptosis of *C. albicans* and *S. cerevisiae* by *P. oleracea*-mediated AgNPs may be due to the generation of reactive oxygen species and decreased production of hydroxyl radicals initiated by the phytoconstituents capped on the synthesized AgNPs [178].

*In vivo* analysis of biochemical and histological parameters provides evidence for the antibacterial effect of AgNPs synthesized using *Leucas aspera* on fish models (*Aeromonas hydrophila* and *Catla catla*) [179]. Antimicrobial activity of *Sphaeranthus amaranthoides* extract synthesized silver

nanoparticles was reported to be enhanced due to the destabilization of the outer membrane, blocking bacterial respiration, and depletion of intracellular ATP leads to the denaturation of bacterial cell wall. The variation in the growth inhibition against the Gram +ve and Gram –ve bacteria might be due to the permeability of cell membrane [180]. AgNPs synthesized using *Vinca rosea* leaf extract showed promising inhibition against *Staphylococcus aureus*, *Lactobacillus*, *Escherichia coli*, and *Pseudomonas fluorescens* at 10  $\mu$ L concentration [181].

*Mukia scabrella* synthesized AgNPs showed 81.81%, 90%, and 63.23% antibacterial activity against nosocomial Gram negative bacterial pathogens *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter*, respectively [182]. The highest zone of inhibition (16 mm) was obtained for the AgNPs synthesized using *Citrus sinensis* and *Centella asiatica* against *Pseudomonas aeruginosa* compared to that of AgNPs produced using *Syzygium cumini* and *Solanum trilobatum* [183]. *Datura alba* (Nees) leaf derived silver nanoparticles showed better inhibitory zone (20 mm) against *Clostridium diphtheriae* and cell death is reported to be accompanied by the protein denaturation and rupture of the bacterial cell wall [184].

AgNPs synthesized using methanol extract of *Solanum xanthocarpum* berry indicates a stronger anti-*H. pylori* activity and a noncompetitive inhibition was concluded from Lineweaver-Burk plots [185]. *Desmodium triflorum* extract aided silver nanoparticles inhibited the growth of *Staphylococcus* and *E. coli* by 62 and 88%, respectively, at 14–60  $\mu$ g/cm<sup>3</sup> concentrations after 24 hours, while 100  $\mu$ g/cm<sup>3</sup> concentration showed almost 100% inhibition [186]. *Gelidiella acerosa* extract synthesized AgNPs are highly active against tested fungal species at a 50  $\mu$ L concentration against *Mucor indicus* (22.3 mm) and *Trichoderma reesei* (17.2 mm) compared to the standard antifungal agent Clotrimazole [187].

Maximum inhibitory zones (25 and 27 mm) were noted for *Ocimum sanctum* leaf extract aided AgNPs against *Proteus vulgaris* and *Vibrio cholerae*, respectively. The leaf extract nanoparticles of *Vitex negundo* showed a minimum inhibition rate against the aforesaid bacterial pathogens [188]. Silver nanoparticles using leaf broth of *Gliricidia sepium* at 50  $\mu$ L concentration showed 3 mm zone of inhibition for *Staphylococcus aureus* and 2 mm for *Escherichia coli*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* [189].

**8.2. Larvicidal Activity.** The larvicidal activity of *Leucas aspera* aided synthesized AgNPs shows maximum efficacy at LC<sub>50</sub> values of 8.5632, 10.0361, 14.4689, 13.4579, 17.4108, and 27.4936 mg/L and LC<sub>90</sub> values of 21.5685, 93.03928, 39.6485, 42.2029, 31.3009, and 53.2576 mg/L, respectively, against the fourth instar larvae of *A. aegypti* [190]. AgNPs synthesized using *Drypetes roxburghii* (Wall.) showed 100% mortality in second instar larvae of *Anopheles stephensi* at 5 ppm concentration and 100% mortality in all instars of *Culex quinquefasciatus* and *Anopheles stephensi*, respectively, at double the concentrations [191]. AgNPs of size 25–30 nm synthesized using aqueous leaf extract of *Nerium oleander*

showed highest mortality against both larvae and pupae of *Anopheles stephensi* [192].

The exposure of the larvae to varying concentrations of *Pedilanthus tithymaloides*-AgNPs showed 100% mortality from first to fourth instars and pupae of *A. aegypti* after 24 h. Lethal concentration (LC<sub>50</sub>) values of AgNPs were found to be 0.029, 0.027, 0.047, 0.086, and 0.018% against the larval and pupal stages, with no mortality in the control [193]. Synthesized AgNPs using *Sida acuta* are reported to have significant activity against the vector mosquitoes *A. stephensi*, *A. aegypti*, and *C. quinquefasciatus*, respectively [194]. The IC<sub>50</sub> values for the antiparasitoid activity of the AgNPs synthesized using aqueous extracts of Ashoka and Neem leaves against *Plasmodium falciparum* are 8 and 30 µg/mL, respectively [195].

*Vinca rosea* synthesized AgNPs did not exhibit any noticeable toxicity on *Poecilia reticulata* after 24, 48, and 72 h of exposure but are reported to possess the potential to control *A. stephensi* and *C. quinquefasciatus* [196]. *Euphorbia hirta* synthesized AgNPs showed highest larval mortality values of LC<sub>50</sub> against larvae and pupae [197]. The adulticidal and larvicidal activity of synthesized AgNPs of *C. quadrangularis* showed 100% mortality against *H. maculata* and *R.(B.) microplus* [198]. Appreciable larvicidal activity of synthesized AgNPs utilizing aqueous extract of *Eclipta prostrata* is reported against *Anopheles subpictus* and *Culex tritaeniorhynchus* [199].

**8.3. Anticancer Activity.** Silver nanoparticles synthesized using *Acalypha indica* Linn show only 40% cell inhibition against human breast cancer cells (MDA-MB-231) [200]. The MCF-7 cells lose their 50% viability at 5 µg/mL for the AgNPs produced by *Dendrophthoe falcata* (L.f) Ettingsh [201]. Silver-(protein-lipid) nanoparticles prepared using seed extract of *Sterculia foetida* (L.) showed cellular DNA fragmentation against HeLa cancer cell lines [202]. *Datura innoxia*-AgNPs inhibited 50% proliferation of human breast cancer cell line MCF7 at 20 µg/mL after 24 h incubation by suppressing its growth, arresting the cell cycle phases, and reducing DNA synthesis to induce apoptosis [203]. The cytotoxic assays of *Chrysanthemum indicum*-AgNPs showed no toxicity toward 3T3 mouse embryo fibroblast cells at a concentration of 25 µg/mL [204].

The differences in their level of anticancer activity against A375 skin melanoma cells was observed for the AgNPs synthesized using the ethanolic extracts of *Phytolacca decandra*, *Gelsemium sempervirens*, *Hydrastis canadensis*, and *Thuja occidentalis* [205]. *Ficus religiosa* derived AgNPs was effective at a dose 50 µg/mL against the DAL induced mice model (30–35 g) [206]. Silver nanoparticles synthesized using *Origanum vulgare* showed dose dependent response against human lung cancer A549 cell line (LD<sub>50</sub>–100 µg/mL) [207]. The complete apoptosis (95%) was observed at 25 µL/mL of *Alternanthera sessilis*-assisted AgNPs for prostate cancer cell (PC3), whereas 99% growth inhibition was obtained for breast cancer cells (MCF-7) [208].

*Albizia adianthifolia* leaf extract synthesized AgNPs (AA-AgNPs) showed 21% and 73% cell viability for A549 cells

and 117 and 109% normal peripheral lymphocytes after 6 h exposure at 10 µg/mL and 50 µg/mL extract, respectively. This indicates that the AgNPs are nontoxic to the normal PLs cells [209]. The 50% cell inhibition of A549 cells was obtained at 43 µg/mL of AA-AgNPs and induces cell death by the generation of ROS resulting in apoptosis [210]. The nuclear condensation, cell shrinkage, and fragmentation are noticed for MCF-7 cells treated with *Sesbania grandiflora* mediated AgNPs (20 µg/mL) after 48 h in Hoechst staining. These changes indicate the activation of DNA repair due to the cleavage of the substrates [211].

The cell death (100%) of the HeLa cell line was observed with 100 µg of AgNPs synthesized using the root of *Morinda citrifolia* [212]. Longer exposures to *Eucalyptus chapmaniana*-AgNPs (0.02 mmol/mL) resulted in 85% cell death after 24 h incubation [213]. The viability (50%) of A375 cells was found at different concentrations of AgNPs synthesized using *Phytolacca decandra*, *Gelsemium sempervirens*, *Hydrastis canadensis*, and *Thuja occidentalis* [205]. AgNPs produced using extracts of *Aloe*, *Magnolia* leaves, and *Eucalyptus* leaves at concentrations 2–4 ppm were found to be noncytotoxic to Human Embryonic Kidney 293 cells as analyzed by the automated InQ Plus equipment [214].

The viability of HL-60 cells decreased to 44% after 6 h treatment with the *Rosmarinus officinalis*-AgNPs at 2 mM and cell death increased to 80% after 24 h incubation [215]. Cytotoxic activity was extremely sensitive to the size of the nanoparticles produced using *Iresine herbstii* leaf and the viability measurements decreased with increasing dosage (25–300 µg/mL) against the HeLa cell line [216]. The piperidine, piperlongumine, and piperlonguminine present in *Piper longum* may be responsible for the synthesis of silver nanoparticles and exhibited a significant cytotoxic effect (94.02%) at 500 µg/mL on HEP-2 cell lines [217]. The stem latex of *Euphorbia nivulia* capped AgNPs solubilizes the AgNPs in water and acts as a biocompatible vehicle for the transport of nanosilver to human lung carcinoma cells (A549) [218]. Aloe Vera-conjugated AgNPs treated with Human Dermal Fibroblasts (HDF) cells showed no cytotoxicity but possess excellent antibacterial activity on *E. coli* even at very low concentration [219].

**8.4. Wound Healing Activity.** Silver nanoparticles synthesized *in situ* within the network of peptide fibers using UV irradiation inhibited the bacterial growth of *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. AgNPs-containing hydrogels on HDFa cells did not show any significant influence on cell viability [220]. AgNPs hydrogel derived using *Arnebia nobilis* root extract investigated for wound healing activity in an excision animal model exert a positive effect due to their antimicrobial potential and provided a novel therapeutic direction for wound treatment in clinical practice [221]. *Indigofera aspalathoides* mediated AgNPs were tested for wound-healing applications following excision in animal models [222]. AgNPs derived from *Chrysanthemum morifolium* added to clinical ultrasound gel used on an ultrasound probe were found to exhibit bactericidal activity contributing to the sterility of the instrument [223].

*In vitro* study of the AgNPs-based dressing, Acticoat Flex 3 applied to a 3D fibroblast cell culture and to a real partial thickness burn patient showed that AgNPs greatly reduce mitochondrial activity and cellular staining techniques revealed nuclear integrity with no signs of cell death [224]. AgNPs drive the differentiation of fibroblasts into myofibroblasts and promote wound contraction, thereby increasing the wound healing efficacy [225]. The reduction in wound inflammation with modulation in liver and kidney functions was observed during skin wound healing by the positive effects of silver nanoparticles through their antimicrobial properties [226]. AgNPs play a role in dermal contraction and epidermal reepithelialisation during wound healing, contributing to increased rate of wound closure [227].

AgNPs prepared extracellularly using the fungus *Aspergillus niger* are reported to modulate cytokines involved in wound healing in the excision rat model [228]. A significant decrease in wound-healing was observed in an average time of 3.35 days for the AgNPs incorporated onto the cotton fabric and dressings and bacterial clearance was also increased from infected wounds with no adverse effects [229–236]. Silver nanoparticles exert antimicrobial properties causing reduction in wound inflammation and modulation of fibrogenic cytokines [19].

**8.5. Medicinal Textiles and Devices.** AgNPs synthesized using *A. dubius* fabricated on the cotton cloth and perspiration pad samples showed high resistance towards *Corynebacterium*, a sweat bacterium [237]. Antibacterial activity of gauze cloth discs incorporated with AgNPs produced by green mature thalli of *Anthoceros* showed antimicrobial activity against *Pseudomonas aeruginosa* [238]. *Curcuma longa* tuber powder capped silver nanoparticles exhibited minimum bactericidal concentration for *Escherichia coli* BL-21 strain at 50 mg/L. The immobilization onto the cotton cloth using sterile water is reported to show better bactericidal activity when compared to polyvinylidene fluoride immobilized cloth [239]. The incorporation of *Azadirachta indica* synthesized silver nanoparticles into cotton cloth results in antibacterial effect against *E. coli* [240].

## 9. Miscellaneous Applications

*Manilkara zapota* leaf extract mediated synthesis of AgNPs and showed acaricidal activity at  $LC_{50}$  3.44 mg/L against *Rhipicephalus (Boophilus) microplus* [241]. AgNPs synthesized using *Jatropha gossypifolia* extract showed higher amoebicidal activity against *Acanthamoeba castellanii trophozoites* [242]. The nonlinear refraction and absorption coefficients values of AgNPs synthesized using *Coriandrum sativum* extract measured by Z-scan technique with ns laser pulses showed superior optical nonlinearity compared to those synthesized through other procedures [243].

**9.1. Water Treatment.** Stable AgNPs synthesized using *Anacardium occidentale* fresh leaf extract at 80°C bud as a novel probe for sensing chromium ions [Cr(VI)] in tap water [244].

The population of bacteria decreased when the concentration of silver nanoparticles prepared using *Prosopis juliflora* leaf extract (10 mg) was treated with 100 mL of sewage after 6 h and increases as the time of incubation increases [245].

**9.2. Catalytic Activity.** The size dependent, catalytic activity of the synthesized AgNPs using Kashayam, Guggulutiktham, was established in the reduction of Methylene Blue (MB) by  $NaBH_4$  [246]. *Acacia nilotica* pod mediated AgNPs modified glassy carbon electrode showed greater catalytic activity on the reduction of benzyl chloride compared to those of glassy carbon and metallic Ag electrode [247]. Photocatalytic degradation of methyl orange was measured spectrophotometrically using *Ulva lactuca* synthesized AgNPs as nanocatalyst under visible light illumination [248]. The synthesized AgNPs using *Gloriosa superba* extract act through the electron relay effect and influence the degradation of methylene blue at the end of the 30 min [249]. Hydrogen peroxide reduction proceeds rapidly with the excellent catalytic activity of polydispersed silver nanoparticles produced using *Triticum aestivum* (khopali ghahu) extract [250]. The reduction of 4-nitrophenol (4-NP) to 4-aminophenol (4-AP) efficiently carried out in the presence of *Breynia rhamnoides*-AgNPs and  $NaBH_4$  and found to be depend upon the nanoparticle size or the stem extract concentration [251].

## 10. Recent Techniques in the Synthesis of Nanoparticles

**10.1. Pulsed Laser Ablation Techniques.** It is a simple technique compared to other physical and chemical methods and widely used for fabrication of nanomaterials such as noble metals, alloys, oxides, and semiconductors. In this method, the output of a pulsed laser (nano, pico, or femto second) is focused on the surface of the target material immersed in the liquid. The main characteristic of Pulsed Laser Ablation in Liquid (PLAL) is the production of well-defined crystalline nanoparticles through a single step process without any posterior thermal treatment [252–254].

AgNPs of size ranging from 15.1 to 4.3 nm were synthesized by laser ablation of an Ag target in deionised water at a relatively high laser fluence of  $15 \text{ J/cm}^2$  which increased linearly with an increase in water layer thickness and reached maximum value of 14 mm thickness [255]. AgNPs of size (22.08 and 10.5 nm) were prepared in the organic compound (ethylene glycol) and biopolymer (chitosan), respectively, by ablation of a pure Ag plate for 30 min using A Q-Switched Nd: YAG pulsed laser ( $\lambda = 532 \text{ nm}$ , 360 mJ/pulse) [256]. Laser ablation was used for the synthesis of AgNPs (6–12 nm) on a high purity silver bulk in distilled water by optimizing the effect of laser fluences [257]. Fragmentation of AgNPs at an absorption of 355 nm laser light was found to be highly effective which was synthesized by laser (Nd: YAG,  $\lambda = 1064 \text{ nm}$ ) ablation of a silver target immersed in various concentrations of NaCl solutions as well as in water showed [258].



## 11. Conclusion

The above review encompasses the various methods of synthesis of silver nanoparticles and its gamut of applications. This review spotlights the research work on plant-assisted synthesis of AgNPs, an emerging area in the field of nanotechnology. The steady increase in the publications on the aforesaid topic was explored for the benefit of the future researchers. New insights about the pharmacological applications such as anticancer, larvicidal, medical textiles, and devices are gleaned with these exotic silver nanoparticles. Hence, these biogenically synthesized silver nanoparticles will result in a significant payoff for the field of bionanomedicine.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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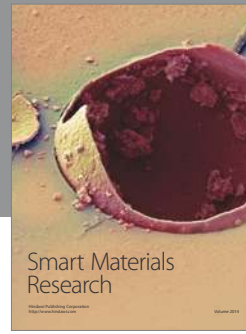
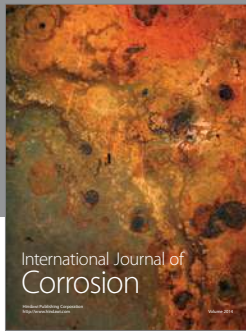
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