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# Facile green synthesis and applications of silver nanoparticles: a state-of-the-art review

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In the field of nanotechnology, the development of reliable and eco-friendly methods for the synthesis of NPs is crucial. The conventional methods for the synthesis of NPs are costly, toxic, and not ecofriendly. To overcome these issues, natural sources such as plant, bacteria, fungi, and biopolymers have been used to synthesize AgNPs. These natural sources act as reducing and capping agents. The shape, size, and applications of AgNPs are prominently affected by the reaction parameters under which they are synthesized. Accessible distributed data on the synthesis of AgNPs include the impact of different parameters (temperature and pH), characterization techniques (DLS, UV-vis, FTIR, XRD, SEM, TEM and EDX), properties and their applications. This review paper discusses all the natural sources such as plants, bacteria, fungi, and biopolymers that have been used for the synthesis of AgNPs in the last ten years. AgNPs synthesized by green methods have found potential applications in a wide spectrum of areas including drug delivery, DNA analysis and gene therapy, cancer treatment, antimicrobial agents, biosensors, catalysis, SERS and magnetic resonance imaging (MRI). The current limitations and future prospects for the synthesis of inorganic nanoparticles by green methods are also discussed herein.

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## 1. Introduction

Nanoscience and nanotechnology are highly interdisciplinary branches conducted at the nanoscale, which is about 1 to 100 nm. The physicist Richard Feynman presented a talk entitled "There is Plenty of Room at the Bottom" on December 29, 1959 at the California Institute of Technology at a meeting of the American Physical Society Feynman RP. There's plenty of room at the bottom: an invitation to enter a new field of physics.[1] In his keynote address, Feynman described manipulation technology at the atomic scale. After a decade, in his expedition of ultraprecise fabrication, Professor Norio Taniguchi framed the term nanotechnology. Nanotechnology has turned into a mainstream and vital innovation in recent years. Nanotechnology itself addresses NPs that are nuclear or atomic aggregates described by a size of under 100 nm. Nanotechnology alludes to the term for the assembling, depiction, control, and utilization of structures to control the size and shape at the nanoscale.[2] Materials in the nanoscale have exceptional contrasting properties to that of similar materials in bulk. These distinctions are due to the basic and physical properties of metal molecules and surface-to-volume proportion to nanotechnology progression, where countless nanomaterials display characteristic properties.[3]

Around 5000 years back, numerous Egyptians, Persians, Greeks and Romans utilized silver in several structures to store nourishment items.[4] During ancient periods, silverware was used in household daily activity due to its antimicrobial activity. There are records regarding the therapeutic applications of silver in the literature as early as 300 BC. Until the revelation of antimicrobials by Alexander Flemming, silver was ordinarily utilized as an antimicrobial specialist. In the Hindu religion, to date, silverware is favored for making the "panchamrit" utilizing *Ocimum sanctum*, curd, and different ingredients. The restorative properties of different metals are referenced in the old Indian Ayurvedic prescription book named "Charak Samhita[5]".

In the past, AgNPs have attracted considerable attention from analysts. Due to the uncommon attributes of AgNPs, they are used in different fields such as biomedical (fast diagnosis, imaging, tissue regeneration and drug delivery, and development of new medical products),[7] textile industry,[8] food packaging,[9] cosmetic industry,[10] catalysis,[11] sensors,[12] biology, coatings,[13] plasmonics (SERS),[14] optoelectronics,[15] antimicrobial activities,[16] DNA sequencing,[17] SERS,[18] climate change and contamination control,[19] clean water technology,[20] energy generation, and information storage. Also, due to their remarkable protection against a wide scope of microorganisms and medicinal properties, AgNPs are utilized as anti-infection agents, tranquilizers conveyance agents, water treatment, farming, *etc.*[21] Furthermore, due to their high conductivity, AgNPs have found application in electronic devices, inks, adhesives, pastes, *etc.*[22] Generally, the synthesis of AgNPs is

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carried out using physiochemical techniques such as autoclaving, gamma-ray radiation, use of microemulsions, electrochemical techniques, chemical reduction, laser ablation, microwave irradiation, and photochemical reduction.[23–33] Fig. 1 presents the various techniques used for the synthesis of NPs.

The above methods have a high yield, but simultaneously they have limitations such as the use of toxic chemicals, and high functional cost and energy requirement. To overcome the limitations of physiochemical methods, alternative cost-effective methods involving plant extracts, microorganisms and natural polymers have been used for the synthesis of AgNPs. The combination of green chemistry and nanotechnology has extended the range of cytogenetically and biologically compatible metallic NPs.[4]

Over the previous decade, few review concentrating on the green synthesis of AgNPs have been published.[34] The majority of them concentrated on a few plants (aloe leaf,[35] cherry extract,[36] *Coffea arabica* seed,[37] *Trianthema decandra*,[38] *Macrotyloma uniflorum*,[39] and *Rosa rugosa*[40]), biopolymers[41] (chitosan[42,43]) and microbial sources[44] for the synthesis of AgNPs. Several characterization procedures (DLS, UV-vis, FTIR, XRD, SEM, TEM and EDX) have been employed to investigate information regarding the source, shape, size and properties of AgNPs with respect to different applications. The present review, in contrast to the prior reviews, focuses on the synthetic methods, parameters, characterization techniques, applications, and anticipated antibacterial components from different green ways for the synthesis of AgNPs.

## 2. Green synthesis

The basic requirement for the green synthesis of AgNPs is silver nitrate and a natural reducing agent.[10–30] Generally, a natural reducing agent or different components present in the cell work as stabilizing or capping agents, and thereby the need to include these agents from outside is minimized.[4] The traditional strategies for the production of NPs are costly, harmful, not environment-friendly. Thus, to overcome these issues, specialists have adopted green methods for the synthesis of NPs.[45] Natural resources and their constituents have been utilized to synthesize NPs. Green synthesis can be classified as: (a) from plants and their extracts, (b) from bacteria, (c) from fungi and (d) from biopolymers. Various reducing agents have been used for the synthesis of AgNPs, which are shown in Table 1 with the general mechanism for the synthesis of AgNPs. The green synthesis *via* plants and plant extracts, bacteria, fungi, and biopolymers is described in the next sections of this review.

### 2.1 Synthesis of AgNPs from plants

The plant-based synthesis of AgNPs is generally adopted more compared to methods that use microorganisms since it can be improved easily, less bio-threatening and do not include the step of cell culture growth.[46–50] All the parts of a plant (leaves, fruits, roots, seeds, and stems) contain biomolecules (*e.g.* enzymes, alkaloids, polysaccharides, tannins, terpenoids, phenols, and vitamins), which are of great therapeutic value and, despite their complex structures, are good for the

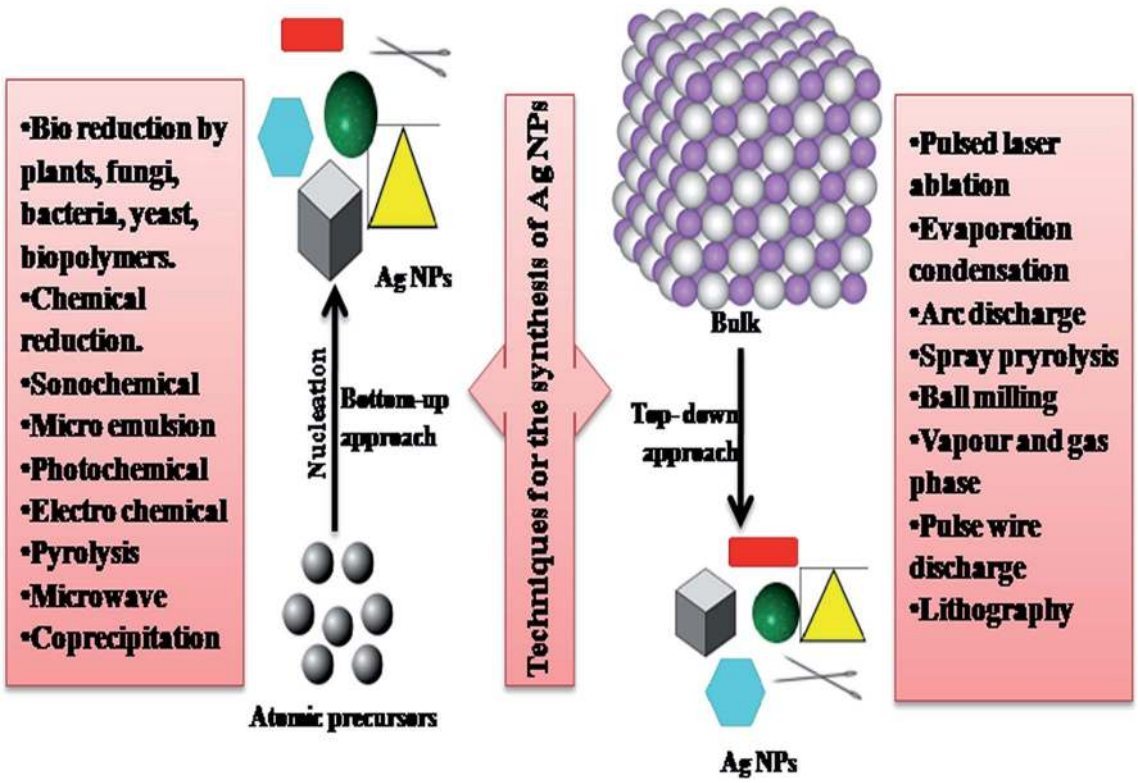


Fig. 1 Representation of various techniques for the synthesis of NPs.[6]

Table 1 Various constituents of plant, bacteria, fungi, and biopolymer responsible for the reduction of silver nitrate to AgNPs

| Source | Components responsible for the reduction of silver nitrate | Mechanism for the synthesis |
|---|---|---|
| Plants | Flavanoids, terpenoids, alkaloids, polyphenols, alcohol, phenolic acids, antioxidants, vitamins | Electrostatic interaction between the functional groups of respective constituent of plant extract and $Ag^+$ ion |
| Fungi | Proteins, enzymes, NADH, NADPH, peptides, nitrogenous biomacromolecules, napthoquinones, anthraquinones | Intracellular and extracellular synthesis of AgNPs |
| Biopolymers | Chitosan, lignin, polypeptides, alginate, cellulose, protein | Electrostatic interaction between $Ag^+$ ion and polar groups attached to polymer |

environment.[51] Plant extract replaces all toxic chemicals such as trisodium citrate and sodium borohydride ($NaBH_4$). The extract from plants assists well in the synthesis of NPs due to the formation of AgNPs stabilized by the flavonoid and terpenoid components present in leaf broth, while the reduction of silver ions is favored by the polyol and water-soluble heterocyclic components of leaf broth.[52] The extract of plant *Salvia spinosa* grown under *in vitro* conditions was used for the first time to synthesize AgNPs.[53] The first report on the formation of AgNPs by a living plant system *Alfalfa sprouts* was presented by Gardea Torresdey *et al.* (2003). Alfalfa roots can absorb Ag from agar medium and transfer them in the same oxidation state to the shoots of the plant. These Ag atoms are converted to AgNPs in the shoots.[54] Harekrishna Bar *et al.* (2009) reported the use of the latex of *Jatropha curcas* as the reducing and capping agent to synthesize AgNPs.[55] Sithara *et al.* synthesiszed AgNPs by using leaf extract of *Acalypha hispida* and these AgNPs were used for the detection of $Mn^{2+}$ ions.[56] Gavhane *et al.* (2012) reported the use of the extract of Neem and Triphala to synthesize AgNPs, which were characterized using EDX, TEM, and NTA. TEM and NTA revealed the size of the AgNPs was in the range of 43 nm to 59 nm and they were spherical in shape.[57] Ahmad and Sharma (2012) utilized (pineapple juice) *Ananas comosus* as a stabilizing and reducing agent for the synthesis of AgNPs.[58] Charusheela Ramteke *et al.* (2012) synthesized antibacterial AgNPs using the leaf extract of (Tulsi) *Ocimum sanctum*.[59] Roy *et al.* (2014) used *Malus domestica* fruit extract as a reducing and capping agent to synthesize AgNPs with an average diameter of 20 nm. The formation of the NPs was characterized by UV-vis spectroscopy, their morphology and distinctive phases were analyzed by TEM and XRD, and biomolecules for the reduction and stabilization of NPs were identified *via* FTIR spectroscopy.[60] Velmurugan *et al.* (2015) synthesized AgNPs using peanut shell extract and compared their antifungal activity and characteristics with that of commercial AgNPs.[61]

Prem Jose Vazhacharickal *et al.* (2015) synthesized AgNPs using Curry leaf (*Murraya koenigii*) as the reducing and capping agent, which exhibited good antibacterial activity.[62] M. Firdaus *et al.* (2017) reported the synthesis of AgNPs using aqueous fruit extract from (*Carica papaya*) papaya as the reductant under sunlight irradiation without additional capping agents. The AgNPs were characterized *via* UV-vis spectrophotometry and FTIR spectroscopy. A green environmental sensor was developed due to the good selectivity of AgNPs towards the hazardous heavy metal mercury in aqueous solution.[63] Jerushka S. Moodley *et al.* (2018) reported the antimicrobial potential of synthesized AgNPs using leaf extracts of *Moringa oleifera* and utilized sunlight irradiation as the primary source of energy.[64] Yu C. *et al.* (2019) synthesized AgNPs from the leaf extract of *Eriobotrya japonica* (Thunb) and utilized them in the catalytic degradation of reactive dyes.[65] The most suitable choice to synthesize AgNPs is plant-like angiosperms. Medicinally important plants such as *Boerhaaviadiffusa*,[66] *Tinosporacordifolia*,[67] *Terminalia chebula*,[68] aloe vera[69], *Ocimumtenuiflorum*,[70] *Catharanthus roseus*,[71] *Emblica officinalis*[72], *Azadirachtaindica*,[73] *common spices Piper nigrum*,[74] *Cocos nucifera*[75], *Cinnamon zeylanicum*[76] and some tropical weeds such as *Partheniumhystero-phorus*[77] have been utilized to synthesize AgNPs. Plants that produce essential oils (*Mentha piperita*) and alkaloids (*Papaver somniferum*) have also been used to synthesize AgNPs. The have been a few cases in which chemicals such as sodium-dodecyl sulfate were used externally to stabilize AgNPs.[78] All the plant extracts act as both reducing agents and capping agents. The proteins metabolites[79] and chlorophyll[80] present in the extract of plants act as stabilizing agents to synthesize AgNPs. Fig. 2 presents the mechanism for the synthesis of AgNPs from plants. Other synthetic procedures, conditions, characterization and application of AgNPs are discussed below (Table 2).

### 2.2 Green synthesis of AgNPs from bacteria

NPs of noble metals such as Ag and Gold have been synthesized utilizing either intra or extracellular inorganic materials created by bacteria.[130] Fig. 3 shows the synthetic procedure for the synthesis of AgNPs from the biomass of bacteria. Slawson *et al.* (1992) reported that AgNPs are biocompatible in a few bacteria, which are Ag-resistant.[131] Pooley (1982) reported that bacteria aggregate Ag on the bacterial cell walls, and recommended the use of bacteria to industrially recover Ag from ore.[132] First, Klaus *et al.* (1999) synthesized AgNPs using the biomass of the *Pseudomonas stutzeri* AG259 bacteria (Ag resistant). The amount of AgNPs accumulated by the bacteria cells was up to 200 nm.[133] Kalimuthu *et al.* (2008) reported the synthesis of AgNPs with a size of 50 nm by adding silver nitrate aqueous solution to the biomass of *B. licheniformis*. A whitish-yellow to brown color confirmed the formation of AgNPs stabilized by the nitrate of enzymes.[134] Nanda and Saravanan (2009) also synthesized AgNPs utilizing culture supernatants of *Staphylococcus aureus*.

 

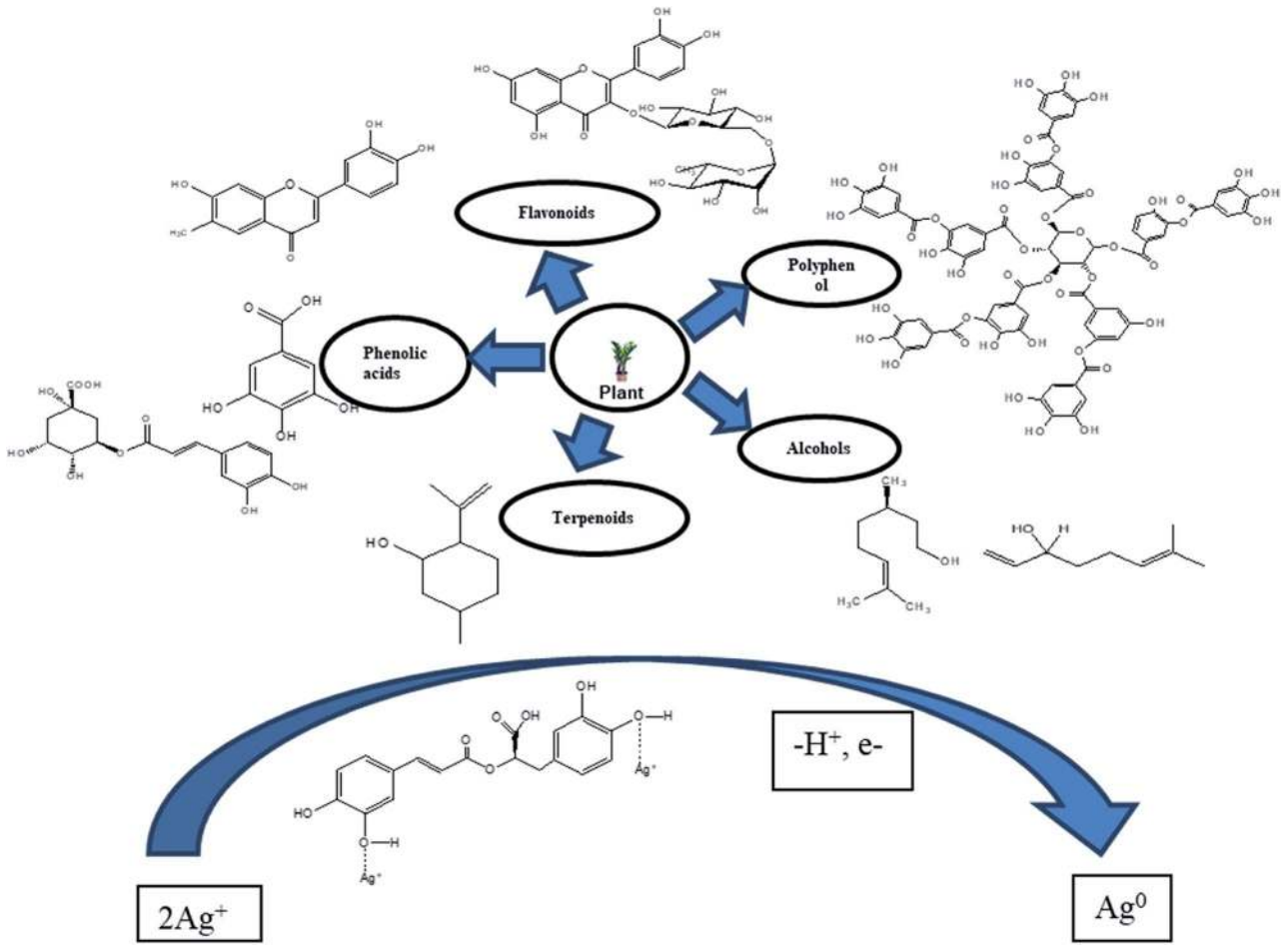


Fig. 2 Mechanism for the synthesis of AgNPs from plant sources.

However, the culture supernatants from *Enterobacteriaceae* can be used for the quick synthesis of AgNPs.[135] Shivaji *et al.* (2011) synthesized AgNPs utilizing culture supernatants of psychrophilic bacteria.[136] Monowar *et al.* (2018) reported the extracellular synthesis of AgNPs utilizing the extract of an endophytic bacterium from *Pantoeaananatis*.[137] Samadi *et al.* (2009) reported the use of *Proteus mirabilis* PTCC 1710 bacteria to synthesize AgNPs. During the incubation of bacteria, different types of broth are used for advancement in extracellular and intracellular synthesis. The mechanism for the green synthesis of AgNPs from bacteria is shown Fig. 3.

The choice of bacteria in the green synthesis of AgNPs and an appropriate method are important for their large scale production.[138] Mokhtari *et al.* (2009) reported the synthesis of AgNPs *via* photosynthesis by adding a solution of silver nitrate to the culture supernatant of *Klebsiella pneumonia*, and showed visible-light irradiation prompted the synthesis of AgNPs with a size of 3 nm[139]. According to reports by Lee and Shehata and Marr (Lee 1996; Shehata and Marr 1971), AgNPs were also produced *via* the reduction of silver ions using culture supernatants of bacteria. It should be noted that the growth of bacteria depends on the nutrients in the culture medium (glucose, phosphate or tryptophan).[140] Shahverdi *et al.* (2007) reported the use of *Enterobacter cloacae* (*Enterobacteriaceae*), *Escherichia coli* and *Klebsiella pneumonia* for the fast synthesis of AgNPs, which formed AgNPs within a few minutes of Ag ions reacting with the cell filtrate.[141] Kharissova *et al.* (2013) highlighted that bacteria kept on growing after the synthesis of AgNPs. However, compared to conventional methods, the utilization of bacteria for the reduction of $Ag^+$ ions leads to a slow formation rate and limited range of shapes and sizes of AgNPs.[142] Therefore, fungi-based NPs and reducing agents involving plants and plant extracts have been investigated for the synthesis of AgNPs (Table 3).

### 2.3 Synthesis of AgNPs from fungi and yeast

Organic matter provides unique traits for the synthesis of NPs with advanced properties. Fungi are the main choice of microorganisms for the synthesis of NPs due to their vast range of advantages over yeast, bacteria, plants, and physicochemical techniques.[144] Fig. 4 presents the mechanism for the synthesis of AgNPs using fungi.

Fungi can synthesize metal NPs since they secrete enzymes and proteins, which are used to reduce metal salts. The large-scale synthesis of NPs from distinct fungal strains has been implied due to their growth even *in vitro*[145]. Xue B. *et al.* (2016)

Table 2 Synthesis using plant extracts generates NPs with well-defined shapes, structures, and morphologies compared to that obtained using the bark, tissue, and entire plant

| S. no. | Reducing agents (green sources) | Applications | Operating conditions | Characterization techniques used | Particle characteristics | Reference |
|---|---|---|---|---|---|---|
| 1 | Aqueous leaf extract of aloe vera | Antibacterial activity | $AgNO_3$ (1 mM), extract: $AgNO_3$ (1 : 10), stir 20 min, incubated for 24 h | UV-vis, FTIR, TEM, | Size: (36.61 ± 4.88 nm), shape: spherical | 35 |
| 2 | Cherry extract | Antioxidant | $AgNO_3$ (1 mM), extract: $AgNO_3$ (1 : 10) | FTIR, UV-vis, DLS, TEM, XRD, TGA and DTA | Size: (61.1 ± 39.2 nm) (for blue light), shape: spherical, crystal - FCC | 36 |
| 3 | *Coffea arabica* seed extract | Antibacterial activity on *E. coli* and *S. aureus* | $AgNO_3$ (0.02 M, 0.05 M, and 0.1 M), volume of extract kept constant for each solution | SEM-EDX, TEM, XRD, UV-vis, DLS | Size: (20 to 30 nm), shape: spherical and ellipsoidal | 37 |
| 4 | Plant extract of *Trianthema decandra* | Antimicrobial activity | $AgNO_3$ (1 mM), extract: $AgNO_3$ (1 : 5, 1 : 10, 1 : 15) | EDX, FTIR, UV-vis, SEM | Size: (36 to 74 nm) | 38 |
| 5 | Seed extract of *Macrotyloma uniflorum* | | $AgNO_3$ (0.59 mM), extract: $AgNO_3$ (1 : 60) | TEM, XRD, UV-vis, FTIR | Size: (12 nm) | 39 |
| 6 | Fruit extract of *Tanacetum vulgare* | | $AgNO_3$ (1 mM), extract: $AgNO_3$ (1.8 : 50) | TEM, XRD, EDX, FTIR, zeta potential, UV-vis, FTIR | Size: (16 nm), shape: spherical | 40 |
| 7 | Leaf extracts of *Rosa rugosa* | | $AgNO_3$ (1 mM), extract: $AgNO_3$ (2.5 : 60) | UV-vis, TEM, XRD, FTIR, zeta potential, EDX | Size: (12 nm), shape: spherical | 40 |
| 8 | *Saccharum officinarum* | High antimicrobial activity against biofilm-forming bacteria and fungi. Reduce cytotoxicity on mammalian somatic and tumoral cells | $AgNO_3$ (14, 24, 52, and 104 mM), CS : $AgNO_3$ : VC (5 : 1 : 1), stirred for 12–15 h with heat | UV-vis, TEM, EDS | Size: (<10 nm) | 42 |
| 9 | Seed extract of *Nelumbo nucifera* | Antibacterial and antifungal | $AgNO_3$ (1 mM), seed extract: $AgNO_3$ (1 : 19) | UV-vis, FTIR, TEM, XRD, SEM | Size: (5.03 to 16.62 nm), shape: spherical | 43 |
| 10 | *Eriobotrya japonica* (Thunb.) leaf extract | Catalytic degradation of reactive dyes | Different ratios of leaf extract and silver salt solution (1 : 1, 1 : 2, and 1 : 10, v/v) | UV-vis, XRD, TEM, SEM, FTIR, EDX | Size at different temperatures: 20 °C: 9.26 ± 2.72, 50 °C: 13.09 ± 3.66, 80 °C: 17.28 ± 5.78 nm | 65 |
| 11 | Sapota pomace extract (*Manilkara zapota*) | Good antibacterial activity against Gram-positive and Gram-negative bacteria | $AgNO_3$ (7 mM), mixed with extract in ratio 1 : 0.5 (v/v), temperature 20 °C | UV-vis, XRD, FTIR, DLS, TEM, zeta potential | Size: (8 to 16 nm), shape: spherical, moderate stability (zeta potential of −13.41) | 81 |
| 12 | Pomegranate peel extract (*Punica granatum*) | Antibacterial activity against *Staphylococcus*, *Pseudomonas aeruginosa* and *Escherichia coli* pathogens | $AgNO_3$ (1 mM), mixed with extract (incubated for 24 h) | UV-vis, FTIR, SEM | Size: (5 to 50 nm), UV-vis: 371 nm | 82 |
| 13 | *Saccharum officinarum* extract and chitosan | Antibacterial against *Bacillus subtilis*, *Klebsiella planticola*, *Streptococcus faecalis*, *Pseudomonas aeruginosa* and *Escherichia coli* | $AgNO_3$ (1 mM), extract: $AgNO_3$ (9 : 1), incubated at 37 °C, till change in color | UV-vis, TEM, SEM, EDS, FTIR | Size: (10 to 60 nm), UV-vis: 460 nm | 83 |
| 14 | *Arbutus unedo* (strawberry) leaf extract | Cost effectiveness, medical and pharmaceutical applications | $AgNO_3$ (1 mM), $AgNO_3$:extract (1 : 1); temperature 80 °C, stir at 1000 rpm | UV-vis, EDS, TEM, XRD | Size: (2 to 20 nm), shape: spherical, geometry: FCC | 84 |
| 15 | Pomegranate leaf extract | Antibacterial, anticancer activity on human cervical cancer cells[86] | $AgNO_3$ (1 mM), $AgNO_3$:extract (9 : 1) | UV-vis, FTIR, SEM, XRD, EDX | Size: (10 to 30 nm), geometry cubic | 85 |
| 16 | Walnut seed extract | Used in photocatalytic degradation of effluent dye | $AgNO_3$ (1 mM), $AgNO_3$ : Extract (10 : 1) | UV-vis, XRD, FTIR, TEM | Size: (80 to 90 nm), shape: spherical, UV-vis: 420 nm, crystalline | 87 |
| 17 | *Cinnamomum camphora* leaf extract | | $AgNO_3$ (1 mM), heat: 30 °C, stir: 150 rpm | UV-vis, XRD, TEM, SEM, AFM, FTIR | Size: (55 to 80 nm), shape: spherical and triangular | 88 |

Table 2 (*Contd.*)

| S. no. | Reducing agents (green sources) | Applications | Operating conditions | Characterization techniques used | Particle characteristics | Reference |
|---|---|---|---|---|---|---|
| 18 | Pomegranate peel extract | Photocatalytic degradation of methylene blue | $AgNO_3$ (1 mM), pH: 8, temperature: (21 ± 5 °C) | UV-vis, XRD, FTIR, EDS | Size: (57.7 to 142.4 nm) | 89 |
| 19 | *Azadirachta indica* aqueous leaf extract | Antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* | $AgNO_3$ (1 mM to 5 mM) (1–5 mL) of extract was added to 10 mL of $AgNO_3$ solution | FTIR, UV-vis, DLS, photoluminescence, TEM | Size: (34 nm), shape: spherical and irregular | 90 |
| 20 | Grape (*Vitis vinifera*) fruit extract | Antibacterial activity against *Bacillus subtilis* and *Escherichia coli* | $AgNO_3$ (20 mM) extract: $AgNO_3$ solution (1 : 1) | UV-vis, DLS, EDX, TEM | Size: (19 nm), shape: spherical | 91 |
| 21 | *Alpinia katsumadai* seed extract | Free radical scavenging, antibacterial and antioxidant | $AgNO_3$ (10 mM) extract: $AgNO_3$ (1 : 10) pH: 10, stir: 200 rpm for 90 min | UV-vis, FETEM, EDX, SAED, XRD, FTIR | Size: (12.6 nm), shape: spherical | 92 |
| 22 | Apple extract | Antibacterial against Gram-negative and Gram-positive bacteria with MIC of 125 mg $mL^{-1}$ | $AgNO_3$ (0.1 M) extract: $AgNO_3$ (1 : 9) stir and heat at 80 °C | XRD, DLS, FTIR, UV-vis | Size: (30.25 ± 5.26 nm), crystalline | 93 |
| 23 | *Berberis vulgaris* leaf and root extract | Antibacterial activity against *Escherichia coli* and *Staphylococcus* bacteria | $AgNO_3$ (0.5, 1, 3, 10 mM) extract: (3, 5, 10, 15, 30 mL) contact time: (1, 2, 6, 12, 24 h) | XRD, TEM, UV-vis, DLS, | Size: (30 to 70 nm), shape: spherical | 94 |
| 24 | Cinnamon zeylanicum bark extract and powder | Bactericidal activity | 100, 500 and 1000 mg of CBP added to 50 mL of 1 mM aqueous $AgNO_3$ solution and incubated in the dark at 25 °C and shaken at 160 rpm. For CBPE, 1, 2.5 and 5 mL extract added to 50 mL of 1 mM aqueous $AgNO_3$ solution | UV-vis, TEM, EDX, XRD, zeta potential | Size: (31 and 40 nm), quasi-spherical, and small, rod-shaped | 95 |
| 25 | *Lippia nodiflora aerial* extract | Antioxidant and antibacterial against human pathogenic bacteria, cytotoxic against MCF-7 breast cancer cell lines | $AgNO_3$ (1 mM), extract: $AgNO_3$ solution (1 : 19), heat from 30 °C to 95 °C for 10 min | UV-vis, FTIR, XRD, SEM-EDX, TEM, zeta potential | Size: (30 to 60 nm) | 96 |
| 26 | Andean blackberry fruit extract | Antioxidant | $AgNO_3$ (1 mM), extract: $AgNO_3$ solution (1 : 10), keep at 25 °C | UV-vis, TEM, DLS, XRD, FTIR | Size: (12 to 50 nm), shape: crystalline and spherical | 97 |
| 27 | Aqueous broccoli extract | High toxicity against MCF-7 cell line | $AgNO_3$ (1 mM), extract: $AgNO_3$ solution (1 : 19), pH: (6 to 7) | UV-vis, FTIR, XRD, SEM, TEM, EDAX | Size: (40 to 50 nm), FCC structure | 98 |
| 28 | *Pinus merkusii* cone flower extract | | $AgNO_3$ (0.1 M), extract: $AgNO_3$ solution (2 : 1), heated at 60 °C | FTIR, UV-vis, TEM | Size: (9 to 23 nm), shape: spherical | 99 |
| 29 | Curcuma longa tuber (turmeric) powder and extract | Immobilization on cotton cloth for bactericidal activity | 100, 500 and 1000 mg of CLP added to 50 mL of 1 mM aqueous $AgNO_3$ solution and incubated in the dark at 25 °C in a rotary shaker at 160 rpm. 1, 2.5 and 5 mL extract added to 50 mL of 1 mM aqueous $AgNO_3$ solution | UV-vis, TEM, XRD | Size: (21 and 30 nm) | 100 |
| 30 | Garlic extract (*Allium sativum*) | Nontoxic to VSMCs and NIH 3T3 fibroblasts | $AgNO_3$ (0.98 mM), extract solution (1.0 mL to 2.5 mL) added to 51 mL of $AgNO_3$ solution | TEM, UV-vis, EDX, ATR-FTIR, zeta potential, HPLC | Size: (4 to 6 nm) | 101 |
| 31 | Ginger extract (*Zingiber officinale*) | Antibacterial activity against *Escherichia coli*, | $AgNO_3$ (1 mM) extract (20%): $AgNO_3$ solution | UV-vis, XRD | Size: (2.89 nm), shape: spherical | 102 |

Table 2 (*Contd.*)

| S. no. | Reducing agents (green sources) | Applications | Operating conditions | Characterization techniques used | Particle characteristics | Reference |
|---|---|---|---|---|---|---|
| | | *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Bacillus cereus* and *Proteus vulgaris* | (1 : 9), temperature (27 ± 2 °C) | | | |
| 32 | Aqueous seed extract of *Manilkara zapota* (L.) | Antimicrobial activity against *Candida* species | 10% concentration of MZSE was added to 0.01 M $AgNO_3$, heated at 80 °C | EDX, DLS, TEM, XRD, UV-vis | Size: (40 to 100 nm) | 103 |
| 33 | Leaf extract of avocado | Antibacterial activity | $AgNO_3$ (5 mM), extract: $AgNO_3$ solution (1 : 9), kept in the dark for 24 h | FTIR, XRD, SEM, UV-vis | Size: (35.6 nm), shape: spherical | 104 |
| 34 | *Origanum vulgare* L. plant extract | Antimicrobial activity | $AgNO_3$ (0.5 mM), 1 mL of plant extract added to 49 mL of $AgNO_3$ solution and stirred for 2 h at 85–90 °C | FTIR, UV-vis, XRD, TEM, EDX | Size: (12 nm), FCC structure | 105 |
| 35 | Root extract of *Croton sparsiflorus* | Antimicrobial activity | $AgNO_3$ (1 M) | UV-vis, SEM | Size: (30 to 50 nm), shape spherical | 106 |
| 36 | Roots extract of *Coleus forskohlii* | Antimicrobial activity | $AgNO_3$ (1 mM), extract: $AgNO_3$ solution (1 : 20), incubated for 24 h at 28 °C | UV-vis, EDS, FTIR, SEM, XRD | Size: (82.46 nm), shape: needle | 107 |
| 37 | Lemon leaf extract | Antimicrobial activity | $AgNO_3$ (2 mM), extract: $AgNO_3$ solution (1 : 9), keep in the dark at room temp | FTIR, UV-vis, TEM, SEM, AFM | Size: (Smaller than 100 nm range), shape: multi-shaped | 109 |
| 38 | Banana peel extract | Antimicrobial activity | $AgNO_3$ (1.75 mM), extract: $AgNO_3$ (1 : 50 (v/v)) | UV-vis, XRD, SEM, EDX | Size: (23.7 nm), crystalline | 110 |
| 39 | *Valerian officinalis* aqueous extract | | $AgNO_3$ (5 mM), plant powder (0.25, 0.50, 0.75 and 1.0 g) 50 mL distilled water | UV-vis, XRD, SEM, TEM | Size: (22 nm), shape: spherical, crystalline | 111 |
| 40 | *Tectona grandis* seed extract | Antimicrobial activity against microorganisms | $AgNO_3$ (1 mM), $AgNO_3$:seed extract (1 : 9) | UV-visible XRD, FTIR SEM/EDS, FESEM, TEM | Size: (10 to 30 nm), shape: spherical, crystalline | 112 |
| 41 | Extracts of *Ananas comosus* | | $AgNO_3$ (10 mM), pineapple juice: $AgNO_3$ (1 : 10) | XRD, UV-vis, EDAX, TEM | Size: (12 nm), FCC crystalline | 113 |
| 42 | Extract of saffron (*Crocus sativus* L.) | Antibacterial activity | $AgNO_3$ (2 mM), extract: $AgNO_3$ solution (1 : 4) | UV-vis, FTIR, XRD, TEM | Size: (15 nm), shape: spherical | 114 |
| 43 | Onion (*Allium cepa*) extract | Antibacterial activity | $AgNO_3$ (0.1 mM), extract: $AgNO_3$ solution (1 : 10), constant stirring at 50–60 °C | UV-vis, DLS, TEM | Size: (33.6 nm), shape: spherical | 115 |
| 44 | *Thymus kotschyanus* plant extract | Antioxidant, antibacterial and cytotoxic effects | $AgNO_3$ (1 mM), extract: $AgNO_3$ (1 : 10), stir for 30 min in the dark | UV-vis, FTIR, EDX, XRD, TGA, SEM, TEM, AFM | Size: (50 to 60 nm) | 116 |
| 45 | *D. carota* (carrot) extract | | $AgNO_3$ (0.5 mM), extract: $AgNO_3$ (1 : 6) | XRD, UV-visible FTIR, TEM | Size: (20 nm), shape: spherical | 117 |
| 46 | *Garcinia mangostana* stem aqueous extract | Antimicrobial activity | $AgNO_3$ (1 mM), extract: $AgNO_3$ (3 : 17) | UV-vis, XRD, SEM, EDX | Size: (30 nm) | 118 |
| 47 | Olive leaf extract | Antibacterial activity | $AgNO_3$ (1 mM), extract (2–9 mL) added to $AgNO_3$ solution | TEM, UV-vis, FTIR, TG, XRD | Size: (20 to 25 nm), shape: spherical | 119 |
| 48 | Extract of *Chenopodium ambrosioides* | | $AgNO_3$ (1 mM and 10 mM), extract: $AgNO_3$ (0.5, 1, 2, 3 and 5 mL : 5) | UV-vis, TEM, FTIR | Size: (4.9 ± 3.4 nm), FCC | 120 |
| 49 | Aqueous leaf extract of *Acalypha indica* | Antifungal effect against *Phytopathogen Colletotrichum capsici* | $AgNO_3$ (1 mM), extract: $AgNO_3$ (1 : 9), incubate at 37 °C | UV-vis, antifungal | | 121 |
| 50 | Ficus benghalensis leaf extract | Antibacterial activity | | UV-vis, TEM-EDX, XRD | | 122 |

Table 2 (*Contd.*)

| S. no. | Reducing agents (green sources) | Applications | Operating conditions | Characterization techniques used | Particle characteristics | Reference |
|---|---|---|---|---|---|---|
| 51 | Litchi chinensis leaf methanolic extract | Strong muscle relaxant, analgesic and anti-inflammatory activities | $AgNO_3$ (1 M), extract: $AgNO_3$ (1 : 1 and 1 : 10) | UV-vis | | 123 |
| 52 | *Salvia leriifolia* leaf extract | Antibacterial activity against 9 bacteria | $AgNO_3$ (1 mM), extract: $AgNO_3$ (1 : 24) | SEM, AFM, XRD, FTIR | Size: (27 nm), shape: pherical | 124 |
| 53 | *Glycyrrhiza glabra* root extract | Treatment of gastric ulcer | $AgNO_3$ (1 mM), extract: $AgNO_3$ (1 : 49) | UV-vis, TEM, XRD, FTIR | Size: (19 nm), crystalline | 125 |
| 54 | *Pimpinella anisum* seed extract | Antimicrobial activity and cytotoxicity on human neonatal skin stromal cells and colon cancer cells | $AgNO_3$ (3 mM), extract: $AgNO_3$ (1 : 100) | UV-vis, FTIR, XRD, EDX, TEM | Size: (15 nm) | 126 |
| 55 | *Glycyrrhiza uralensis* root extract | Antimicrobial agent against *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella enterica* | $AgNO_3$ (1 mM), extract: $AgNO_3$ (1 : 1), heated in oil bath at 80 °C, change in color is observed | UV-vis, TEM, SAED, XRD, DLS, FTIR | Size: (5 to 15 nm), shape: spherical | 127 |
| 56 | Orange peel | Antimicrobial activity | $AgNO_3$ (1 mM), $AgNO_3$:orange peel extract (1 : 1), pH above 7 | UV-vis, FTIR, DLS, XRD, zeta potential, TEM | Size: (48.1 ± 20.5 nm) | 128 |
| 57 | *Citrus recticulata* fruit peel extract | Antibacterial activity against *Streptococcus pyogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Salmonella Paratyphi* and *Klebsiella pneumoniae* | $AgNO_3$ (1 mM), extract: $AgNO_3$ (1 : 20) | UV-vis, FTIR, XRD, SEM, EDX | Size: (24 nm) | 129 |

reported morphological and molecular methods to synthesize AgNPs under optimized conditions, *i.e.*, the substrate concentration of 1.5 mM, alkaline pH, reaction temperature of 55 °C, and reaction time of 10 h, utilizing the fungal strain of *Arthroderma fulvum*. The synthesized AgNPs were found to be crystalline in nature and the particle size was optimized to be ~15.5 ± 2.5 nm. Antifungal activity was observed against fungal strains, including *Candida*, *Fusarium*, and *Aspergillus*.[146] Honary S. *et al.* (2013) evaluated a green synthetic method for the extracellular production of AgNPs using *Penicillium citrinum* isolated from soil. The synthesized NPs were found to be spherical in shape with an average diameter of 109 nm.[147] A

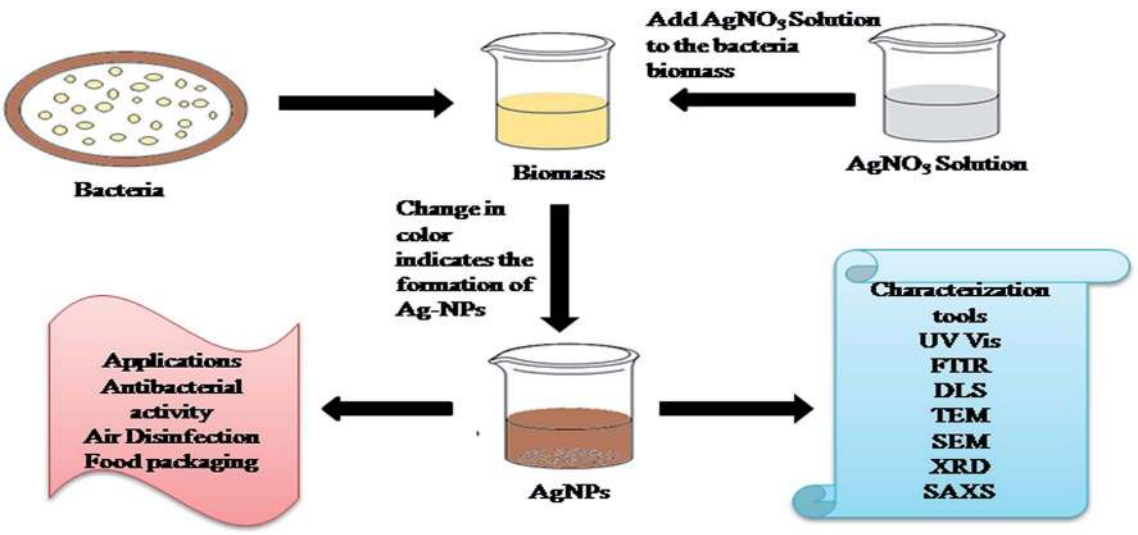


Fig. 3 Mechanism for the green synthesis of AgNPs from bacteria.

Table 3 Synthetic conditions, applications, size and characterization techniques for AgNPs using various strains of bacteria

| S. no. | Bacteria | Application | Conditions | Characterization | Size | Reference |
|---|---|---|---|---|---|---|
| 1 | *Psychrophilic* bacteria | Stable for 8 months in the dark | 1 mL of 1 mM $AgNO_3$ was added to 25 mg of the washed cell, and incubated under a fluorescent lamp (CFL) of 9 W | UV-vis spectroscopy, transmission electron microscopy, atomic force microscopy | Size: (6 to 13 nm) | 136 |
| 2 | Endophytic bacterium, *Pantoea ananatis* | Antimicrobial against multi-drug resistant bacteria | Reaction mixture of cell free extract and 100 mL of 0.1 mM $AgNO_3$ solution (2%, v/v) exposed to bright sunlight, pH (7) | UV-vis, TEM, SEM, FTIR, zeta potential | Size: (8.06 to 91.32 nm) | 137 |
| 3 | Culture supernatant of *Klebsiella pneumonia* | | $AgNO_3$ (1 mM), supernatant 1% (v/v) | XRD, UV-vis, TEM, EDS | Size: (3 nm) | 139 |
| 4 | Culture supernatants of *Enterobacteriaceae* | | $AgNO_3$ (1 mM), supernatant (1%, v/v) | UV-vis, EDS, TEM | Size: (52.5 nm) | 141 |
| 5 | Biomass of bacterial exopolysaccharide | Used in degradation of azo dye | $AgNO_3$, (9 mM) | UV-vis, TEM, SEM, AFM, XRD, TGA-DTA, Raman spectroscopy | Size: (35 nm), shape: spherical | 143 |

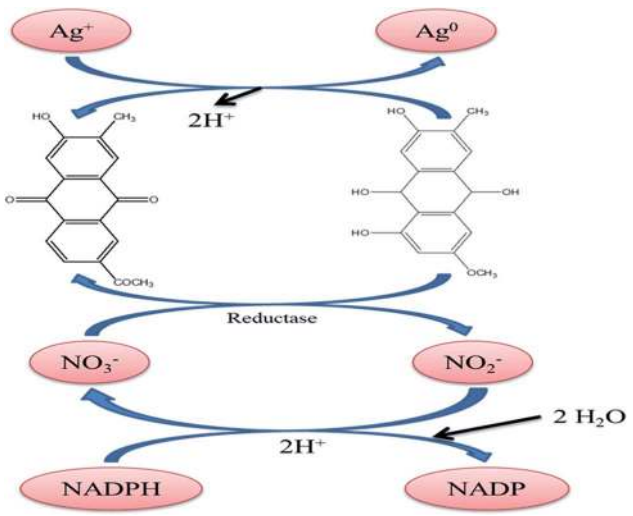


Fig. 4 Mechanism for the synthesis of AgNPs using fungi.

controlled and up-scalable green method for the synthesis of AgNPs with a well-defined morphology utilizing the cell-free aqueous filtrate of a non-pathogenic and suitable biocontrol agent *Trichoderma asperellum* was reported for the first time.[148] Verma VC *et al.* (2010) prepared AgNPs utilizing *Aspergillus clavatus* and demonstrated their antimicrobial potential.[149] AgNPs were synthesized by Li G. *et al.* (2012) using culture supernatants of *Aspergillus terreus* for the reduction of Ag ions.[150]

Subashini G. and Bhuvaneswari S. (2018) reported the synthesis of AgNPs from fungi and their applications in various fields of biology.[151] AgNPs synthesized using *Fusarium oxysporum* were optimized by Birla SS *et al.* (2013) using different media, pH, temperature, light intensity, filtrate volume, salt concentration, and quantity of biomass.[152] Neethu S. *et al.* (2018) reported the extracellular green synthesis of AgNPs utilizing the biomass of Penicillium *polonium*.[153] Khan MN *et al.* (2015) utilized aqueous *Raphanussativus* root extract as a reducing and capping agent for the synthesis of silver nanomaterials for the first time.[154] Ma L. *et al.* (2017) utilized the supernatant of the fungus strain *Penicillium aculeatum* Su1 to synthesize extracellular AgNPs.[155] Al-Bahrani R. *et al.* (2017) reported the green synthesis of AgNPs utilizing the aqueous extract of basidiocarps of oyster mushroom, *Pleurotus stratus*[156]. Jalal M. *et al.* (2018) studied the extracellular green synthesis of AgNPs using the supernatant of *Candida glabrata* isolated from oropharyngeal mucosa of human immunodeficiency virus (HIV) patients and evaluated them for antibacterial and antifungal potential against human pathogenic bacteria and fungi.[157] Eugenio M. *et al.* (2016) reported the biosynthesis of Ag NPs using yeast strains.[158] Otari SV *et al.* (2014) synthesized AgNPs utilizing the culture supernatant of phenol degraded broth as the reducing agent.[159] Ishida K. *et al.* (2014) studied the synthesis and antifungal activity of AgNPs synthesized utilizing the aqueous extract of the fungus *Fusarium oxysporum*.[160] More details on the synthesis of AgNPs from fungi and yeast are discussed in Table 4.

### 2.4 Green synthesis of AgNPs using biopolymers

Nearly all of the wide varieties of biopolymers used for the synthesis of AgNP play the dual role of reducing and stabilizing agent except for the use of starch as a capping agent.[163] Fig. 5 presents the synthesis of AgNPs from various sources of biopolymers. Leung TC *et al.* (2010) synthesized AgNPs within 10–15 min by utilizing carboxymethylated-curdlan or fucoidan as reducing and stabilizing agents. Heating the reaction mixture at 100 °C led to the formation of AgNPs with a size in the range of 40–80 nm.[164] Regiel Futyra A *et al.* (2017) reported that biopolymers enhanced the antimicrobial activity of AgNPs. Chitosan and ascorbic acid were utilized as the reducing and capping agent, respectively, for the synthesis of AgNPs with

 

Table 4 Synthetic conditions, applications, size and characterization techniques for the synthesis of AgNPs using various strains of fungi

| S. no. | Reducing fungus | Application | Optimization conditions | Characterization techniques | Shape and size | Reference |
|---|---|---|---|---|---|---|
| 1 | Arthroderma fulvum | Antifungal against *Candida*, *Aspergillus* spp., and *Fusarium* spp. | $AgNO_3$ (1.5 mM) alkaline pH, reaction temperature 55 °C, and reaction time of 10 h | UV-vis, XRD, TEM | Size: (15.5 ± 2.5 nm) crystalline | 146 |
| 2 | *Penicillium citrinum* isolated from soil | Presence of amide linkage groups found in the fungal extract | Dark compartment at 28 °C, 24 h, membrane filter (0.45 *f*Ê) | FTIR, photon correlation spectroscopy (PCS), SEM, fluorescence spectroscopy, UV-vis | Size: (109 nm), shape: spherical | 147 |
| 3 | *Trichoderma asperellum* | AgNPs formed were highly stable for 6 months | $AgNO_3$ (1 mM), 5 days incubated at 25 °C with biomass of *Trichoderma asperellum* | UV-vis, FTIR, TEM, XRD, SERS | Size: (13–18 nm) | 148 |
| 4 | *Aspergillus clavatus* (AzS-275), an endophytic fungus | Antimicrobial against *Candida albicans*, *Pseudomonas fluorescens* and *Escherichia coli* | $AgNO_3$ (1 mM), cell biomass: $AgNO_3$ (1 : 9), incubated at 25 °C on a rotary shaker (150 rpm) for 72 h | UV-vis, FTIR, XRD, TEM, AFM | Size: (10 to 25 nm) extracellular, polydispersed spherical or hexagonal | 149 |
| 5 | Biomass of *Aspergillus terreus* | Antifungal and antibacterial | $AgNO_3$ 10 mM, NADH, biomass: $AgNO_3$ solution (5 : 1), incubated for 24 h at 28 °C | XRD, TEM, UV-vis | Size: (1 to 20 nm), shape: spherical | 150 |
| 6 | Raphanus sativus | Antimicrobial activity | *Raphanus sativus* root extract, (1.0–6.0 mL) added to $AgNO_3$ solution | DLS, TEM, EDX, XRD, FTIR, SEM | Size: (3.2 to 6 nm) | 154 |
| 7 | Cell-free filtrate of the fungus strain penicillium aculeatum Su1 | Antimicrobial activity, drug delivery vehicle or anticancer drug for clinical treatment. | $AgNO_3$ (10 mM) | TEM, XRD, FTIR | Size: (4 to 55 nm), FCC crystalline | 155 |
| 8 | Pleurotus ostreatus | Inhibitory activity against pathogenic bacteria | (1–6 mg $mL^{-1}$) of aqueous extract of *P. ostreatus* was added to 5 mL, of 1 mM aqueous silver nitrate, kept at 28 ± 2 °C in the dark and incubated for 6, 12, 18, 24, 30, 36 and 40 h | SEM, TEM, EDX, FTIR | Size: (<40 nm) | 156 |
| 9 | *Candida glabrata* | Antimicrobial activity against clinical strains of bacteria and fungi | $AgNO_3$ (1 mM) supernatant (20 mL) kept at room temperature overnight | FTIR, UV-vis, TEM | Size: (2 to 15 nm) | 157 |
| 10 | Biomass of *Trichoderma viride* (fungi) | Antibacterial activity against human pathogenic bacteria | $AgNO_3$ (10 mM), biomass: $AgNO_3$ (5 : 1), incubated for 24 h at 25 °C | UV-vis, TEM, SEM, | Size: (1 to 50 nm), shape: globular | 158 |
| 11 | Biomass of thermophilic *Bacillus* sp. AZ1 | Antimicrobial activity against human pathogenetic bacteria | $AgNO_3$ (1 mM) | SEM, EDX, TEM, UV-vis | Size: (7 to 31 nm), shape: spherical | 161 |
| 12 | Biomass of *Aspergillus niger* | Antimicrobial activity | $AgNO_3$ (10 mM), biomass of fungi:$AgNO_3$ (5 : 1), incubated for 24 h at 28 °C | UV-vis, XRD, TEM | Size: (1 to 20 nm), shape: spherical | 162 |

a size smaller than 10 nm.[165] Biogenic AgNPs were synthesized using *Nigella sativa* extract (NSE), which exhibited potential antioxidant activity. The TEM image showed biphasic spherical AgNPs with an average particle size of 8 nm. The effect of the AgNPs on the sustained release and film-forming capacity of chitosan was then evaluated.[166] Ahmad MB *et al.* (2011) synthesized AgNPs in an aqueous medium. The reduction of $AgNO_3$ was carried out using chitosan and polyethylene glycol (PEG). PEG and chitosan were utilized as the polymeric stabilizer and solid support, respectively.[167] Vasileva *et al.* (2011) synthesized stable and uniform starch-stabilized silver NPs with an average diameter of 14.4 ± 3.3 nm using ultrasound-mediated silver nitrate reduction by D-glucose. UV-vis spectroscopy, HR-TEM, XRD, TG/DTA, and DSC were used to characterize the starch-stabilized silver NPs completely. These NPs exhibited catalytic activity for the reduction of $H_2O_2$. Induced by the catalytic decomposition of $H_2O_2$, the degradation of the AgNPs caused a significant change in the absorbance strength

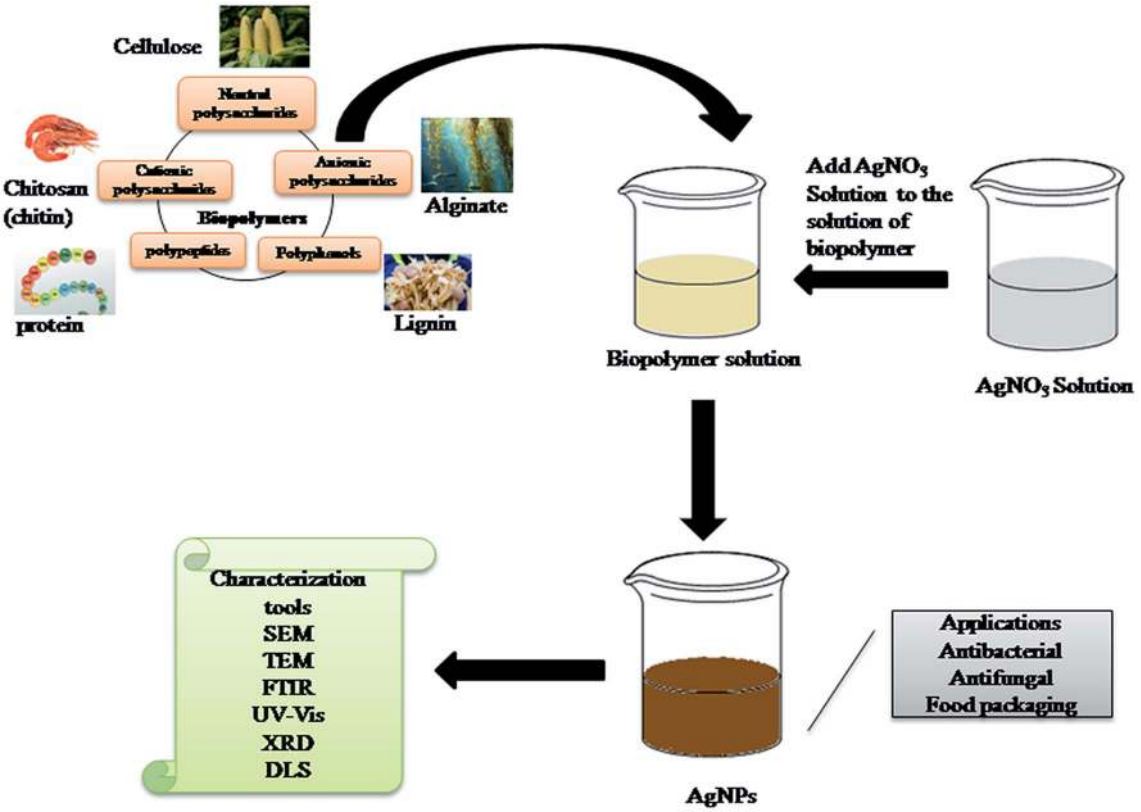


Fig. 5 Procedure for the synthesis of AgNPs using biopolymers.

of the localized surface resonance band depending on the concentration of $H_2O_2$. Subsequently, the optical sensor based on improvised plasmon resonance was characterized and calibrated. Good sensitivity and a linear response over the wide concentration range of $10^{-1}$ to $10^{-6}$ mol $L^{-1}$ $H_2O_2$ were established.[168]

Atta A *et al.* (2014) reported a green synthetic method involving the reduction of $Ag^+$ ions in aqueous acidic solution in the presence of polyvinyl alcohol modified with thiol groups (PVA-SH). AgNPs were stabilized by coating different types of citrate-reduced AgNPs with different weight ratios of PVSH derivatives (1–3 wt%). The as-prepared AgNPs were characterized *via* UV-vis spectroscopy, TEM/EDS, DLS and XRD combined with Rietveld analysis. The changes in the particle size, shape and hydrodynamic diameter of the AgNPs were determined using TEM, XRD and UV-visible spectroscopy after different durations of exposure to synthetic stomach fluid (SSF) and 1 M HCl. The data showed that for more than 90 days, these AgNPs were highly stable against SSF, which was not previously reported in the literature.[169] Wu Q. *et al.* (2008) synthesized glutathione-capped AgNPs with adjustable sizes. These particles could be bound covalently to other functional molecules and displayed sensitive optical properties to particle size and surface modification. The AgNPs with a diameter of ~6 nm prevented the proliferation of human K562 cells with leukemia, implying their potential cancer activity.[170] The procedure for the synthesis of AgNPs using biopolymers is shown in Fig. 5.

Si S. *et al.* (2007) synthesized AgNPs at pH 11 utilizing synthetic oligopeptides containing tryptophan residue at the C-terminus. The tryptophan residue in the peptides, possibly through electron transfer, is responsible for reducing metal ions to the respective metals.[171] Oligopeptides based on L-valine with the chemical structure Z–(L-Val) 3–OMe and Z–(L-Val) 2–L-Cys(S-Bzl)–OMe formed stable organogels in butanol. Both peptides are effective gelators, but they crystallize more readily than Z–(L-Val) 2–L-Cys(S-Bzl)–OMe. These two peptides are capable of forming mixed fibers, including gel butanol. The fibers can be mineralized using DMF as a reducing agent with AgNPs. The Z–(L-Val) 2–L-Cys(S-Bzl)–OMe fraction of the sulfur-containing peptide controlled the shape and size of the resulting NPs. Small spherical particles were distributed throughout the fiber at a high Z–(L-Val) 2–L-Cys(S-Bzl)–OMe content. A lower Z–(L-Val) 2–L-Cys(S-Bzl)–OMe content led to an increase in particle size and more complex forms such as plate-like and silver-like raspberry particles. The interactions between peptide and silver ions or silver particles occur through the complexation of silver ions to the sulfur atom of the thioether moiety and were shown to be the key interaction in controlling the formation of the particles.[172]

Kasthuri J. *et al.* (2009) reported the synthesis of quasi-sphere AgNPs using apiine as the reduction and stabilization agent. The size and shape of the NPs could be controlled by varying the ratio of metal salts to apiine compound in the reaction medium. UV-vis-NIR, TEM, FTIR spectroscopy, XRD

 

and TGA were used to characterize the synthesized NPs. The interaction between the NPs and the carbonyl group of the apiine compound was confirmed using FT-IR spectroscopy. The average size of the AgNPs was found to be 39 nm *via* TEM invetigation.[173] Safaepour M. *et al.* (2009) synthesized evenly dispersed AgNPs with a uniform size and shape in the range of 1 to 10 nm using geraniol. The cytotoxicity analysis of the AgNPs showed a direct dose–response relationship, where higher concentrations resulted in increased cytotoxicity. The AgNPs were able to inhibit the growth of the Fibrosarcoma-Wehi 164 cell line by less than 30% at a concentration of 1 μg $mL^{-1}$.[174] The aqueous solution of AgNPs exhibited different SPR when prepared at different pH values. PEG was used as a reducing and stabilizing agent to synthesize AgNPs since it is ecofriendly, which produced monodispersed particles with a diameter of less than 10 nm. The colloids exhibited activity against Gram-positive and Gram-negative bacteria and fungi. Biodegradable starch played the role of a capping agent in the synthesis of AgNPs. The analysis showed that a starch layer was coated on NPs. The diameters of the particles ranged from 5–20 nm. XRD analysis showed the face-centered cubic structure of the NPs. In many fields of science, ion-exchangeable polymers act as capping agents. These often-used polymers contain phosphonic acid groups with a low molecular weight. Polymer complexation to $Ag^+$ occurs, and then the metal ions are reduced to NPs. In the presence of an ion-exchange polymer, AgNPs were stabilized. The morphology of the surface indicated the formation of cubes and rectangular prism structures. Copolymers such as CD, grafted with PAA, helped to synthesize AgNPs initiated by potassium persulfate.[175]

Maity D. *et al.* (2011) used poly(methyl vinyl ether co maleic anhydride) (PVM/MA) as a reducing and capping agent. The synthesized NPs were stable for a month at room temperature and surrounded by 5–8 nm sheath of PVM/MA.[176] A variety of factors influenced the formation of NPs, such as acidity, initial concentration of starting materials, and molar ratio of reactants. Some dispersing agents prevented the accumulation of NPs and helped in the analysis of morphology, particle size, composition of elements, *etc.* The NPs were non-aggregated, and possessed a face-centered cubic (FCC) structure, and spherical shape. Ascorbic acid or citrate was used to reduce ions, which resulted in an average particle size of approximately 10.2–13.7 nm. The zeta potential ranged from 40–42 mV and was primarily influenced by the acidity and size of the NPs.[177] When reacted with ammonium hydroxide, formaldehyde produced a polymer that affected the way silver was bound to the substrate. In unfavorable conditions for the synthesis of the polymer, the NPs formed were concentrated and possessed a gold-silver plasmon resonance (498 nm).[177]

# 3. Synthetic mechanism and characteristics of AgNPs

The synthesis of AgNPs can be carried out using natural sources such as carbohydrates, fat, phenols flavonoids, proteins, enzymes and coenzymes, terpenoids, gum, alkaloids, and sugars to reduce $Ag^+$ ions. Depending on the organism/extract used, the active ingredient responsible for the reduction of $Ag^+$ ions varies. Electrons are required for the nano transformation of AgNPs from acid (ascorbic acid) dehydrogenation and keto–enol conversion in mesophytes or both mechanisms in xerophyte plants. A similar reduction process can be performed by microbial cellular and extracellular oxidoreductase enzymes. The major constituents of fungi and bacteria such as quinones, NADH, nitroreductase enzyme, and proteins are responsible for the reduction and stabilization of AgNPs. It is assumed that the electrostatic interactions between the carboxylic group attached to the surface of fungal cell and $Ag^+$ ions result in the formation of AgNPs, and proteins prevent the AgNPs from agglomerating.[178] The major source of nitrogen used by bacteria is nitrate, which is reduced to nitrite by the enzyme nitrate reductase and NADH. This metabolic activity for the formation of ammonium and nitrile can be utilized in the green synthesis of AgNPs *via* the intracellular reduction of $Ag^+$ ions. Proteins effectively prevent the agglomeration and increase in particle size caused by particle collisions, which maintain the high stability of colloidal AgNPs.[155] Fig. 6 shows a schematic of the reaction mechanism for the synthesis of AgNPs from various sources. Based on the tautomerization in flavanoids, the possible mechanism for the synthesis of AgNPs is shown in Fig. 6(a). Fig. 6(b) presents the reaction mechanism for the synthesis of AgNPs involving the reduction of $Ag^+$ ions due to the oxidation of NADH to $NAD^+$.

## 3.1 Separation of AgNPs from suspension and their characterization

Researchers mainly use the centrifugation technique to obtain the pellet or powder form of synthesized AgNPs. AgNPs suspensions have also been dried to obtain the product in powder form. Some common techniques for the characterization of AgNPs include UV-vis spectroscopy, SEM, TEM, FTIR, XRD, and EDAX. DLS study is used for AgNPs synthesized from bio-polymers rather than plant extracts and microorganisms. UV-visible spectroscopy is considered the primary characterization technique to monitor the synthesis and stability of synthesized AgNPs. Due to the unique optical properties of AgNPs, they show strong interaction with light at specific wavelengths. The band gap in AgNPs is very low, and as a result electrons move freely, causing an SPR band due to the collective oscillation of electrons of AgNPs in resonance with light.[179–181] Zeta potential values show the stability of synthesized AgNPs. Dubey *et al.* reported that AgNPs show a lower zeta potential value in acidic pH, and higher values in more basic pH solutions. It was observed that the absorbance peak became sharp with an increase in reaction time.[40] The XRD analysis of the AgNPs shows diffraction peaks at 38.13°, 44.21°, 64.47°, 77.37°, 81.47°, 98.01°, 110.56° and 114.80°, which confirms the FCC structure of AgNPs.[40,43,93] XRD and EDAX study also confirm the purity of the synthesized AgNPs. FTIR analysis reveals the functional groups responsible for the reduction and stabilization of AgNPs. TM Nguyen *et al.* analyzed the presence of protein *via* FTIR in the seeds of *Nelumbo nucifera*. The synthesized spherical AgNPs using the seed extract of *Nelumbo nucifera*

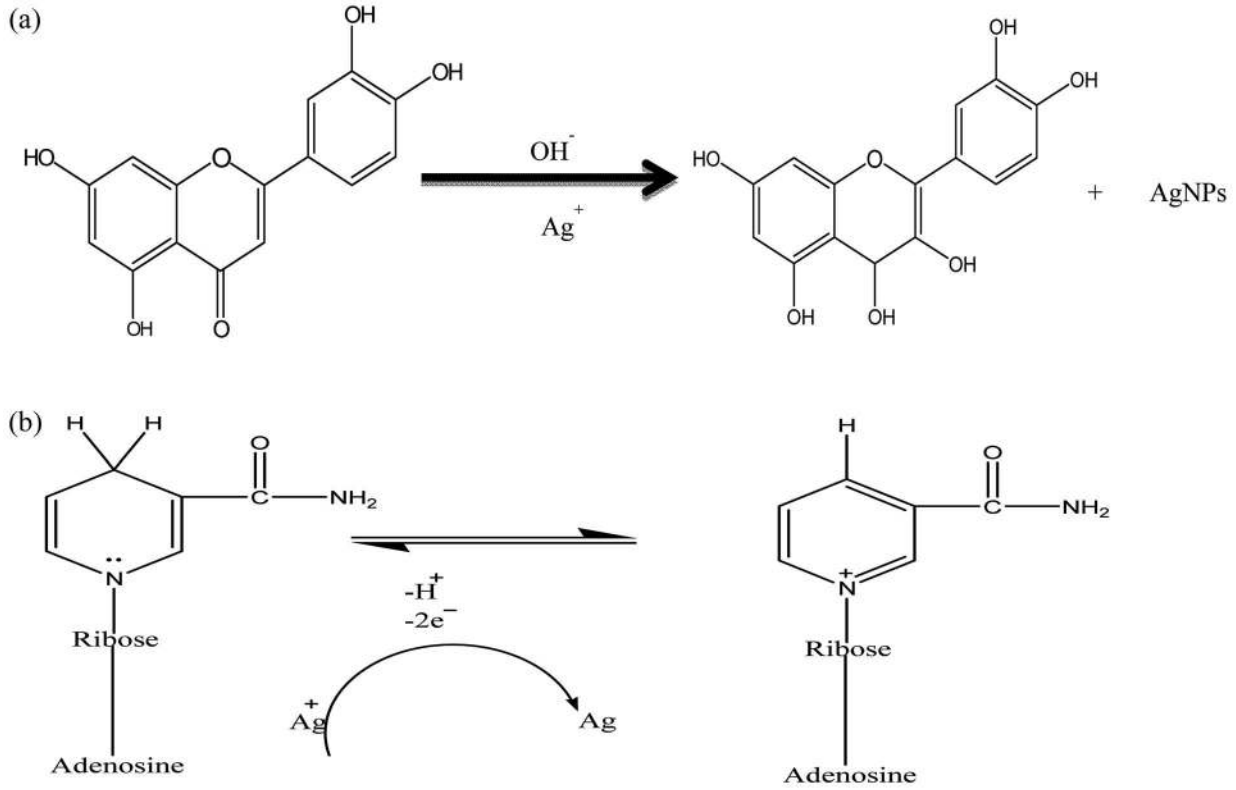


Fig. 6 (a) Reaction mechanism for the synthesis of AgNPs due to the flavanoids[92] present in plant extract. (b) Reaction mechanism for the synthesis of AgNPs due to NADH present in fungi and bacteria.

showed cytotoxicity against Gram-negative bacteria.[43] ZA Ali *et al.* reported that AgNPs synthesized using apple extract were stable due to the presence of the ethylene group in apple extract. These AgNPs showed antibacterial activity against multidrug-resistant bacteria.[93] Honary S. *et al.* studied the presence of amide and ester linkages in *Penicillium citrinum*, which are responsible for the formation of AgNPs. The presence of a fluorescence emission band at 414 nm showed that the AgNPs were bound with protein and the protein also present in its native form in solution.[147] DLS study on the suspension of AgNPs was used to calculate the average particle size and particle distribution of the synthesized AgNPs. SEM, TEM and AFM were used to study the surface morphology, size and various shapes of AgNPs.[36,109,116] Koyla H. *et al.* synthesized globular polycrystalline AgNPs using the leaf extract of spinach.[108] Hamelian M. *et al.* synthesized plate- or rod-shaped AgNPs using the plant extract of *Thymus kotschyanus*.[116] Fig. 8 presents micrographs of the AgNPs synthesized by various methods under different optimization conditions. TGA was used to determine the effect of $AgNO_3$ and L-cystine on the organic composition of the AgNPs. It was also used to determine the amount of organic material in the synthesized AgNPs and to predict their thermal stability.

### 3.2. Factors affecting the microstructure and application of silver nanoparticles

It has been reported that material properties are influenced by the structure (micro or nano) of the materials.[182,183] The major physical and chemical parameters that affect the AgNP synthetic process are the temperature of the reaction, concentration of metal salt, content of extracts, pH of the reaction mixture, duration of the reaction and agitation. Parameters such as metal ion concentration, extract composition and reaction period have a major impact on the size, shape and morphology of AgNPs, where different experimental conditions lead to changes in the color of the reaction mixture. Yangqing He *et al.* reported that with an increase in the concentration of silver nitrate from 1 to 10 mM, the intensity of SPR peaks increased, which implies that at higher precursor salt concentration, more AgNPs were formed. A minor blue shift was observed for a higher concentration of $Ag^+$ ions in the range of 425 to 418 nm. FTIR analysis revealed the presence of flavonoids and proteins, which were responsible for the reduction and stabilization of the AgNPs. These nanoparticles showed cytotoxicity against human gastric carcinoma, acted as free radical scavengers and showed antimicrobial activity.[92] AgNPs synthesized from grape and tomato showed good antioxidant antibacterial and protein kinase inhibitory activity.[91] Most authors reported the suitability of basic medium for the synthesis of AgNPs due to the better stability of the synthesized NPs observed.[89,92,98] Some other benefits reported under basic pH are fast growth rate, good yield and monodispersity, as well as enhanced reduction process. By altering the pH, nearly spherical shaped AgNPs are converted into spherical AgNPs. The AgNPs formed in more basic pH (>11) and in acidic pH (<7) are unstable and agglomerate in the medium. Synthetic conditions such as

 

stirring time and temperature are significant. Many researchers have used temperatures ranging from 20–100 °C to synthesize AgNPs from biopolymers and plant extracts; however, microorganisms die at high temperatures, and therefore they require a temperature of up to 40 °C. The rate of synthesis of AgNPs increases with an increase in temperature (30–90 °C) and encourages the production of small-size NPs. On average, the temperature range of 25–37 °C is considered suitable for the synthesis of AgNPs. Fig. 7 presents SEM images of AgNPs synthesized from different sources.[95,99,116] Several reports demonstrated that AgNPs absorb electromagnetic radiation in the visible range from 380 to 450 nm, which is known as LSPR excitation. Honary S. *et al.* synthesized spherical monodispersed AgNPs with a size of 109 nm using *Penicillium citrinum*. PCS spectra show a polydispersity index (PDI) of 0.01. For a broad size distribution of AgNPs, the PDI is greater than 0.7, and the PDI should be between 0.01 and 0.5 for good monodispersity of AgNPs.[147] Based on the symmetry in the shape of AgNPs, many researchers reported with a decrease in symmetry, charge separation increases, and consequently the primary SPR peak shows a red shift, while due to the snipping of the corners of asymmetric AgNPs, a blue shift observed. In general for spherical AgNPs, they have more SPR peaks than irregular AgNPs.[88,97]

### 3.3 Indication for the formation of AgNPs

The literature review has reported the appearance of colorless $AgNO_3$ solution to yellow or brown-yellow solution as the indication that AgNPs have been synthesized. UV-vis data showed the maximum absorbance wavelength for the synthesized AgNPs to be in the range of 400–460 nm.[10–30] The absorbance data also helps to analyze the effect of pH, concentration of metal ions, and extract content on the size and stability of AgNPs. In most of the studies, SEM morphological analysis revealed spherical AgNPs, whereas few authors reported irregular, triangular, hexagonal, isotropic, polyhedral, flower, pentagonal, anisotropic and rod structures.[37,40,88,91,92,149] Fig. 7 shows the SEM images of AgNPs with different shapes. The formation of face-centered cubic (FCC) crystalline-structured AgNPs has been reported by nearly all researchers using XRD studies. In some cases, AgNPs have been reported to show cubic and hexagonal structures also. EDAX is used to determine the elemental composition in nanomaterials. Depending on the reducing agent and other operating conditions, the stability of AgNPs may vary from 1 day to 1 year. Compared to plant extracts, the reaction mixture for the synthesis of AgNPs using microorganisms and bio-polymers is continually agitated to prevent agglomeration. The agitation of the reaction mixture achieved through the application of an external mechanical force can accelerate the formation of NPs.

## 4. Applications of silver nanoparticles

AgNPs have been used extensively as anti-bacterial agents in the health industry, food storage, textile coatings and a number of environmental applications. It is important to note that despite decades of use, the evidence for the toxicity of silver is still unclear. Products made with AgNPs have been approved by a variety of accredited bodies, including the US FDA, US EPA,

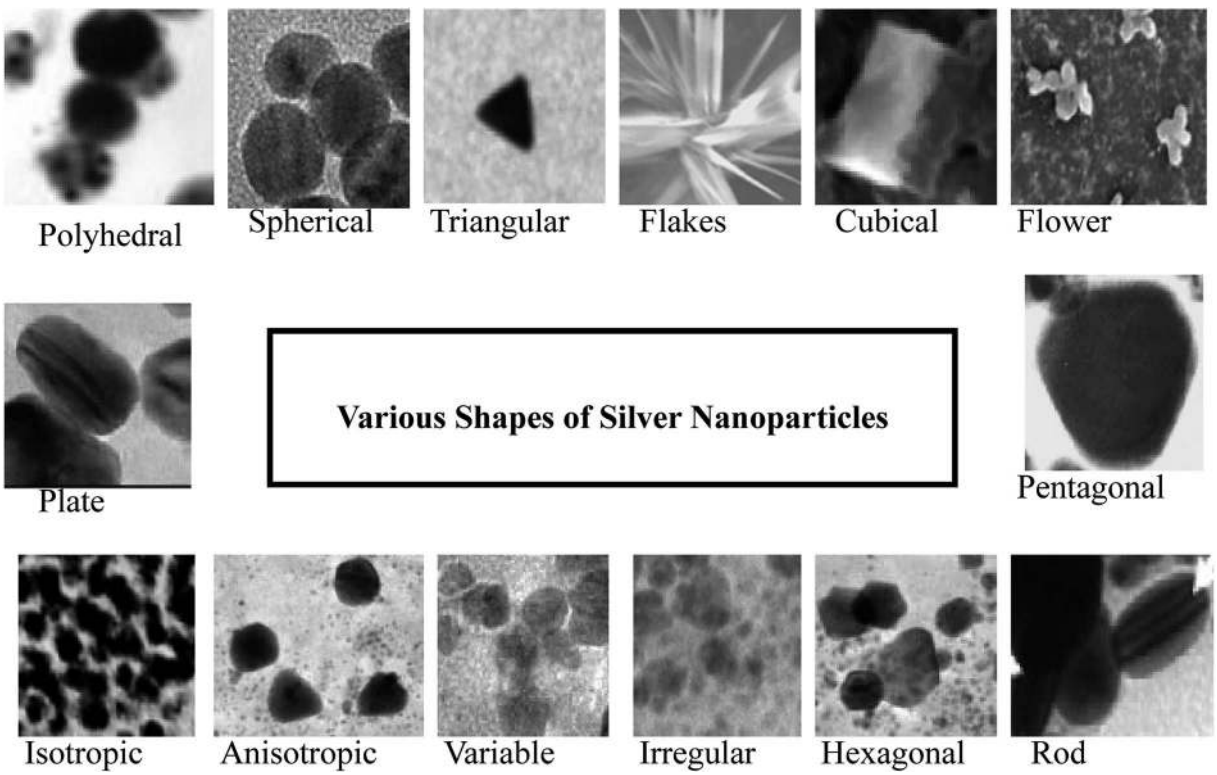


Fig. 7 SEM images of AgNPs synthesized from different sources.[4]

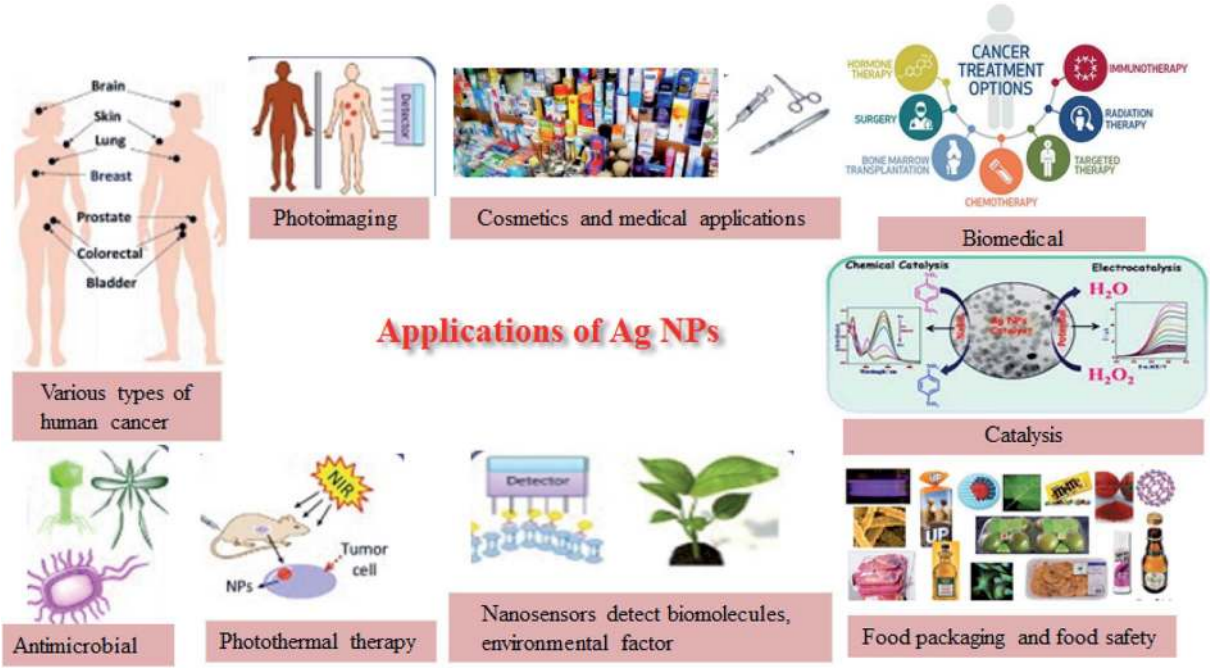


Fig. 8 Various applications of AgNPs.

and Japan SIAA, and in Korea.[181] In addition, AgNPs are incorporated into nanoscale sensors due to their electrochemical properties, which can provide faster response times and lower detection limits. AgNPs are used as antibacterial agents, ranging from disinfecting medical devices and home appliances to water treatment. The textile industry has been encouraged to use AgNPs in various textile fabrics. Silver nanocomposite fibers with AgNPs incorporated into the fabric have been prepared in this direction.[184] Cotton fibers with AgNPs are highly antibacterial against *Escherichia coli*. The catalytic activities of NPs differ from that of bulk materials, for example, high catalytic activity for the decoloration of monochrome black T dye in the presence of sodium borohydride (from 19.74% to 86.05%) and a light source (from 41.96% to 80.11%).[185] The optical properties of metallic NPs depend primarily on their surface plasmon resonance, where the plasmon refers to the collective oscillation of free electrons within the metallic NPs.[193] The plasmon resonant peaks and line widths are well known to be sensitive to the size and shape of the NPs. AgNPs are used in agriculture to increase crop production, plant nutrition, and defend against diseases.[189] The various applications of AgNPs are presented in Fig. 8.

Chen Yu *et al.* reported the application of AgNPs in catalysis, which enhanced the reduction rate of $NaBH_4$ in the reduction of azo dye.[65,186] Due to the enhanced electromagnetic field on the surface of AgNPs, AgNPs are broadly used in nanomedicine including diagnostics, biomedicines, nanoelectronics and molecular imaging. AgNPs act as nanoantennas due to the increase in their resonant SPR peak with an increase in the intensity of the electromagnetic field. AgNPs act as sensors with Raman spectroscopy to identify any molecule due specific vibrational modes.[50,187] Due to the antimicrobial action of AgNPs they are used in food packaging to prevent microbial infections.[188,191] AgNPs are used in nanosensors to analyze contaminations, colors or flavors, drinking water and for clinical diagnostics.[189] AgNPs have found application in agriculture also. Plant productivity can be enhanced *via* the communication of nanotechnology-based smart plant sensors with actuate electronic device, where these sensors optimize and automate water and agrochemical allocation, and enable high-throughput plant chemical phenotyping. Ag NPs are used in plant nutrition and defense against diseases,[192] where AgNPs can be delivered with pesticides to crops to enhance the production of crops in agriculture.[190] AgNPs are extensively used as therapeutic agents as antifungal, antimicrobial, anti-inflammatory and antiviral agents. Due to the antimicrobial action of AgNPs, they can be used in drug delivery to reduce the dose of drugs, improve specificity and decrease toxicity.[88,193]

## 5. Conclusion

In conclusion, considering environmental concerns for the synthesis of AgNPs, the green approach is preferred over conventional methodologies. The conventional synthetic methods for AgNPs require a huge amount of energy and hazardous chemicals (hydrazine or borohydride as reduction agents) and may lead to the formation of hazardous by-products. The use of biodegradable polymers, sugars or polyphenols from plant extracts, enzymes and bacteria under ambient conditions may lead to the sustainable synthesis of AgNPs with a uniform size. AgNPs formed in more basic pH (>11) are stable and in acidic pH (<7) are unstable and agglomerated. This implies that the size and stability of AgNPs are dependent on pH. Using composites based on PEG and Ag

 

CMC, Ag nanorods exhibiting luminescent properties, specific size and shape can be easily obtained using microwave irradiation. The synthesis of AgNPs is quite easy and simple using plants and their extract compared to other sources such as fungi and bacteria. The size and morphology of AgNPs varies with a variation in reaction parameters. The simple use of vitamins such as vitamin B2, B1, and C may produce NPs at ambient temperature in aqueous media. In addition, new biomimetic techniques have proven to be beneficial for the preparation of AgNPs, although there are still some inherent safety concerns. The green methods for the synthesis of AgNPs using bio-renewable materials seems to be promising because they require non-toxic chemicals for the reduction of silver salt. This review provides a wide spectrum of all the natural resources such as plants, bacteria, fungi, and biopolymers for the production of AgNPs in the last ten years.

## Abbreviations

| | |
|---|---|
| AgNPs | Silver nanoparticles |
| NPs | Nanoparticles |
| PEG | Polyethylene glycol |
| SERS | Surface-enhanced Raman scattering |
| DLS | Dynamic light scattering |
| TEM | Transmission electron microscopy |
| SEM | Scanning electron microscopy |
| XRD | X-ray diffraction spectroscopy |
| EDAX | Energy-dispersive X-ray spectroscopy |
| FTIR | Fourier transform infrared spectroscopy |
| CD | Cyclodextrin |
| PAA | Polyacrylic acid |
| CMC | Carboxy methyl cellulose |
| NTA | NP tracking analysis |
| PCS | Photon correlation spectroscopy |
| SPR | Surface plasmon resonance |
| TGA | Thermo-gravimetric analysis |

## Conflicts of interest

The authors do not have any conflict of interest.

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