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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.

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 nano-materials 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 Fleming, 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 Samhita⁵”.

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), biopolymers⁴¹ (chitosan^{42,43}) and microbial sources⁴⁴ 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

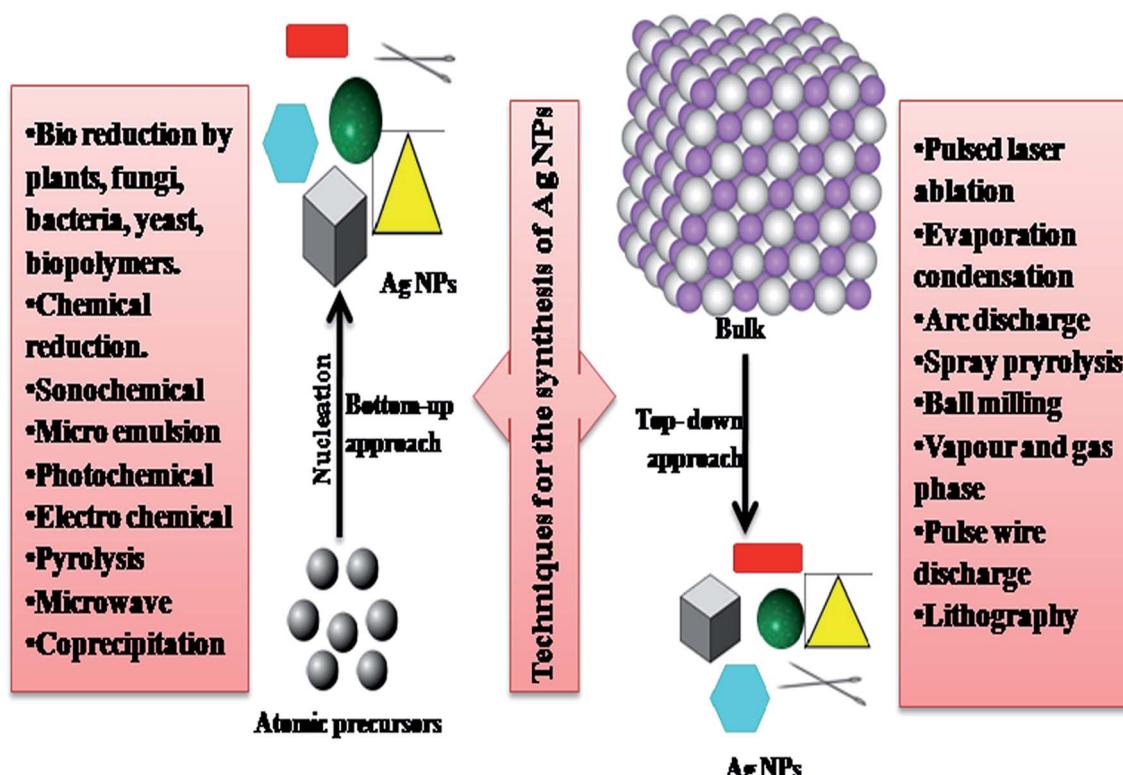


Fig. 1 Representation of various techniques for the synthesis of NPs.⁵

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, naphthoquinones, 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.* synthesized 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 *Boerhaavia diffusa*,⁶⁶ *Tinospora cordifolia*,⁶⁷ *Terminalia chebula*,⁶⁸ aloe vera⁶⁹, *Ocimum tenuiflorum*,⁷⁰ *Catharanthus roseus*,⁷¹ *Embllica officinalis*,⁷² *Azadirachta indica*,⁷³ common spices *Piper nigrum*,⁷⁴ *Cocos nucifera*,⁷⁵ *Cinnamom zeylanicum*⁷⁶ and some tropical weeds such as *Parthenium hysterophorus*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. There 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 metabolites⁷⁹ and chlorophyll⁸⁰ 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*.



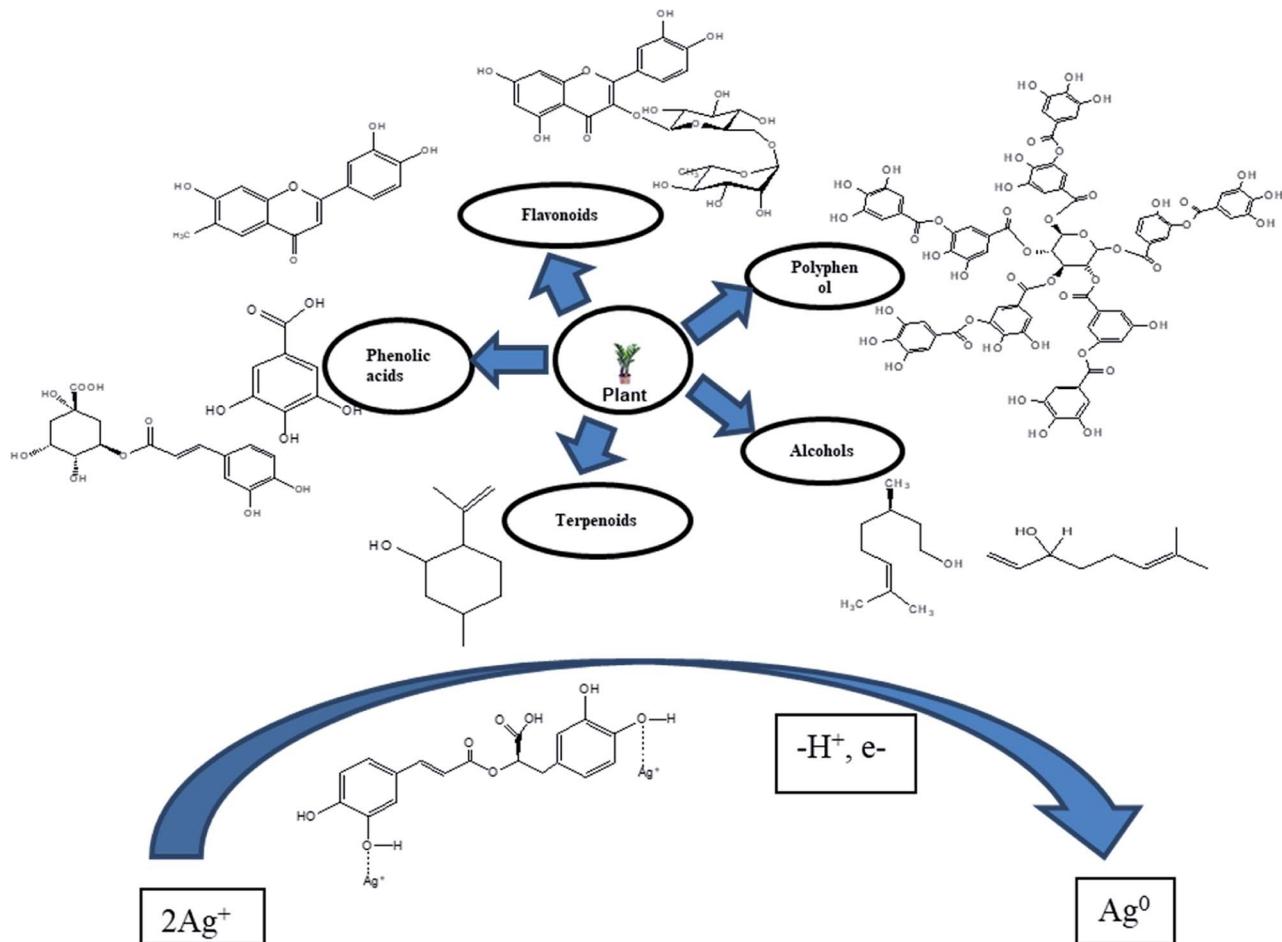


Fig. 2 Mechanism for the synthesis of AgNPs from plant sources.

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 *Pantoea ananatis*.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 nm¹³⁹. 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 micro-organisms 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. Reducing agents (green no. sources)	Applications	Operating conditions	Characterization techniques used	Particle characteristics	Reference
1 Aqueous leaf extract of <i>aloe vera</i>	Antibacterial activity	AgNO ₃ (1 mM), extract: AgNO ₃ (1 : 10), stir 20 min, incubated for 24 h	UV-vis, FTIR, TEM, XRD, TGA and DTA	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 <i>Coffea arabica</i> seed extract	Antibacterial activity on <i>E. coli</i> and <i>S. aureus</i>	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 <i>Trianthema decandra</i>	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 <i>Macrotyloma uniflorum</i>		AgNO ₃ (0.59 mM), extract: AgNO ₃ (1 : 60)	TEM, XRD, UV-vis, FTIR	Size: (12 nm)	39
6 Fruit extract of <i>Tanacetum vulgare</i>		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 <i>Rosa rugosa</i>		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 <i>Saccharum officinarum</i>	High antimicrobial activity against biofilm-forming bacteria and fungi. Reduce cytotoxicity (5 : 1 : 1), stirred for 12–15 h with heat on mammalian somatic and tumoral cells	AgNO ₃ (14, 24, 52, and 104 mM), CS : AgNO ₃ : VC	UV-vis, TEM, EDS	Size: (<10 nm)	42
9 Seed extract of <i>Nelumbo nucifera</i>	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 <i>Eriobotrya japonica</i> (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 (<i>Manilkara zapota</i>)	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 (<i>Punica granatum</i>)	Antibacterial activity against <i>Staphylococcus</i> , <i>Pseudomonas aeruginosa</i> and <i>Escherichia coli</i> 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 <i>Saccharum officinarum</i> extract and chitosan	Antibacterial against <i>Bacillus subtilis</i> , <i>Klebsiella planticola</i> , <i>Streptococcus faecalis</i> , <i>Pseudomonas aeruginosa</i> and <i>Escherichia coli</i>	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 <i>Arbutus unedo</i> (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 <i>Cinnamomum camphora</i> 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. Reducing agents (green no. 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 <i>Azadirachta indica</i> aqueous leaf extract	Antibacterial activity against <i>Staphylococcus aureus</i> and <i>Escherichia coli</i>	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 (<i>Vitis vinifera</i>) fruit extract	Antibacterial activity against <i>Bacillus subtilis</i> and <i>Escherichia coli</i>	AgNO ₃ (20 mM) extract: AgNO ₃ solution (1 : 1)	UV-vis, DLS, EDX, TEM	Size: (19 nm), shape: spherical	91
21 <i>Alpinia katsumadai</i> 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 <i>Berberis vulgaris</i> leaf and root extract	Antibacterial activity against <i>Escherichia coli</i> and <i>Staphylococcus</i> 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, zeta potential	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 <i>Lippia nodiflora</i> 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 <i>Pinus merkusii</i> 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 (<i>Allium sativum</i>)	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 (<i>Zingiber officinale</i>)	Antibacterial activity against <i>Escherichia coli</i> , (20%): AgNO ₃ solution	AgNO ₃ (1 mM) extract	UV-vis, XRD	Size: (2.89 nm), shape: spherical	102



Table 2 (Contd.)

S. Reducing agents (green no. sources)	Applications	Operating conditions	Characterization techniques used	Particle characteristics	Reference
		<i>Klebsiella pneumoniae</i> , <i>Pseudomonas aeruginosa</i> , <i>Bacillus cereus</i> and <i>Proteus vulgaris</i>	(1 : 9), temperature (27 ± 2 °C)		
32	Aqueous seed extract of <i>Manilkara zapota</i> (L.)	Antimicrobial activity against <i>Candida</i> 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	<i>Origanum vulgare</i> 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 <i>Croton sparsiflorus</i>	Antimicrobial activity	AgNO ₃ (1 M)	UV-vis, SEM	Size: (30 to 50 nm), shape: spherical 106
36	Roots extract of <i>Coleus forskohlii</i>	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	<i>Valerian officinalis</i> 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	<i>Tectona grandis</i> 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 <i>Ananas comosus</i>		AgNO ₃ (10 mM), AgNO ₃ (1 : 10) pineapple juice: AgNO ₃ (1 : 10)	XRD, UV-vis, EDAX, TEM	Size: (12 nm), FCC crystalline 113
42	Extract of saffron (<i>Crocus sativus</i> 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 (<i>Allium cepa</i>) 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	<i>Thymus kotschyanus</i> 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	<i>D. carota</i> (carrot) extract		AgNO ₃ (0.5 mM), extract: AgNO ₃ (1 : 6)	XRD, UV-visible FTIR, TEM	Size: (20 nm), shape: spherical 117
46	<i>Garcinia mangostana</i> 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 <i>Chenopodium ambrosioides</i>		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 <i>Acalypha indica</i>	Antifungal effect against <i>Phytopathogen</i>	AgNO ₃ (1 mM), extract: AgNO ₃ (1 : 9), incubate at 37 °C	UV-vis, antifungal	121
50	<i>Ficus benghalensis</i> leaf extract	Antibacterial activity		UV-vis, TEM-EDX, XRD	122



Table 2 (Contd.)

S. Reducing agents (green no. sources)	Applications	Operating conditions	Characterization techniques used	Particle characteristics	Reference
51 <i>Litchi chinensis</i> 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 <i>Salvia leyiifolia</i> leaf extract	Antibacterial activity against 9 bacteria	AgNO ₃ (1 mM), extract: AgNO ₃ (1 : 24)	SEM, AFM, XRD, FTIR	Size: (27 nm), shape: spherical	124
53 <i>Glycyrrhiza glabra</i> 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 <i>Pimpinella anisum</i> 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 <i>Glycyrrhiza uralensis</i> root extract	Antimicrobial agent against <i>Escherichia coli</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> and <i>Salmonella enterica</i>	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 <i>Citrus reticulata</i> fruit peel extract	Antibacterial activity against <i>Streptococcus pyogenes</i> , <i>Staphylococcus aureus</i> , <i>Bacillus subtilis</i> , <i>Escherichia coli</i> , <i>Salmonella Paratyphi</i> and <i>Klebsiella pneumoniae</i>	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 *Arthroderra 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

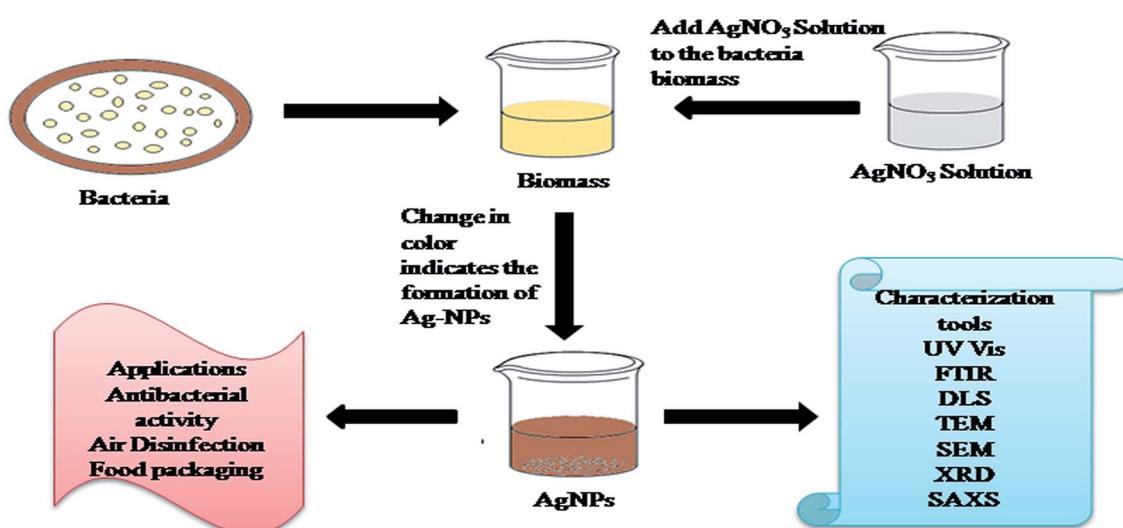


Fig. 3 Mechanism for the green synthesis of AgNPs from bacteria.



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	<i>Psychrophilic</i> 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, <i>Pantoea ananatis</i>	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 <i>Klebsiella pneumonia</i>		AgNO ₃ (1 mM), supernatant 1% (v/v)	XRD, UV-vis, TEM, EDS	Size: (3 nm)	139
4	Culture supernatants of <i>Enterobacteriaceae</i>		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

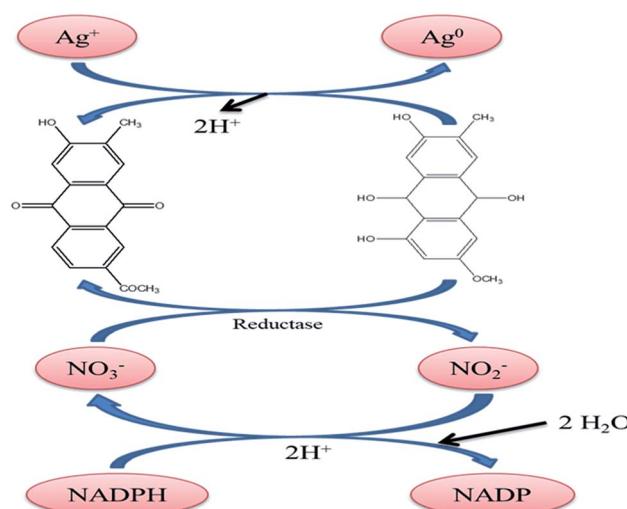


Fig. 4 Mechanism for the synthesis of AgNPs using fungi.

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 *Raphanus sativus* 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-curdan 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	<i>Arthroderma fulvum</i>	Antifungal against <i>Candida</i> , <i>Aspergillus</i> spp., and <i>Fusarium</i> 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	<i>Penicillium citrinum</i> isolated from soil	Presence of amide linkage groups found in the fungal extract	Dark compartment at 28 °C, 24 h, membrane filter (0.45 μm)	FTIR, photon correlation spectroscopy (PCS), SEM, spherical fluorescence	Size: (109 nm), shape: spherical	147
3	<i>Trichoderma asperellum</i>	AgNPs formed were highly stable for 6 months	AgNO_3 (1 mM), 5 days incubated at 25 °C with biomass of <i>Trichoderma asperellum</i>	UV-vis, FTIR, TEM, XRD, SERS	Size: (13–18 nm)	148
4	<i>Aspergillus clavatus</i> (AzS-275), an endophytic fungus	Antimicrobial against <i>Candida albicans</i> , <i>Pseudomonas fluorescens</i> and <i>Escherichia coli</i>	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 <i>Aspergillus terreus</i>	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	<i>Raphanus sativus</i>	Antimicrobial activity	<i>Raphanus sativus</i> 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 <i>penicillium aculeatum</i> 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	<i>Pleurotus ostreatus</i>	Inhibitory activity against pathogenic bacteria	(1–6 mg mL ⁻¹) of aqueous extract of <i>P. ostreatus</i> 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	<i>Candida glabrata</i>	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 <i>Trichoderma viride</i> (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 <i>Bacillus</i> 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 <i>Aspergillus niger</i>	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



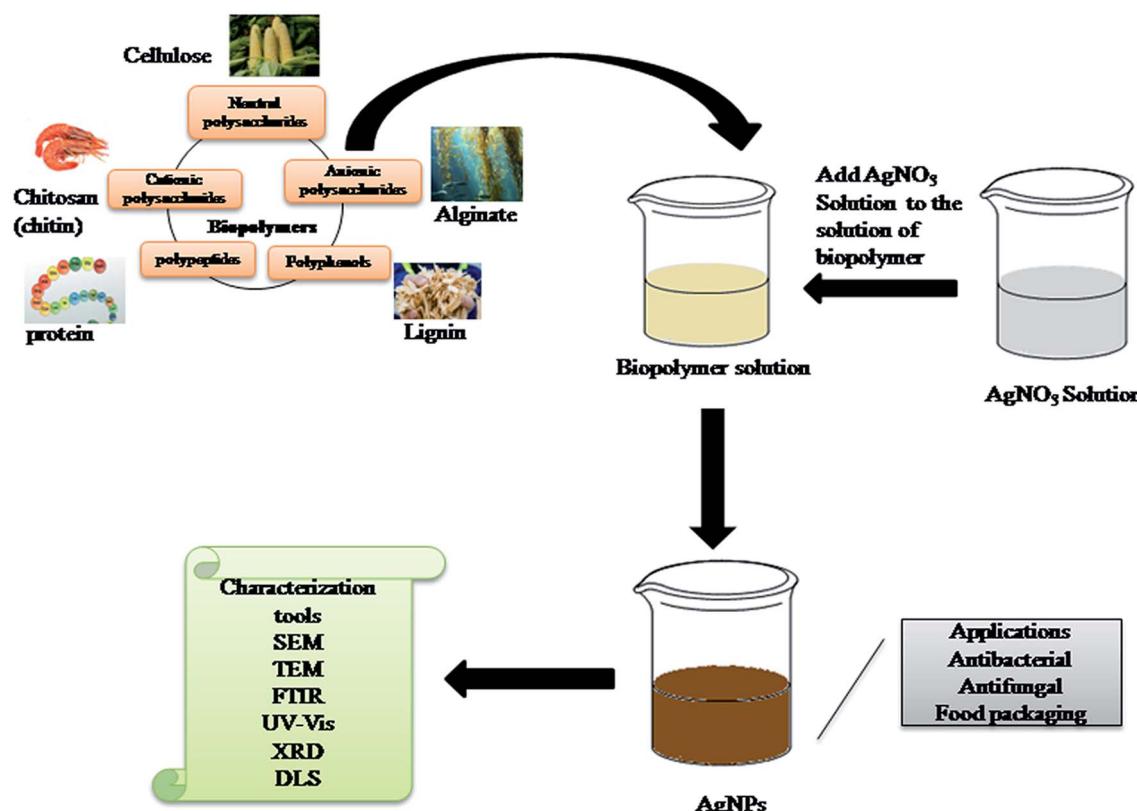


Fig. 5 Procedure for the synthesis of AgNPs using biopolymers.

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 investigation.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 $\mu\text{g mL}^{-1}$.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 flavonoids, 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*



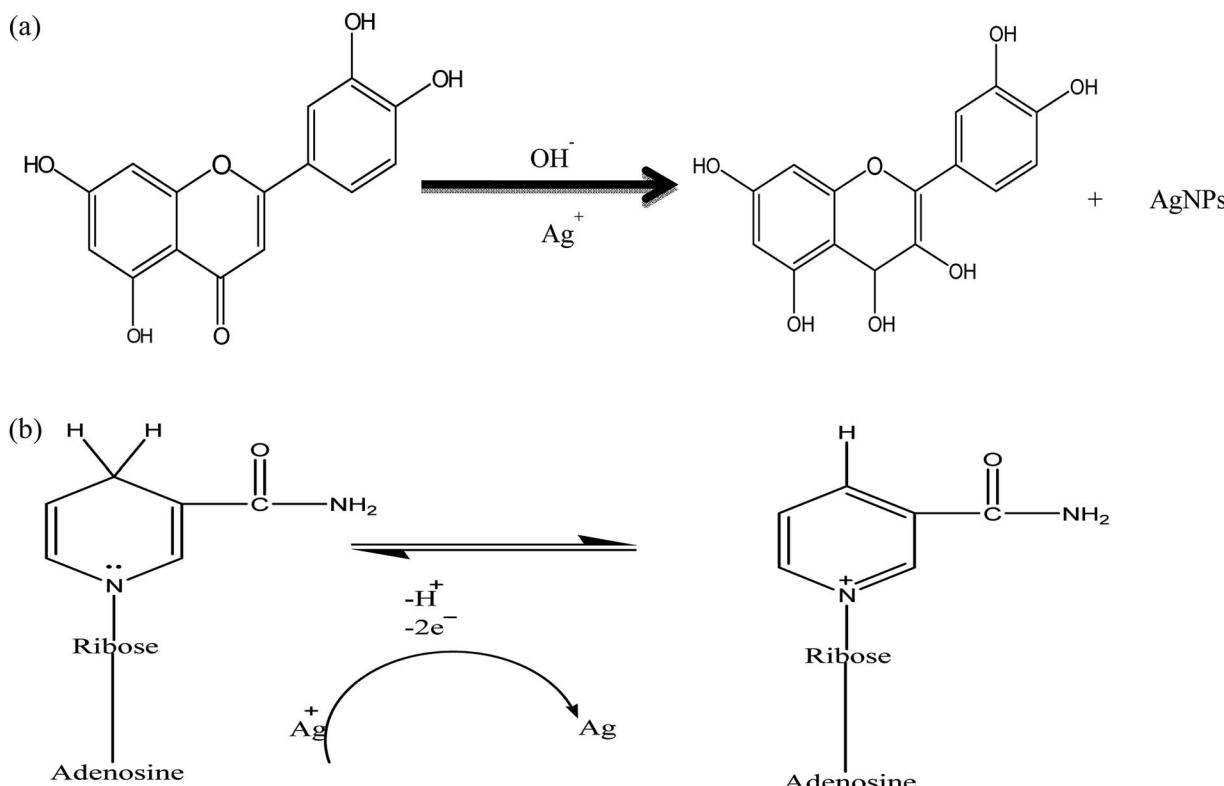


Fig. 6 (a) Reaction mechanism for the synthesis of AgNPs due to the flavanoids⁹² present in plant extract. (b) Reaction mechanism for the synthesis of AgNPs due to NADH present in fungi and bacteria.

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 kotschyuanus*.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,

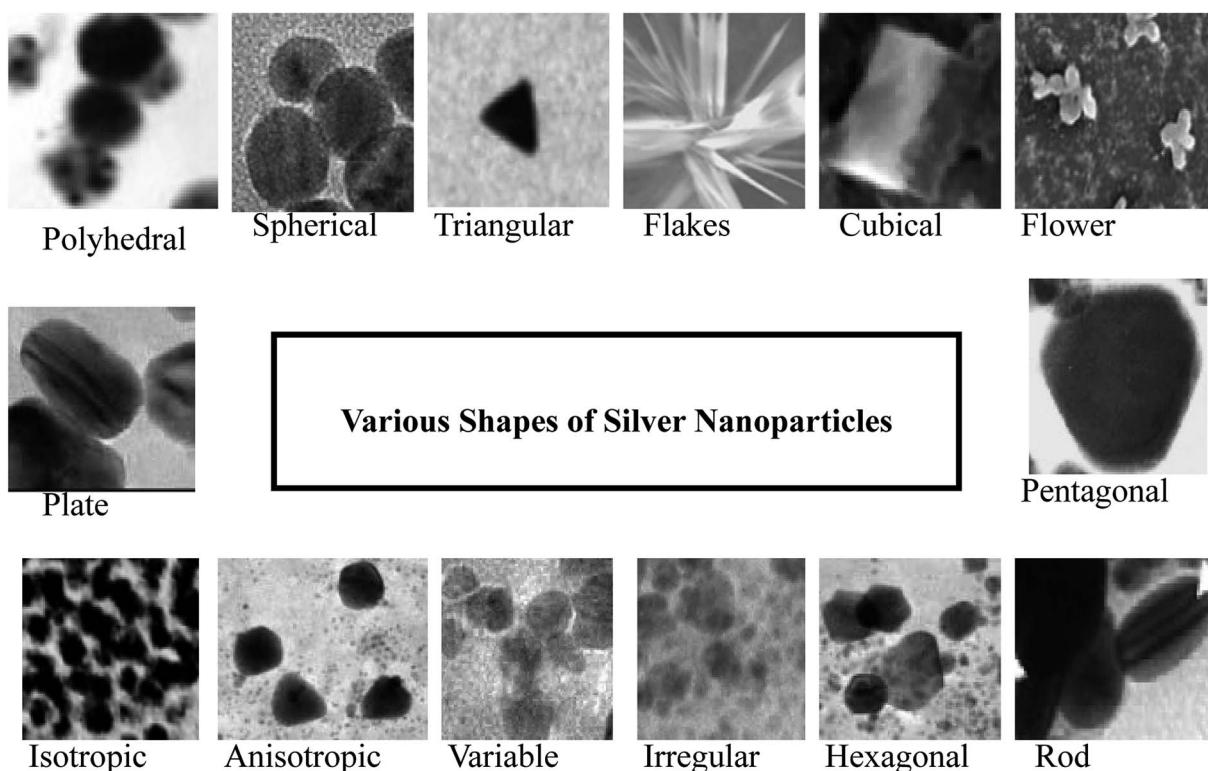


Fig. 7 SEM images of AgNPs synthesized from different sources.⁴



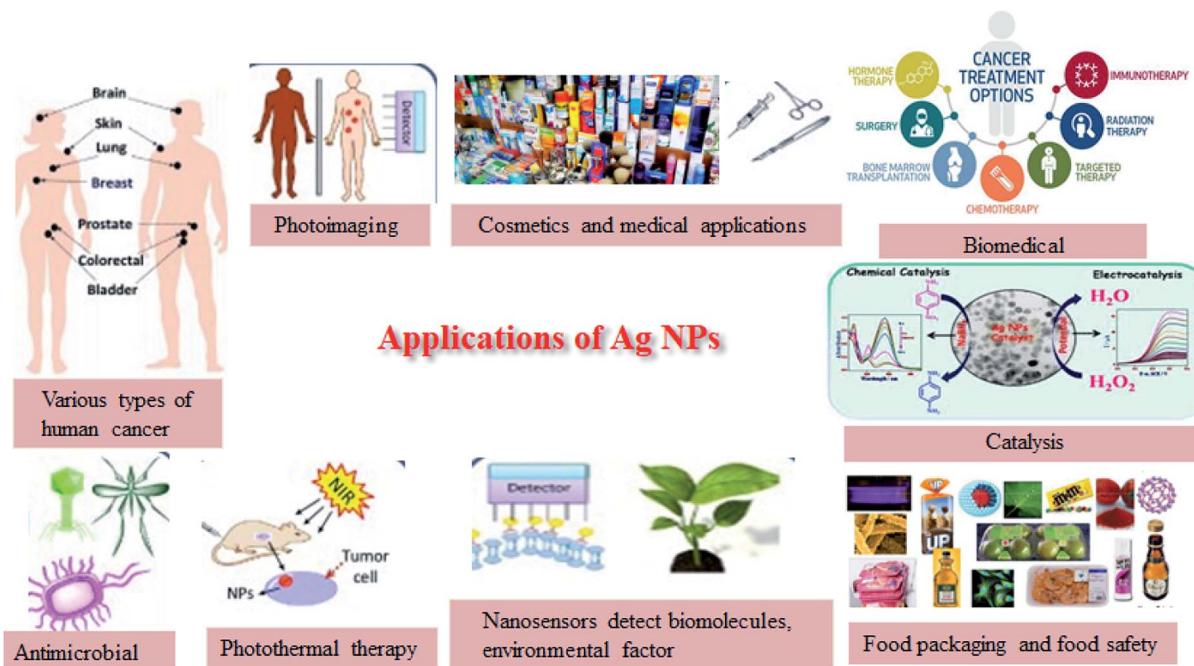


Fig. 8 Various applications of AgNPs.

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 NaBH₄ 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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