



# A review of the phytochemical mediated synthesis of AgNP (silver nanoparticle): the wonder particle of the past decade

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## Abstract

Silver nanoparticle (AgNP) has been one of the most commonly used nanoparticles since the past decade for a wide range of applications, including environmental, agricultural, and medical fields, due to their unique physicochemical properties and ease of synthesis. Though chemical and physical methods of fabricating AgNPs have been quite popular, they posed various environmental problems. As a result, the bioinspired route of AgNP synthesis emerged as the preferred pathway for synthesis. This review focuses extensively on the biosynthesis of AgNP-mediated through different plant species worldwide in the past 10 years. The most popularly utilized application areas have been highlighted with their in-depth mechanistic approach in this review, along with the discussion on the different phytochemicals playing an important role in the bio-reduction of silver ions. In addition to this, the environmental factors which govern their synthesis and stability have been reviewed. The paper systematically analyses the trend of research on AgNP biosynthesis throughout the world through bibliometric analysis. Apart from this, the feasibility analysis of the plant-mediated synthesis of nanoparticles and their applications have been intrigued considering the perspectives of engineering, economic, and environmental limitations. Thus, the review is not only a comprehensive summary of the achievements and current status of plant-mediated biosynthesis but also provides insight into emerging future research frontier.

**Keywords** Silver nanoparticle · Plant-mediated synthesis · Bibliometric analysis · Phytochemicals

## Introduction

Nanotechnology is a new branch of colloidal science that has gained immense importance over the past decade (Mondal et al. 2021). Nanotechnology is concerned with the study of materials at the nanoscale where the fundamental structural units of this novel technology are nanoparticles (NPs) which have sizes ranging from 1 to 100 nm in at least one dimension (Ajitha et al. 2015; Mondal et al. 2019; Kumar et al. 2021b). Many different types of NPs have been synthesized, modified and applied constructively in a wide variety of disparate fields including drug delivery, environmental remediation, material engineering food industries, and medicine (Shaikh et al. 2020). In some fields, advancements have been remarkable, including the use of semiconductor NPs for water splitting (Hisatomi et al. 2014), various environmental applications (Mondal et al. 2021), medical application (Gujrati et al. 2014), and application in electronic field like sensor (Ahmad et al. 2011). Amongst the metallic NPs, silver nanoparticles (AgNPs) have been applied most beneficially across a variety of diverse application

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fields due to their unique biological, chemical, and physical properties including excellent catalytic activity, chemical stability, high electrical conductivity, optical and thermal properties (Shaikh et al. 2018). Consequently, amongst the metallic NPs, AgNPs have become one of the most significant nanomaterials attracting tremendous research interest. Thus, AgNPs have been successfully used in various fields, including antibacterial, antifungal, antiviral, anti-inflammatory activities, composite fibers, cryogenic superconducting materials, electronic component, food industry, health care, industrial purposes, medical, photocatalytic degradation of dye and can also be easily assimilated into cosmetic products (Ajitha et al. 2014b; Shaikh et al. 2018, 2020; Mondal et al. 2019, 2021). In addition, due to the presence of Surface Plasmon Resonance (SPR) phenomenon, AgNPs have attracted unparalleled attention as color-based biosensors (Ahmed et al. 2016).

Initially research focused on the development of robust methods for AgNP synthesis. Today a wide variety of synthetic methods have been established including chemical methods (Iravani and Zolfaghari 2013), electrochemical (Lim et al. 2006), microwave-assisted synthesis (Darmanin et al. 2012), photochemical reductions (Remita et al. 2007) and physical synthesis (Ashkarran 2010). All of these methods have their specific own limitations, such as the use of toxic chemicals as reducing and/or stabilizing agent, the requirement for high vacuum technology or other expensive equipment, production of impure AgNPs of very low yield, and high operating costs (Shaikh et al. 2018). For these reasons there is still a need to develop alternative synthetic routes for AgNP.

A holistic view of the current emerging trends in AgNP synthesis was identified through an open-access database search engine (dimensions), and VOSviewer software in the present review. In the past decade, biological routes for AgNP synthesis have become increasingly popular due to the advantages of providing a one-step synthesis of non-toxic, eco-friendly NPs without the need for preservation or additional maintenance of cultures (Shaikh et al. 2020). Till date, numerous plant species have been utilized for the biosynthesis of AgNP worldwide. Hence this review attempts to summarize the wide variety of plant species and their biochemicals, responsible for the synthesis of AgNP and critically evaluates their limitations and feasibility for real applications.

## Applications of biosynthesized AgNP

### Antibacterial activity of AgNP

Silver nanoparticles have been extensively used in food storage, the health industry, as textile coatings, and in some

environmental applications as an antibacterial agent. Several accredited bodies including the US EPA, US FDA, SIAA of Japan, Korea's Testing and many research institutes have approved products containing AgNP within certain ranges for food storage, health industry, and textile coatings (Gupta et al. 2018). The antibacterial properties exhibited by AgNP tend to depend on several parameters such as shape, size, pH, temperature and most importantly the capping agent used (Ahmed et al. 2016; Edhari et al. 2021). The antibacterial properties of biologically synthesized AgNP were investigated by several methods including the following: the disk diffusion method (Jyoti et al. 2016), the Agar well diffusion assay (Nayak et al. 2015), Kirby–Bauer (Mariselvam et al. 2014) and standard plate count (Zhang et al. 2014).

Several pathogenic Gram (–)ve bacteria such as *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella pneumonia*, and Gram (+) ve like *Staphylococcus aureus*, *Bacillus pumilis*, *Bacillus subtilis*, *Streptococcus pyogenes* were tested to investigate their antimicrobial activity (Table S1) (Rao et al. 2016). Results indicated a concentration-dependent inhibition of bacterial growth for *B. subtilis* and *E. coli*, with a Minimum Inhibitory Concentrations (MIC) of 6.25–12.5 and 12.5–25  $\mu\text{g mL}^{-1}$ , and where no growth was observed in plates above 12.5 and 25  $\mu\text{g mL}^{-1}$ , respectively (Rao et al. 2016). Similarly, the MIC for some several other bacterial strains like *Plesiomonas shigelloides*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Vibrio alginolyticus*, and *Klebsiella pneumonia* were routinely found to be at AgNP doses < 15  $\mu\text{g mL}^{-1}$  (Okafor et al. 2013).

### Mechanism of antibacterial activity of biosynthesized AgNP

The exact mechanism of interaction between AgNP and the constituents of the outer membrane of *E. coli* is only partially known. However, since *E. coli* cells are made up of soft bases like phosphorus and sulfur, acid–base reactions between the metallic nanoparticles and the microbial cells are believed to lead to cell death (Prabhu and Poulouse 2012). Since basic sulfur and phosphorus are major components of DNA and AgNP interacts with such soft bases (Hatchett and White 1996), exposure to AgNP may stop DNA replication, resulting in protein formation inhibition (Feng et al. 2000). Betina (1966) showed after treatment with AgNP the protein (DNA and/or ribosomal protein) became denatured due to the formation of a bond between  $\text{Ag}^+$  and the proteins functional groups. Some researchers claim that the antibacterial properties of AgNP were due to electrostatic attraction between positively charged nanoparticles and negatively charged bacterial cells (Stoimenov et al. 2002). It is assumed that the cell wall degrades when AgNP interacts with binding biomolecules of the cell wall which finally causes cell death (Stoimenov et al. 2002).

Another probable mechanism often proposed following AgNP exposure is that cell death around the wall occurs due to disorganization of cytoplasmic membrane and linkages of several biomolecules like carbohydrates, amino acids, and protein (Patil et al. 2012). It is well known that AgNPs can form complexes with nucleic acids via interaction with the nucleosides groups of nucleic acids which results in antimicrobial activity (Ahmed et al. 2016).

### Degradation of toxic dyes using AgNPs

The number of commercially available dyes is currently estimated to exceed 1 million, where at least 10,000 different dyes are routinely used in the cosmetic, dyeing, leather, paper, pharmaceutical plastic, printing and textile industries (Shaikh et al. 2021). As a result, about 0.7 million tons of dye wastes are commonly released in effluent annually (Shaikh et al. 2020), where non-biodegradable bi-products (dyes and dyestuff) pose major environmental threats to the biosphere. This is one of the most alarming environmental problems, where AgNP particles have exhibited considerable mitigation options (Ghazal et al. 2020). This is an area where the role of AgNP for the degradation of toxic industrial dyes from wastewater by either catalytic or photocatalytic degradation has received much attention (Table 1).

#### Catalytic degradation

Relative to equivalent bulk materials of the same mass, AgNPs show improved catalytic activities due to a relatively greater surface area with a lower volume. Normally, the large difference in redox potential between the electron donor and the electron acceptor makes reaction difficult by limiting electron transfer between acceptor and donor due to the limited passage of electrons (Tripathi et al. 2013). However, for AgNPs in presence of borohydride ion, an electron donor helps to cross the activation energy barrier in the catalytic degradation reaction (Varadavenkatesan et al. 2019).

#### Mechanism of catalytic dye degradation using AgNPs

The Bond Dissociation Energy (BDE) plays a significant role in most chemical reactions because it is often necessary to break existing bonds to form new ones. In a typical degradation system,  $\text{NaBH}_4$  acts as an electron donor and releases electrons to the dye, which acts as an electron acceptor and receives the electron (Varadavenkatesan et al. 2019). When AgNP is present in this system, it acts as a catalyst by helping the “electron-shuttling” process, more efficiently passing electrons to the acceptor (dyes) from the donor ( $\text{NaBH}_4$ ) but via AgNP. This electron relay process causes surface alteration of AgNPs which visually appears as blue spectral shift of the surface plasmon resonance band of AgNPs (Pradhan

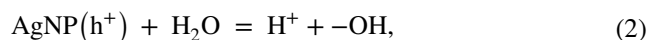
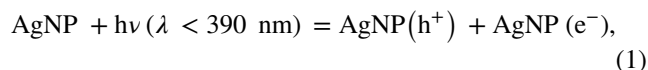
et al. 2002). The spectral blue shift results in the resonance band of AgNP overlapping the absorption peak of the dye (corresponds to  $n-\pi^*$  and/or  $\pi-\pi^*$  transitions of dye) (Varadavenkatesan et al. 2019). Thus, electron transfer becomes easier and smoother when a catalyst has an intermediate redox potential between that of the acceptor and the donor (Tripathi et al. 2013). This is shown visually in Fig. 1.

#### Photocatalytic dye degradation

Biosynthesized AgNPs are often very effective photocatalytic degraders of toxic dyes due to surface plasmon resonance (SPR) where photocatalytic degradation efficiency increases as the metal nanoparticle size decreases (Table 1). Photocatalysis is the mutual competition between separation and recombination of electron–hole pairs [valence band (VB) and conduction band (CB)]. Photocatalytic activity was generally increased by increasing the number of electron–hole pairs on the surface of the charge carrier. During photocatalytic degradation, when light photons hit the valence electrons of AgNPs, they gained energy and thereafter the valence shell emitted highly energetic electrons, which generated active radicals which acted as potent oxidizing agent to completely degrade dye to non-hazardous products like  $\text{CO}_2$ ,  $\text{H}_2\text{O}$ ,  $\text{NH}_4^+$ , and  $\text{NO}_3^-$  (Shaikh et al. 2018, 2020).

#### Mechanism of photo-catalytic dye degradation using AgNPs

Under visible and/or UV irradiation the VB electrons of the metal are excited and promoted to the CB, creating conduction electron ( $e^-_{\text{CB}}$ ) in the CB and a positive hole ( $h^+_{\text{VB}}$ ) in the VB (Eq. 1) (Sinha et al. 2014) (Fig. 2). These photogenerated species ( $h^+_{\text{VB}}$  and  $e^-_{\text{CB}}$ ) generate highly reactive radicals capable of dye degradation (Shaikh et al. 2018). For example,  $h^+_{\text{VB}}$  can dissociate water ( $\text{H}_2\text{O}$ ) into hydrogen ( $\text{H}^+$ ) and hydroxyl ( $^-\text{OH}$ ) ion, where the hydroxyl ( $^-\text{OH}$ ) ion is subsequently converted into a hydroxyl radical ( $\cdot\text{OH}$ ) (Eq. 2 and 3). Simultaneously,  $e^-_{\text{CB}}$  can convert dissolved oxygen ( $\text{O}_2$ ) into a superoxide radical anion ( $\cdot\text{O}_2^-$ ) (Eq. 4) (Saravanakumar et al. 2016), which can thereafter also react with  $\text{H}_2\text{O}$  to produce both  $\cdot\text{OH}$  and the hydroperoxyl radical ( $\text{HO}_2\cdot$ ) (Eq. 5) (Shaikh et al. 2020). These three photogenerated radicals ( $\cdot\text{OH}$ ,  $\cdot\text{O}_2^-$ , and  $\text{HO}_2\cdot$ ) all contribute to the degradation of complex dyes into simple non-toxic fragments like ammonium ( $\text{NH}_4^+$ ), carbon dioxide ( $\text{CO}_2$ ), nitrate ( $\text{NO}_3^-$ ), and water ( $\text{H}_2\text{O}$ ) (Eq. 6) (Tahir et al. 2015).

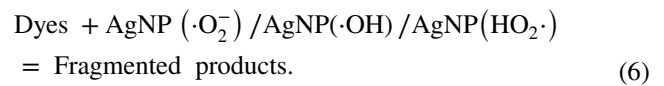
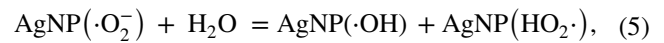
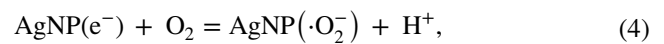
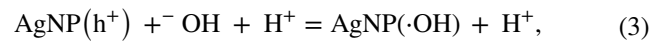


**Table 1** Degradation (catalytic and photocatalytic) of dye using AgNPs

Sl. No.	Plant	Plant part	Size (nm)	shape	Degradation	Dye	Concentration	Efficiency	References
1	<i>Azadirachta indica</i>	Leaf extract	11–35	Spherical	Photocatalytic	Congo red	20 mg L <sup>-1</sup>	90%	Shaikh et al. (2018)
2	<i>Shorea robusta</i>	Leaf extract	12–37	Spherical	Photocatalytic	Rhodamine B	21 mg L <sup>-1</sup>	90.41%	Shaikh et al. (2020)
3	<i>Gmelina arborea</i>	Fruit extract	8–32	Spherical	Catalytic	Methylene Blue		100%	Saha et al. (2017)
4	<i>Camellia japonica</i>	Leaf extract	12–25	Spherical	Photocatalytic	Eosin-Y	10 mg L <sup>-1</sup>	> 97%	Karthik et al. (2017)
5	<i>Ficus panda</i>	Leaf extract	12–36	Spherical	Catalytic	Methylene blue			Tripathi et al. (2013)
6	<i>Areca catechu</i>	Nut extract	18.2–24.3	Spherical	Catalytic	Methylene blue, Eosin-yellowish, and methyl orange			Rajan et al. (2015)
7	<i>Polygonum hydropiper</i>	Leaf extract	45–70	Spherical	Catalytic	methylene blue			Bonnia et al. (2016)
8	<i>Mussaenda erythrophylla</i>	Leaf extract	~100	Spherical	Catalytic	Methyl orange			Varadavenkatesan et al. (2016)
9	<i>Litchi chinensis</i>	Aqueous extract	4–8	Spherical	Catalytic	Methylene blue		99.24%	Khan et al. (2016b)
10	<i>Amaranthus gangeticus</i> Linn	Leaf extract	11–15	Spherical	Catalytic	Congo red	10 <sup>-3</sup> M		Kolya et al. (2015)
11	<i>Hypnea musciformis</i>	Aqueous extract	2–55.8	Spherical	Photocatalytic	Methyl orange			Ganapathy Selvam and Sivakumar (2015)
12	<i>Prangos ferulacea</i>	Roots extract	79–200	Spherical	Photocatalytic	New Fuchsin, Methylene Blue, and Erythrosine B		96.5%, 96.0%, and 92%, respectively	Mavaei et al. (2020)
13	<i>Nepeta leucophylla</i>	Stem extracts	15–25	Spherical	Photocatalytic	Methylene blue		82.80%	Singh and Dhaliwal (2020)
14	<i>Convolvulus arvensis</i>	Leaf extract	28	Spherical	Catalytic	Methylene blue			Hamedi et al. (2017)
15	<i>Achillea millefolium</i> L.	Aqueous extract	20	Spherical	Catalytic	Methyl Orange and Methylene Blue			Khodadadi et al. (2017)
16	<i>Trigonella foenum-graecum</i>	Seeds extract	22–32	Spherical	Catalytic	Methylene blue and eosin Y			Vidhu and Philip (2014a)
17	<i>Polygonum Hydro-piper</i>	Leaf extract	45–70	Spherical	Catalytic	Methylene Blue			Bonnia et al. (2016)
18	<i>Zanthoxylum armatum</i>	Leaf extract	15–50	Spherical	Catalytic	Safranine O, Methyl red, Methyl orange, and Methylene blue			Jyoti and Singh (2016)
19	<i>Cassia auriculata</i>	Flower Extract	10–35	Spherical and triangle	Catalytic	methyl orange			Muthu and Priya (2017)
20	<i>Litchi chinensis</i>	Aqueous extract	4–8	Spherical	Photocatalytic	Methylene blue		99.24%	Khan et al. (2016b)
21	<i>Saraca Indica</i>	Flower Extract	16–20	Spherical	Catalytic	Methylene blue			Vidhu and Philip (2014b)
22	<i>Centella asiatica</i>	Leaf extract	30–50	Spherical	Catalytic	Methyl red, methyl orange, and phenyl red		98.49%, 98.84%, and 99.62% respectively	Raina et al. (2020)
23	<i>Clitoria ternatea</i>	Pod extract	62.51	Spherical	Catalytic	Methylene blue	12 mg L <sup>-1</sup>		Varadavenkatesan et al. (2019)

Table 1 (continued)

Sl. No.	Plant	Plant part	Size (nm)	shape	Degradation	Dye	Concentration	Efficiency	References
24	<i>Alpinia officinarum</i>	Rhizome extract	20–80	Hexagonal	Photocatalytic	Malachite green and methylene blue	10 mg L <sup>-1</sup>	91%	Li et al. (2020)
25	<i>Mentha aquatica</i>	Leaf extract	8–14	Spherical	Catalytic	Methylene blue		83%	Nouri et al. (2020)
26	<i>Prosopis juliflora</i>	Leaf extract	30	Spherical	Photocatalytic	Rose bengal dye		85%, 70%, and 78%, respectively	Mahini et al. (2020)
27	<i>Crataegus pentagyna</i>	Fruit extract	15–60	Spherical	Photocatalytic	Rhodmine B, eosin Y, and methylene blue		96%	Ebrahimzadeh et al. (2020)
28	<i>Salvadora persica</i>	Stem extracts	1–6	Spherical	Photocatalytic	Methylene blue	15 mg L <sup>-1</sup>		Tahir et al. (2015)
31	<i>Diospyros lotus</i>	Leaves extract	10–25	Spherical	Catalytic	Methylene Blue	10 <sup>-4</sup> M		Hamedi and Shojaoosadati (2019)



### Biosensing

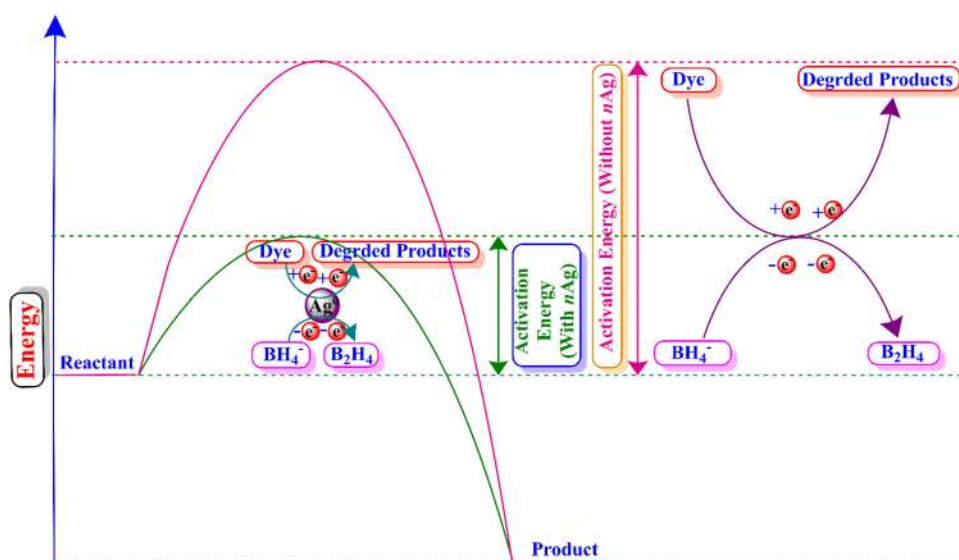
Due to the surface plasmon resonance (SPR) effect and changes induced in nanoparticles' shape and size, triangular AgNPs have recently attracted tremendous attention as a color based biosensor. These AgNP-based nanosensors have two unique characteristics: (i) unique refractive sensitivity and (ii) short-range sensing by the local electromagnetic field, where the combined contribution of these two properties results in sensitivity of ~ 100–1000 pg mm<sup>-2</sup> (Larguinho and Baptista 2012). Bindhu and Umadevi (2014) reported biosynthesis of AgNPs using *Solanum lycopersicums* extract and successfully applied it for the sensing of heavy metal ions (Fe<sup>3+</sup> and Cu<sup>2+</sup>) in water by using a SPR optical sensor. Kirubaharan et al. (2012) prepared AgNPs using an *Azadirachta indica* extract, where the biosynthesized AgNPs exhibited excellent specific metal (Cu<sup>2+</sup>) ion selectivity. This study also showed significant Cu<sup>2+</sup> selectivity even when Cu was present in minimal concentration (0.001 mg L<sup>-1</sup>) in aqueous solution. AgNPs have also been considered in bio-molecular diagnostics, including for biomarker characterization and detection of gene expression profiles, characterization of proteins, DNA or RNA, and nucleic acids (Goyal et al. 2009).

### Biomedical applications of AgNPs

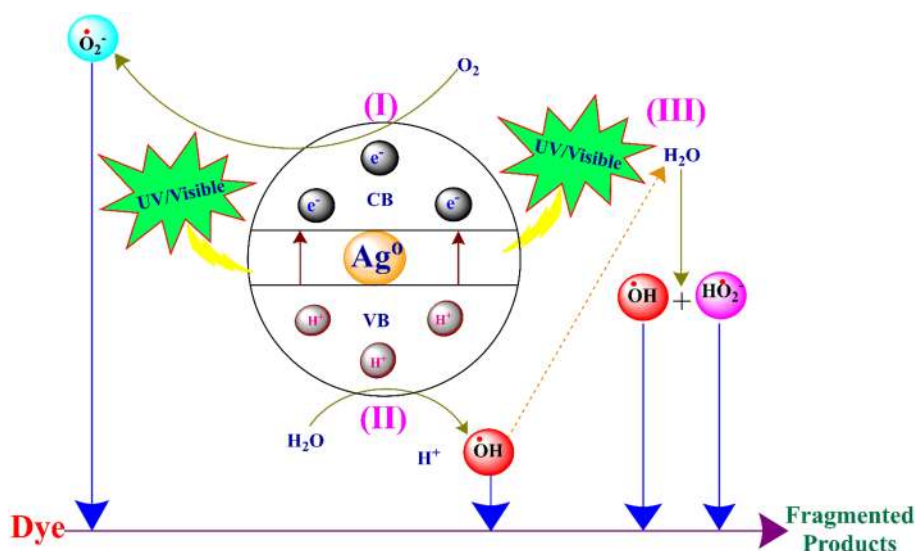
Increasingly, a range of metal nanoparticles, but especially AgNP, have played important roles in the recent development of science and technology (Shamaila et al. 2016). In the past decade, AgNP has attracted tremendous attention in the medical field for precise and selective drug-delivery resulting in enhanced pharmacokinetics, pharmacodynamics, and a high capacity to bind with a wide range of biomolecules (Burduşel et al. 2018). Such specific drug-delivery system has proved extremely useful in providing suitable drug release profiles with maximum therapeutic efficiency and minimal side-effects. Recent improvements of AgNPs via surface modification have also led to



**Fig. 1** Mechanism of catalytic dye degradation using AgNP



**Fig. 2** Mechanism of photo-catalytic degradation of dyes using AgNP



enhanced biocompatibility and stability, which has only enhanced AgNP suitability as a nanoscale drug-delivery system. Indeed, the unique target specificity of AgNP has resulted in its extensive use in nanoparticle-based drug-delivery applications, specifically anti-cancer and anti-tumor drug delivery systems (Philip et al. 2011).

In addition, AgNP has also proven to be a potent antimicrobial and antipathogenic agent, in dental-related nanotechnology-based strategies (Qing et al. 2018). The main aim of using AgNP in dentistry has been to protect against harmful pathogens and protect the oral cavity (Burduşel et al. 2018). For example, the good biocompatibility of AgNP was shown to be useful as a coating material for dental barrier membranes (DBM). Such AgNP coatings have also been shown to prevent pathogenic contamination from dental implants' and poor tooth-brushing techniques. Recently, AgNP has

also been used in tooth stainers in the form of nanosilver diamine fluoride (SDF); however, the effect of this new compound is still unknown (Burduşel et al. 2018). Moreover, Qing et al. (2018) reported AgNP as a coating material (silver-coated prostheses) for an unconventional approach for prophylaxis of tumor-related infections in the Orthopedic and bone-implant-related field.

### Use of AgNP for COVID-19 mitigation

Currently the COVID-19 pandemic is a major urgent topic of scientific endeavour. The current pandemic, coronavirus disease (COVID-19), has spread almost all over the world (over 221 countries), with > 138,057,338 infected people causing the death of > 2,972,992 people. Scientists around the world have done significant studies to develop

therapeutics or vaccines. However, the efficiency of these vaccines is still questionable. A considerable effort has also been given to nano-based anti-viral agents or vaccines, all of which are currently in initial development phases far from public implementation. However, Talebian et al. (2020) proposed metallic nanoparticles (Ag, Cu, TiO<sub>2</sub>) as an alternative to conventional approaches to fighting COVID-19. Among these various nanoparticles, AgNP has already shown significant promising results as a durable and self-sterilizing agent. Talebian et al. (2020) reported AgNP has been used as a photocatalytic coating agent on the surface of COVID-19 test kits and the coating layer of respiratory face masks. AgNP-based fortification equipment and disinfecting agents can provide enhanced protection against SARS-CoV-2 and carrier for antigens or as an adjuvant, making way to develop new generation of vaccines (Rai et al. 2021). However, the main disadvantages with these kits where the chance of false-negative results, poor analytical sensitivity, long response time, and the health impact of the AgNP coated kit on a human are yet to be studied (Vaculovicova et al. 2017). To overcome these challenges, recently, Swiss researchers developed a dual-functional plasmonic biosensor (involving a combination of a localized surface plasmon and photothermal resonance plasmon), as a promising alternative to AgNP for clinical diagnosis of COVID-19 (Qiu et al. 2020).

### Approaches of silver nanoparticle synthesis

There are two main approaches involved in AgNP synthesis, ‘bottom up’ or ‘top down’ (Ahmed et al. 2016). In the top-down approach, nano-sized particles are synthesized from a suitable bulk material by breaking down the parent material via size reduction (Fig. 3). The most common methods employed in the top-down approach include chemical etching, combustion, mechanical/ball milling, sputtering, and thermal/laser ablation (Mittal et al. 2013). The key step in all these methods is the reduction or breakdown of the parent starting material. However, one major limitation of these processes is that they result in AgNPs which have significant surface imperfections due to the alteration of the physical and chemical properties of the starting material (Mittal et al. 2013).

In the ‘bottom up’ approach, a range of mainly physio-chemical, and occasionally biological methods are used in the self-assembly of atoms to develop new nuclei of a particular nanosize. Common bottom-up approaches include aerosol process, atomic/molecular condensation, (electro)chemical precipitation, laser pyrolysis, sol-gel process, spray pyrolysis, vapor deposition, and bio-reduction (Mittal et al. 2013).

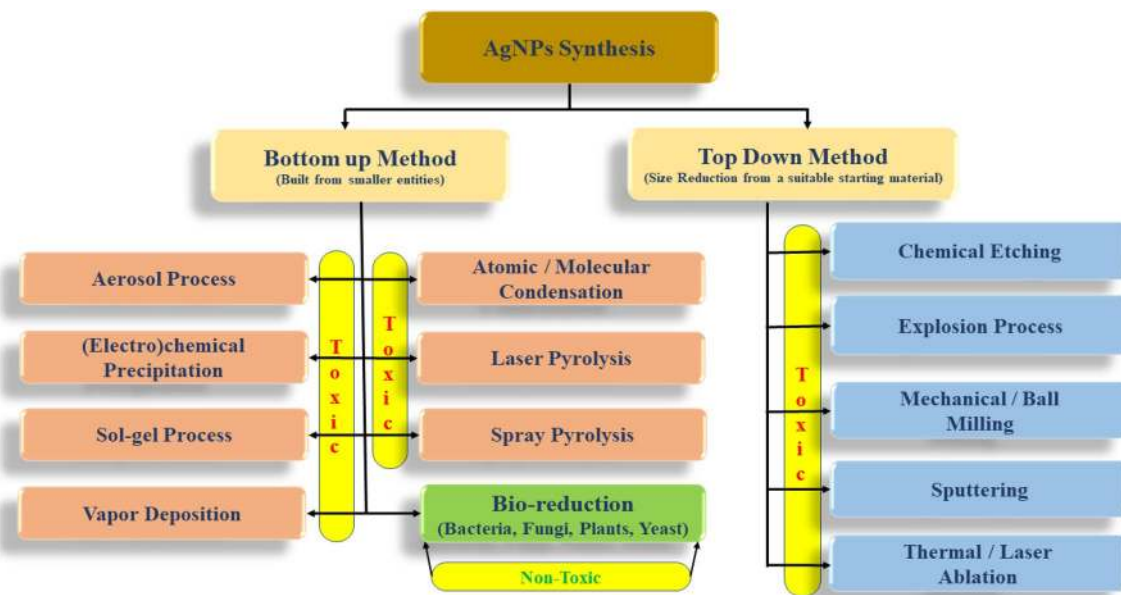


Fig. 3 Different approaches for silver nanoparticles synthesis

## Conventional methods adopted for the biosynthesis of AgNP

Biosynthesis of AgNP mainly involves the following three common steps: 1. collection, 2. Extraction, and 3. reaction, which are briefly described as follows:

- i. Collection and preparation: The first step involves the collection and segregation of the target plant species. The target plant part is then washed with tap water twice or thrice and finally with distilled water to remove any impurities, debris or organic substances adhering to the materials.
- ii. Then the fresh uncontaminated plant parts are either shade-dried for 10–15 days till constant weight and finally powdered with a grinder, or blotted dry, cut into pieces, and boiled with millipore water for 30 min to prepare the plant extract (Joseph and Mathew 2015). Another extraction method involves grinding the fresh plant part after thorough washing with millipore water and/or ethanol. The extract is finally filtered using Whatman filter paper no.1 and stored at 4 °C for further use (Shaikh et al. 2018, 2020; Mondal et al. 2021).
- iii. Reaction: The last step of the biosynthesis process is the reduction process where the plant extract is added to 1–10 mM AgNO<sub>3</sub> to reduce the pure Ag(I) ions to Ag(0) at room temperature normally in a reaction lasting upto 6 h. Typically, the bio-reduction process is monitored by measuring the UV–visible spectrum at 200–800 nm with a regular interval of 0.5 nm, where the AgNP peak arises around 400–450 nm (Mondal et al. 2019, 2021; Shaikh et al. 2020).

## Disadvantages of conventional synthesis methods

While the physiochemical route of AgNP synthesis is useful for large-scale production of high-purity AgNPs with superior physical properties, the top down method does have several disadvantages including the following:

- i. Most physical methods need expensive equipment and high vacuum technology.
- ii. Mechanical/ball milling methods often require substantial amounts of the initial bulk material, which must be subjected to high mechanical energy over an extended period, which tends to result in a high probability of some surface deterioration and contamination due to the use of steel grinding balls.
- iii. During ion sputtering methods, the process of vaporizing solid materials and sputtering through inert gas

ion beams of He, Ne, Ar, Kr, or Xe, can influence the final composition, optical properties, surface morphology, and texture of the nanoparticle produced.

- iv. While applying the laser ablation method, where NPs are synthesized by reduction of size using laser irradiation, the main disadvantage is the low yield due to blockage of the laser path and energy in the colloidal solution (Jamkhande et al. 2019).

Likewise, the bottom up methods also have a range of distinct disadvantages. For example:

- i. Chemical methods need a range of different, often toxic inorganic and/or organic reducing agents, to act as both reducing and, to prevent agglomeration, capping agents This can include ascorbate, elemental hydrogen (H), sodium citrate (Na<sub>3</sub>C<sub>6</sub>H<sub>5</sub>O<sub>7</sub>), sodium borohydride (NaBH<sub>4</sub>), Tollen's reagent [Ag(NH<sub>3</sub>)<sub>2</sub>]NO<sub>3</sub> and *N,N*-dimethyl formamide (C<sub>3</sub>H<sub>7</sub>NO) in both polar or non-polar solvents (Ajitha et al. 2014a).
- ii. Reducing chemicals are often toxic, relatively expensive and can create impurities in the final NPs, which can become secondary pollutants when practically applied.
- iii. Bottom-up methods tend to be relatively expensive, low yield methods, difficult to control leading to low reproducibility (Jamkhande et al. 2019).

To address these major concerns, currently the main focus of AgNP synthesis research has been the synthesis of non-toxic and environment friendly AgNP through biological pathways (Ahmad et al. 2010). This is because biosynthesized AgNP is generally identified as non-toxic with less environmental impacts than other physiochemical methods (Ahmed et al. 2016). Unlike traditional chemical methods, the biosynthesis approach has the advantage of using a single aqueous plant part extract, as both the reducing as well as stabilizing agent rather than a suite of toxic chemicals. While this is a single natural extract, several biomolecules may be involved as either reducing and/or capping biomolecules (Shaikh et al. 2018, 2020). The other main advantage of biosynthesis of AgNPs is that they generally yield large amounts of AgNPs with well-defined size and morphology (Hutchison 2008).

## Biosynthesis of AgNP

Although the mechanism was not well understood, the reduction of metal ions by plant extracts was first identified in the early 1900s (Mittal et al. 2013). Since then, a range of metals have been successfully reduced via a range of



plant materials. However, in the last 35 years, bio-synthesis of AgNP has attracted significant attention using either extracts of plant tissue, plant parts or indeed the whole plant (Fatimah 2016). The bio-reduction process simply involves mixing of a metal solution with plant extracts at room temperature (Mittal et al. 2013). The main objectives during the green synthesis of AgNP is to maximize safety and efficiency and minimize the environmental and societal impact of toxic raw materials. The nature, yield, quality, and characteristics of the produced AgNP are influenced mainly by the relative concentrations of plant extract and metal salt, contact time, temperature, and reaction pH (Dwivedi and Gopal 2010).

The choice of plant extract may also be important because several plants and their respective parts may contain different biomolecules which can act as reducing agent as well as stabilizing agents during bio-reduction (Table 2). These biomolecules may also influence the overall surface characteristics of the AgNPs and also agglomeration in solution due to the numerous possible combinations of biomolecule interactions with the AgNPs (Mittal et al. 2013). Thus, the various different plant types and parts currently used for the biosynthesis of AgNP is briefly discussed below.

### Biosynthesis of AgNP using leaf, root, shoot, flower, and fruit extract

The successful synthesis of AgNP using different plant parts has been summarized in Table 2. Generally most of the AgNP particles synthesized with various plant parts yielded spherical AgNPs with an average size of 5 to 85 nm (Mittal et al. 2013). However, non-spherical AgNPs, i.e. triangular, pentagonal, and hexagonal, were also reported using *Eclipta prostrata* leaf extract; where the particle size ranged between 30 and 60 nm when the reaction occurred at room temperature (Rajakumar and Abdul Rahuman 2011). Similarly, both cubic and irregular AgNPs were also synthesized using the seeds of *Trachyspermum ammi* and *Artocarpus heterophyllus*, respectively (Jagtap and Bapat 2013). At room temperature biosynthesis reaction times ranged between 10 and 300 min. The bio-reduction of the Ag precursor was ascribed to high levels of biomolecules in the different plant parts (leaves, fruits, flowers, seeds, barks and roots). These biomolecules could be very diverse and include a myriad of alcohols, alcoholic compounds, alkaloids, alkynes, allylic benzenes, amide, amino acids, amino acid residues, anthraquinones, ascorbic acid, benzoates, caffeoyl, carbohydrates, carotenes, catechic tannins, diterpenoids, flavonoids, glycosides, iridoids, leucocyanidin, proteins, phenols, phenolic compounds, saponins, steroids, sugars, tannins, terpenoids, triterpenoids, traces of reducing sugars, triterpenes, and vitamin C) which acted both as reducing and/or capping or stabilizing agents (Ebrahimzadeh et al. 2020). Though

the phytochemicals present in the different plant extracts have been identified by several researchers, no one was able to clearly identify one specific biomolecule involved in the bio-reduction of  $\text{Ag}^+$  to AgNP. Biosynthesized AgNP, using various leaf extracts, has generally shown several important properties including excellent antibacterial activities, cytotoxic, mosquitocidal activity, synergistic effects with antibiotics, anticancer effects against human breast cancer cells (MCF-7), and photocatalytic degradation of dye (Shaikh et al. 2020). AgNP synthesized from flower extracts was also shown to have efficient catalytic activity for the reduction of cationic dyes such as methylene blue in the presence of  $\text{NaBH}_4$  by generating active free radicals ( $\cdot\text{OH}$ ,  $\cdot\text{O}_2^-$  and  $\text{HO}_2\cdot$ ) and antibacterial efficiency due to deterioration of the plasma membrane by AgNP penetration through the cell wall causing bacterial cell death in cytotoxic studies using human cell lines (Ocsoy et al. 2017). Fruit extract (*Lycium barbarum*) mediated AgNP also exhibited good optical properties suitable for uses as sensors (Saha et al. 2017).

While Ameen et al. (2019) reported the successful biosynthesis of AgNP using a *Mangifera indica* flower extract, no identification of the specific phytochemicals responsible for reduction was provided. However, other researchers like Hamed and Shojaosadati (2019) did include a general screening and characterization of the phytochemicals responsible for AgNP synthesis when using a *Diospyros lotus* extract, which showed that alkaloids, anthraquinones, flavonoids, saponins, steroids, tannins, and terpenoids were all key components. In fact, of the more than 200 plant species from 86 families extracted and used for AgNP synthesis, most studies have conclusively identified as the specific phytochemical(s) involved, creating a huge knowledge gap regarding the specific phytochemical(s) responsible for either reduction and/or capping during AgNP biosynthesis and the underlying reaction mechanisms. Some attempts to explore the mechanisms by FTIR techniques implicate amide, carboxylate, carbonyl group, proteins, terpenoids, ketones, and aldehydes of *Vigna* sp. seed extract (Mohammadi et al. 2016), but identification of specific biochemicals is a challenge.

### Bio synthesis using other plant parts

Rapid green synthesis of AgNP has also been reported by some other plant parts including pericarp extracts of *Sapindus emarginatus* (Swarnavalli et al. 2017), *Allium stipitatum* (shallot) (Taheri et al. 2015), and apricot tree gum (Rajkuberan et al. 2017), latex extract of *Euphorbia antiquorum* L., (Mariselvam et al. 2014) inflorescence of *Cocos nucifera* (Edison and Sethuraman 2012), and *Musa* sp. (Banana) peel extract (Ibrahim 2015). As with most other plant extract derived NPs most of the biosynthesized AgNP were spherical having a particle size ranging between 4 and

**Table 2** List of plants used in phyto-synthesis of AgNP

Sl no.	Plant	Family	Geographical Origin and distributions	Size of AgNPs	shape	Phytoconstituents responsible for reduction of silver salt	Applications	References
<i>Plant Part: Leaf</i>								
1	<i>Azadirachta indica</i>	Meliaceae	India, Indian subcontinent, Myanmar, Malaysia, Indonesia and Thailand, Fiji, Mauritius, Guyana, Saudi Arabia, Philippines, Egypt, and Australia	34	Spherical	Flavanoids and terpenoids	Photoluminescence	Ahmed et al. (2016)
2	<i>Olea europaea</i> (olive)	Oleaceae	Mediterranean basin	20–25	Spherical	Amine, proteins, oleuropein, apigenin-7-glucoside and/or luteolin-7-glucoside	Antibacterial activity	Khalil et al. (2014)
3	<i>Tephrosia purpurea</i>	Fabaceae	India and Sri Lanka	~20	Spherical	Phenols, alkyl halides, and amide	Antimicrobial activity	Ajitha et al. (2014a)
4	<i>Lantana camara</i>	Verbenaceae	Mexico, Central America, Caribbean, and tropical South America, Australia	17–31	Spherical	Aromatic amines, alcohols, and amide	Antibacterial activity	Ajitha et al. (2015)
5	<i>Platanus orientalis</i> (Oriental Plane)	Platanaceae	Bulgaria, Greece and Iran	10–30	truncated triangular	–	–	Al-Thabaiti et al. (2015)
6	<i>Garcinia mangostana</i> (mangosteen)	Clusiaceae	Malaysia and Nicobar Islands	5–57	Spherical	Ether and aromatic-OH group	Antimicrobial activity	Veerasingam et al. (2011)
7	<i>Urtica dioica</i> Linn	Urticaceae	British Isles	20–30	Spherical	Phytosterols, tannins, diterpenes, phenol, amino acid, proteins, saponins, alkaloids, and	Synergistic effects with antibiotics	Jyoti et al. (2016)
8	<i>Calliandra haematocephala</i>	Fabaceae	Bolivia and Southern Peru	13.54–104.3	Spherical	Enzymes, proteins, flavonoids, terpenoids, and cofactors	Antibacterial activity and Hydrogen peroxide sensing capability	Raja et al. (2017)
9	<i>Tridax procumbens</i>	Asteraceae	Central America	40.6–139	Spherical	Amide, mono chlorinated acyclic, and cyclohexane	Mosquitocidal activity	Ondari Nyakundi and Padmanabhan (2015)

Table 2 (continued)

Sl no.	Plant	Family	Geographical Origin and distributions	Size of AgNPs	shape	Phytoconstituents responsible for reduction of silver salt	Applications	References
10	<i>Biophytum sensitivum</i>	Oxalidiaceae	South East Asia and Madagascar	11–25	Spherical	Flavonoids, polyphenols, phytochelatin, glutathione, metallothioneins, ascorbic acid, dehydroascorbic acid, ascorbates, glutathione, metallothioneins	–	Joseph and Mathew (2015)
11	<i>Camellia japonica</i>	Theaceae	China, Taiwan, Japan, and southern Korea	12–25	Spherical	Amide and alcohols	Photocatalytic degradation and electrocatalytic reduction	Karthik et al. (2017)
12	<i>Eclipta prostrata</i>	Asteraceae	Asia	35–60	Triangles, pentagons, hexagons	Flavonoid, diosmetin, isoflavonoids	Larvicidal activity	Rajakumar and Abdul Rahuman (2011)
13	<i>Moringa oleifera</i>	Moringaceae	Northern India and Pakistan	94.17 ± 1.5	rectangular	Amines, amides, phenols, alcohols, aldehydes, flavonoids, terpenoids	antimicrobial activity	Nayak et al. (2015)
14	<i>Amaranthus gangeticus</i>	Amaranthaceae	Mexico	11–15	Globular and polycrystalline	Amino acids	Antimicrobial and azo dye (Congo red) degradation	Kolya et al. (2015)
15	<i>Psidium guajava</i>	Myrtaceae	American tropics, Philippines, Mexico and Peru	10–90	Spherical	Leucocyanidin, flavonoids, tannins, saponins, carotenes, vitamin C, B6 and carbohydrates	Antibacterial activity	Bose and Chatterjee (2016)
16	<i>Andrographis echioides</i>	Acanthaceae	Southeast Asia, China, America	~68.06	Cubic	Carbohydrates, tannins, saponins, flavonoids, alkaloids, quinones, glycosides, triterpenoids, phenols, steroids, phytosteroids and anthraquinones	Anticancer and antibacterial activities	Elangovan et al. (2015)
17	<i>Chrysophyllum oliviforme</i>	Sapotaceae	Bahamas, Florida, Greater Antilles, and Belize	25	Flower shape	Flavonoids, saponins, catechic tannins, traces of anthraquinones, reducing sugars and phenolic compounds	Antioxidant and anticancer activities	Anju Varghese et al. (2015)

Table 2 (continued)

Sl no.	Plant	Family	Geographical Origin and distributions	Size of AgNPs	shape	Phytoconstituents responsible for reduction of silver salt	Applications	References
18	<i>Azadirachta indica</i>	Meliaceae	India, Indian subcontinent, Myanmar, Malaysia, Indonesia and Thailand, Fiji, Mauritius, Guyana, Saudi Arabia, Philippines, Egypt, and Australia	11–35	Spherical	Flavanones, alkaloids, alkynes, and amide	Photocatalytic degradation of dye	Shaikh et al. (2018)
19	<i>Shorea robusta</i>	Dipterocarpaceae	Southeast Asia and Myanmar	12–37	Spherical	Flavans, flavanone and flavonol	Photocatalytic degradation of dye	Shaikh et al. (2020)
20	<i>Ficus panda</i>	Moraceae	Tropical Southeast Asia	12–36	Spherical	Alkene, alkane, amide, ester and ether	Catalytic degradation of methylene blue	Tripathi et al. (2013)
21	<i>Polygonum Hydropiper</i>	Polygonaceae	Temperate Asia, Europe, North America, Australia, and New Zealand,	45–70	Spherical	Alkene, proteins, amine, carbonyl, and phenolic groups	Catalytic Degradation of methylene blue	Bomma et al. (2016)
22	<i>Zanthoxylum armatum</i>	Rutaceae	China, Nepal, Pakistan, Japan, and Korea	15–50	Spherical	Amide, phenols, proteins	Catalytic Degradation of Safranin O, Methyl red, Methylene orange and Methylene blue	Jyoti and Singh (2016)
23	<i>Mussaenda erythrophylla</i>	Rubiaceae	Indian Subcontinent	37.84–50.75	Spherical	Amide, proteins, alkaloids, flavonoids, terpenoids, aldehydic and ketonic compounds	Catalytic degradation of methyl orange	Varadavenkatesan et al. (2016)
24	<i>Convolvulus arvensis</i>	Convolvulaceae	Temperate and tropical Asia, Africa, and Europe	28	Spherical	amino acids, enzymes, flavonoids, terpenoids, phenols, vitamins, polysaccharides, and proteins	Catalytic, antibacterial and anti-biofilm activities	Hamed et al. (2017)
25	<i>Centella asiatica</i>	Apiaceae	Tropical Asia and Africa	30–50	Spherical	Proteins, flavonoids, polyphenols and terpenoids	Catalytic degradation of methyl red, methyl orange and phenol red	Raina et al. (2020)
26	<i>Mentha aquatica</i>	Lamiaceae	Australia, Europe, Central Asia, and North Africa	8–14	Spherical	Alkene, amid, alcohol, alkaloids, flavonoids, Phenol, proteins, saccharides, steroids, saponins, sugar and tannins	Antibacterial properties and catalytic activity	Nouri et al. (2020)

Table 2 (continued)

Sl no.	Plant	Family	Geographical Origin and distributions	Size of AgNPs	shape	Phytoconstituents responsible for reduction of silver salt	Applications	References
27	<i>Prosopis juliflora</i>	Fabaceae	Peru, Brazil, Africa, Australia, Southeast Asia, northern South America	30	Spherical	Amide, alkanes, alcohols, aromatic compounds, ketones and phenols	Photocatalytic degradation and Antibacterial activity	Malimi et al. (2020)
28	<i>Parkia spectiosa</i>	Fabaceae	Brunei, Indonesia, Malaysia, Thailand and Philippines	26–39	Spherical	Amine, amide, polyphenols and proteins	Antibacterial, antioxidant and photocatalytic activity	Ravichandran et al. (2019)
29	<i>Diospyros lotus</i>	Ebenaceae	Southwest Asia and Southeast Europe	10–25	Spherical	Alkaloids, anthraquinones, flavonoids, saponins, steroids, tannins and terpenoids	Antibacterial catalytic activity	Hamed and Shojaosadati (2019)
30	<i>Allium ampeloprasum</i>	Amaryllidaceae	Northern Africa, Iran, and Portugal	2–43	Spherical, ellipsoidal, hexagonal	Amino acids, proteins, enzymes, vitamins, flavonoids, polysaccharides and organic acids	Antioxidant activity and catalytic reduction of 4-nitrophenol	Khoshnamvand et al. (2019)
<i>Plant part: flower</i>								
31	<i>Matricaria camomila</i>	Asteraceae	Australia, Europe, Afghanistan, India, North and South America, and central Asia	8–35	Spherical	Terpenoids, flavones and polysaccharides	Antibacterial activity	Parlinska-Wojtan et al. (2016)
32	<i>Matricaria camomila</i>	Asteraceae	Australia, Europe, Afghanistan, India, North and South America, and central Asia	~ 5.5	Spherical	Phenolics, carbonyl and amines or alcohol groups	Antibacterial activity	Ocsoy et al. (2017)
33	<i>Allamanda cathartica</i>	Apocynaceae	Brazil, French Guyana, Guyana, Suriname, and Venezuela	39	Spherical	(E,E)-geranyl linalool, n-pentacosane, 1,8-cineole and n-tricosane	Antibacterial activity and Antioxidant	Karunakaran et al. (2016)
34	<i>Millettia pinnata</i>	Fabaceae	India, Sri Lanka, Malaysia, Myanmar, and Australia	16–38	Spherical	Multi-functional aromatic groups	Anti-cholinesterase, Antibacterial and Cytotoxic activities	Rajakumar et al. (2017)
35	<i>Cassia auriculata</i>	Fabaceae	India, Myanmar, Sri Lanka, and Tanzania	10–35	Spherical and triangular	Tannins, flavonoids, glycosides, carbohydrates and polyphenolic	Catalytic degradation of Dye	Muthu and Priya (2017)



Table 2 (continued)

Sl no.	Plant	Family	Geographical Origin and distributions	Size of AgNPs	shape	Phytoconstituents responsible for reduction of silver salt	Applications	References
36	<i>Saraca indica</i>	Fabaceae	India, Sri Lanka, Malaysia, and Indonesia	18–22	Spherical	Flavanones, terpenoids amide, geminal methyls and alkynes	Catalytic degradation of Methylene blue	Vidhu and Philip (2014b)
37	<i>Musa acuminata</i>	Musaceae	Malaysia, Tropical Asia	12.6–15.7	Spherical	Carotenoids, dietary fibre, fatty acids, polyphenol, proteins and vitamins,	Pharmaceutical activity and anticancer efficacy	Valsalam et al. (2019)
38	<i>Mangifera indica</i>	Anacardiaceae	India and Myanmar	10–20	Spherical	Alkaloids, amino acids, flavonoids and proteins	Antibacterial activity	Ameen et al. (2019)
39	<i>Fritillaria</i> sp.	Liliaceae	Mediterranean, North Africa, Eurasia, Southwest Asia, and North America	5–10		Polyphenols and triterpenes	Antibacterial activity	Hemmati et al. (2019)
<i>Plant part: fruit</i>								
40	<i>Lycium barbarum</i>	Solanaceae	China, Asia and Southeast Europe	5–40	Spherical	Tannias, flavanoids, ascorbic acid and alkaloids	–	Dong et al. (2017)
41	<i>Rubus glaucus</i>	Rosaceae	Latin America (Oaxaca to Bolivia) and Andes	12–50	Spherical	Phenolic groups and flavonoids	Antioxidant efficacy	Kumar et al. (2017)
42	<i>Prunus serotina</i>	Rosaceae	America, Guatemala, and Mexico	20–80	Spherical	Chlorogenic acid, Flavonol, Glycosides, Proanthocyanidin and Catechin	Antioxidant efficacy	Kumar et al. (2016)
43	<i>Sambucus nigra</i> L.	Adoxaceae	Europe, Western Asia, northern Africa	12,267	Spherical	Polyphenols	Antioxidant activity	Moldovan et al. (2016)
44	<i>Sterculia acuminata</i>	Malvaceae	Tropical Africa	~ 10	Spherical	Ascorbic acid, gallic acid, phenolic compounds, pyrogallol, methyl gallate and polyphenolic compounds	Green catalyst	Bogireddy et al. (2016)
45	<i>Aegle marmelos</i>	Rutaceae	India, Sri Lanka, Pakistan, and Bangladesh	22.5	spherical, hexagonal, roughly circular	Phytosterols, flavonoids, alkaloids, triterpenoids and amino acids	Antibacterial activity	Velmurugan et al. (2020)

Table 2 (continued)

Sl no.	Plant	Family	Geographical Origin and distributions	Size of AgNPs	shape	Phytoconstituents responsible for reduction of silver salt	Applications	References
46	<i>Terminalia chebula</i>	Combretaceae	Nepal, India, Sri Lanka, Myanmar, Thailand, and China, and Tropical Africa	30	Spherical	Tannins, gallic acid, chebulic acid, chebulic ellagitannins and gallate esters	Optical sensor	Edison et al. (2016a)
47	<i>Gmelina arborea</i>	Lamiaceae	India, Pakistan, Sri Lanka, Thailand, Myanmar, Vietnam and Southern China	8–32	Spherical	Aldehydes, polysaccharides, polyphenols, ketones and proteins	Catalytic degradation of Methylene Blue	Saha et al. (2017)
48	<i>Crataegus pentagyna</i>	Rosaceae	Eastern Europe, Turkey, Bosnia and Herzegovina	15–60	Spherical	Aromatic amine, aliphatic hydrocarbons, alkaloids, flavonoids, carboxylic acid and phenols	Dye degradation and antibacterial application	Ebrahimzadeh et al. (2020)
<i>Plant Part: Seed</i>								
49	<i>Coffea arabica</i>	Rubiaceae	Southwest Ethiopia, South Sudan and Kenya	20–30	Spherical	Aliphatic alkane, carbohydrate and phenolic compounds	Antibacterial activity	Dhand et al. (2016)
50	<i>Vigna</i> sp.	Fabaceae	India, Pakistan, Myanmar, Thailand, Africa, United States, and Cuba	< 70	Spherical	Amide, carboxylate, carbonyl group, proteins, terpenoids, ketones and aldehydes	–	Mohammadi et al. (2016)
51	<i>Mangifera indica</i>	Anacardiaceae	India and Myanmar	14	Spherical and Hexagonal	Phenolic compounds, gallotannins and tannin	BSA protein binding studies	Sreekanth et al. (2016)
52	<i>Pongamia pinnata</i>	Fabaceae	Southeast Asia, Fiji, Japan and Northern Australia	5–30	Spherical	Pongaflavanol, tunicatamol, galactoside and glybanchalcone	Antibacterial activity	Beg et al. (2017)
53	<i>Areca catechu</i>	Areaceae	Philippines, Bangladesh, Cambodia, China, India, Indonesia, Laos, Malaysia, Maldives, New Guinea, Sri Lanka, Taiwan, Thailand, and Vietnam	18.2	Spherical	Polyphenols	Catalytic and antioxidant activity	Rajan et al. (2015)
54	<i>Artocarpus heterophyllus</i>	Moraceae	India and Southeast Asia	10.78	irregular	Lectin—a single major protein	Antibacterial activity	Jagtap and Bapat (2013)

Table 2 (continued)

Sl no.	Plant	Family	Geographical Origin and distributions	Size of AgNPs	shape	Phytoconstituents responsible for reduction of silver salt	Applications	References
55	<i>Embelia ribes</i>	Primulaceae	India	20–30	Spherical	Alkaloids, quinones, proteins, reducing sugars and saponins	In vitro Antioxidant, Antimicrobial and Cytotoxic activities	Dhayalan et al. (2017)
56	<i>Vigna mungo</i>	Fabaceae	India, Bangladesh, Pakistan, and Myanmar	<100		Aliphatic alkane, phenolic compounds, proteins, steroids, flavonoids, ketones, alcohols, amines, carboxylic acids and polyphenols	Antioxidant and anti-coagulant activity	Varadavenkatesan et al. (2017)
57	<i>Trigonella foenum-graecum</i>	Fabaceae	Mediterranean, western Asia and India	22–32	Spherical	saponins, coumarin, fenugreekine, nicotinic acid, sapogenins, phytic acid, scopoletin, trigonelline, gallic acid, tannins and quinones	Degradation of methylochrome, methylene blue and eosin Y	Vidhu and Philip (2014a)
<i>Plant part: Bark</i>								
58	<i>Ficus benghalensis</i>	Moraceae	India and Pakistan	60	Spherical	Flavonoids, terpenoids and phenols	Antimicrobial activity and Antiproliferative response against	Nayak et al. (2016)
59	<i>Azadirachta indica</i>	Meliaceae	India, Indian subcontinent, Myanmar, Malaysia, Indonesia and Thailand, Fiji, Mauritius, Guyana, Saudi Arabia, Philippines, Egypt, and Australia	60	Spherical	Flavonoids, terpenoids and phenols	Antimicrobial activity and Antiproliferative response against	
60	<i>Butea monosperma</i>	Fabaceae	India, Indo-China, Java, Myanmar, Nepal, Thailand, Papua New Guinea, and Sri Lanka	18–50	Spherical	Carboxylic acid, amide, amino acid, protein, tryptophan, hydroxyl and carboxylate groups	Biomedical applications	Pattanayak et al. (2017)
61	<i>Terminalia cuneata</i>	Combretaceae	India, Myanmar, Sri Lanka	25–50	Spherical	Tannins, saponins, triterpenoids, flavonoids, gallic acid, ellagic acid and phytosterols	Catalytic degradation of Direct Yellow-12 dye	Edison et al. (2016b)

Table 2 (continued)

Sl no.	Plant	Family	Geographical Origin and distributions	Size of AgNPs	shape	Phytoconstituents responsible for reduction of silver salt	Applications	References
62	<i>Ziziphus xylopyrus</i>	Rhamnaceae	India, Nepal and Sri Lanka	60–70	Spherical	Reducing agents	–	Sumi Maria et al. (2015)
63	<i>Artocarpus elasticus</i>	Moraceae	Brunei, Indonesia, Malaysia, Myanmar, Philippines, and Thailand	5.81 ± 3.80–19.74 ± 9.70	Spherical	Flavonoids and phenolic compound	–	Abdullah et al. (2015)
64	<i>Butea monosperma</i>	Fabaceae	India, Indo-China, Java, Myanmar, Nepal, Thailand, Papua New Guinea, and Sri Lanka	~ 35	Spherical	carboxylic acid and protein	Antibacterial activity	Pattanayak et al. (2017)
65	<i>Cinnamon zeylanicum</i>	Lauraceae	India and Sri Lanka	31–40	Spherical	Amine, terpenoids, flavones and polysaccharides	Bactericidal activity	Sathishkumar et al. (2009)
66	<i>Dillenia indica</i>	Dilleniaceae	Bangladesh, India, Myanmar, Nepal, Sri Lanka and America	15–35	Spherical	Alkanes, aromatic rings, carbonyl, carboxylic and hydroxyl groups	Catalytic degradation of 4-nitrophenol and methylene blue dye	Mohanty and Jena (2017)
67	<i>Cochlospermum religiosum</i>	Bixaceae	India and Malaysia	20–35	Spherical	Alkene and proteins	Antimicrobial efficacy	Sasikala et al. (2015)
<i>Plant part: Root</i>								
68	<i>Delphinium denudatum</i>	Ranunculaceae	Tropical Africa, Asia, Europe	< 85	Spherical	Proteins, terpenoids, amine, alcohol, ketone, aldehyde and carboxylic acid	Antibacterial and Mosquito larvicidal activities	Suresh et al. (2014)
69	<i>Chelidonium majus</i>	Papaveraceae	Europe and western Asia	15.42	Spherical	–	Antibacterial activity	Alishah et al. (2016)
70	<i>Helicteres isora</i>	Malvaceae	India, Indo-China, Malaysia, Myanmar, Nepal, Northern Australia, Pakistan, Sri Lanka, and Thailand,	16–95	Spherical	Steroids, terpenoids, alkaloids, carbohydrates and phenolic compounds	Antioxidant and antibacterial activity	Bhakya et al. (2016)
71	<i>Diospyros paniculata</i>	Ebenaceae	India	17	Spherical	Phenolics and proteins	Antimicrobial activities	Rao et al. (2016)
72	<i>Amaranthus dubius</i>	Amaranthaceae	Tropical America, Caribbean region and Mexico	18–21	Spherical	Polyphenol compounds and aldehydes	–	Sigamoney et al. (2016)

Table 2 (continued)

Sl no.	Plant	Family	Geographical Origin and distributions	Size of AgNPs	shape	Phytoconstituents responsible for reduction of silver salt	Applications	References
73	<i>Pelargonium endlicherianum</i>	Geraniaceae	Australia, Asiatic Turkey, Asia Minor, Iraq, Madagascar, Namibia, New Zealand, Tasmania, and Yemen		Spherical	Gallic acid, apocynin and quercetin	Antimicrobial activities	Jagtap and Bapat (2013)
74	<i>Waltheria americana</i>	Malvaceae	United States, Caribbean, and South America	7–24	Rectangular flakes	Alkaloids, anthraquinones, glycosides, phenols, tannin, saponins, flavonoids, and terpenoids	Antibiotic and antimicrobial activity	Deshi et al. (2016)
75	<i>Arachis hypogaea</i>	Fabaceae	Argentina, Andes, Bolivia, Brazil, Paraguay, and Uruguay	30	spherical and irregular shaped	2-(4-Methoxyphenyl)-5-(4-methoxyphenyl) thiophene and methyl 2-(N-Benzylimino)-4-chloro-3,3-dimethylbutanoate, 2H-pyran, 2,5-dithienyltetrahydro, didodecyl phthalate, decanoic acid, bis(2-ethylhexyl) phthalate, tetradecanoic acid, dodecanoic acid and phosphonic acid	Antibacterial and clinical applications	Sankaranarayanan et al. (2017)
76	<i>Bergenia ciliata</i>	Saxifragaceae	Afghanistan, Bhutan, India, and Tibet,	25–73	Spherical	Amino acids, flavonoids, proteins, and pigments	Antibacterial activity	Rashid et al. (2019)
77	<i>Bergenia stracheyi</i>	Saxifragaceae	Afghanistan, India, and Tajikistan	25–73	Spherical	Amino acids, flavonoids, proteins, and pigments	Antibacterial activity	
78	<i>Rumex dantatus</i>	Polygonaceae	Eurasia and North Africa	25–73	Spherical	Amino acids, flavonoids, proteins, and pigments	Antibacterial activity	
89	<i>Rumex hastatus</i>	Polygonaceae	Africa, Bangladesh, India, and Myanmar,	25–73	Spherical	Amino acids, flavonoids, proteins, and pigments	Antibacterial activity	



Table 2 (continued)

Sl no.	Plant	Family	Geographical Origin and distributions	Size of AgNPs	shape	Phytoconstituents responsible for reduction of silver salt	Applications	References
<i>Plant Part: Aqueous extract</i>								
80	<i>Artemisia absinthium</i>	Asteraceae	Europe, Italy, Latvia, Lithuania, and Germany	5–20	Spherical	Phenolic compounds and flavonoids	–	Ali et al. (2016)
81	<i>Isatis tinctoria</i>	Brassicaceae	Germany, France, England, Italy, and North America	10–15	Spherical	Saponins and flavonoids	Photo induced Antileishmanial activity	Ahmad et al. (2016)
82	<i>Cirsium japonicum</i>	Asteraceae	Eurasia and northern Africa, and North America	4–8	Spherical	Saponins, proteins and flavonoids	Photocatalytic and electrocatalytic applications	Khan et al. (2016c)
83	<i>Gracilaria birdiae</i>	Gracilariaceae	Brazil, Ceará State, and Espírito Santo State	20.2–94.9	Spherical	Polysaccharide	Antibacterial activity	de Aragão et al. (2019)
84	<i>Crocus sativus</i> L. (Saffron)	Iridaceae	Greece, India Morocco, and Spain	12–20	Spherical	Alcohols, phenolic compounds, terpenoids, flavonoids, glycosides, phenols, tannins	Antibacterial activity	Bagherzade et al. (2017)
85	<i>Cucumis melo</i> (Melon)	Cucurbitaceae	Sudan, Ethiopia, Eritrea, Somalia, Uganda and Tanzania	13–25	Spherical	Proteins, alcohols and phenolic compounds	Feeding deterrent activity against <i>Musca domestica</i>	Gul et al. (2016)
86	<i>Radix Puerariae</i>	Fabaceae	China	10–35	Spherical and oval shape	Aldehydes, alkynes and amines	Catalytic activity	Balwe et al. (2017)
87	<i>Dunaliella salina</i>	Dunaliellaceae	Asia, Europe	15.26	Spherical	Peptide and polypeptidic	anticancer potential	Singh et al. (2017)
88	<i>Litchi chinensis</i>	Sapindaceae	China, Vietnam, Malaysia, and Southeast Asia	4–8	Spherical	Anthocyanins, epicatechin, flavonols, procyanidin A2 and tannins	Photocatalytic degradation of methylene blue	Khan et al. (2016a)
89	<i>Hypnea musciformis</i>	Cystocloniaceae	Asia, Europe	2–55.8	Spherical	Alkene, aliphatic amines, alcohols, amino acids, carbohydrates, fatty acids, phenols, phenolic compounds, and vitamins	Photocatalytic degradation of methyl orange	Ganapathy Selvam and Sivakumar (2015)

Table 2 (continued)

Sl no.	Plant	Family	Geographical Origin and distributions	Size of AgNPs	shape	Phytoconstituents responsible for reduction of silver salt	Applications	References
<i>Other plant parts</i>								
89	<i>Euphorbia antiqorum</i> (Latex)	Euphorbiaceae	Bangladesh, China, India, Indonesia, Iran, Malaysia, Myanmar, Pakistan, Philippines, Sri Lanka, Thailand, and Vietnam	10–50	Spherical	Euphorbia, euphol, isohellanol 24-methylenecycloartanol and cycloeucaenol	Biomedical perspectives as anticancer agents	Rajkubera et al. (2017)
90	<i>Sapindus emarginatus</i> (Pericarp)	Sapindaceae	India and South Asia	5–20	Spherical	Alcohol and Iavanols	Antibacterial activity	Swarnavalli et al. (2017)
91	<i>Cocos nucifera</i> (Inflorescence)	Arecaceae	Caribbean, India, New Zealand, Pacific, Tropical Asia, West Africa,	22	Spherical	Tannin, alkaloids, carbohydrates, terpenoids, saponins, phenolic compounds and reducing sugar	Antibacterial activity	Mariselvam et al. (2014)
92	<i>Punica granatum</i> (Peel)	Lythraceae	Afghanistan, Himalayas Region, India, Iran, and Mediterranean region	30	Spherical	Hydrolysable tannins, chebulic, ellagitannins, gallate esters, gallic acid, and chebulic acid	Catalytic activity on reduction of Methylene blue	Edison and Sethuraman (2012)
93	<i>Musa sp.</i> (Banana) (Peel)	Musaceae	Asia, Africa, Latin-America and Pacific Islands	23.7	Spherical	Pectin, cellulose and hemicelluloses	Antimicrobial activity	Ibrahim (2015)
94	<i>Parkia speciosa</i> Hassk (Pod)	Fabaceae	Brunei, Indonesia, Malaysia, Thailand and Philippines	20–50	Spherical	Phenolic compounds, amide II, amine	Antibacterial activity	Fatimah (2016)
95	<i>Allium stipitatum</i> (shallot) (Gum)	Amaryllidaceae	Afghanistan, Iran, Iraq, Kazakhstan, Kyrgyzstan, Pakistan, Tajikistan, Turkey, Turkmenistan, and Uzbekistan	8–20	Spherical	Quercetin, isorhamnetin and glucose	–	Taheri et al. (2015)
96	<i>Colocasia esculenta</i> (L.) (Stem)	Araceae	Southeast Asia, Japan and Polynestians	13–50	Spherical	Organic compounds containing –OH, –N–H and –C–N groups	Antiarivricidal activity	Mondal et al. (2019)
97	<i>Acorus calamus</i> (Rhizome)	Acoraceae	Asia and Siberia	59.02 ± 1.3	Spherical	Amines, amides, phenols, alcohols, aldehydes, flavonoids, terpenoids	Antimicrobial activity	Nayak et al. (2015)
98	<i>Cucurbita maxima</i> (Petals)	Cucurbitaceae	South America	76.10 ± 0.8	Spherical			

Table 2 (continued)

Sl no.	Plant	Family	Geographical Origin and distributions	Size of AgNPs	shape	Phytoconstituents responsible for reduction of silver salt	Applications	References
99	<i>Nepeta leucophylla</i> (Stem)	Lamiaceae	Iran, Mediterranean region, Himalayan region	15–25	Spherical	Alcoholic compounds, amide, amino acid, proteins, phenols, and phenolic compounds	Photocatalytic degradation of Methylene blue	Singh and Dhaliwal (2020)
100	<i>Achillea millefolium</i> (Peach kernel shell)	Asteraceae	Asia, Europe, Eurasia, and North America	20	Spherical	Cellulose, hemicellulose, and lignin	Catalytic degradation of 4-nitrophenol, Methyl Orange and Methylene Blue	Khodadadi et al. (2017)
101	<i>Clitoria ternatea</i> (Pods)	Fabaceae	Asia, Africa, America, and Pacific Islands	62.51	Spherical	Phenol and phenolic compounds	Degradation methylene blue	Varadavenkatesan et al. (2016)
102	<i>Alpinia officinarum</i> (Rhizome)	Zingiberaceae	China, Europe and Southeast Asia	20–80	Hexagonal	Amides, polypeptides and carbonyl groups	Photocatalytic degradation of malachite green and methylene blue	Li et al. (2020)
103	<i>Solanum tuberosum</i> (Stem)	Solanaceae	Asia, North America, South America, Spain, and UK	10–12	Spherical	Amide, amine, ascorbic acid, amino acids, protein, and thiamine	Photocatalytic degradation of methyl orange	Roy et al. (2015)

60 nm. Biomolecules identified as being involved in bio-reduction included alcohol, aldehydes, alkanes, amines, amide II, amino acids, carbohydrates, carboxylic acid, carbonyl compounds, cellulose, ester, hemicelluloses, hydroxyl group, lycopene, pectin, phenolic compounds, polyphenol, proteins, vitamins (C, K, E), and  $\beta$ -carotene (Ahmed et al. 2016) (Table 2).

The biosynthesized AgNP showed significant antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Vibrio cholera*, (Swarnavalli et al. 2017). It also showed the antibacterial activity towards human pathogens and blood-sucking parasites such as *Aedes aegypti* and *Culex quinquefasciatus* (Rajkuberan et al. 2017), anticancer agents (Rajkuberan et al. 2017), antimicrobial activity against bacterial pathogens of humans such as *Salmonella paratyphi*, *Bacillus subtilis*, *Klebsiella pneumonia*, and *Pseudomonas aeruginosa* (Mariselvam et al. 2014).

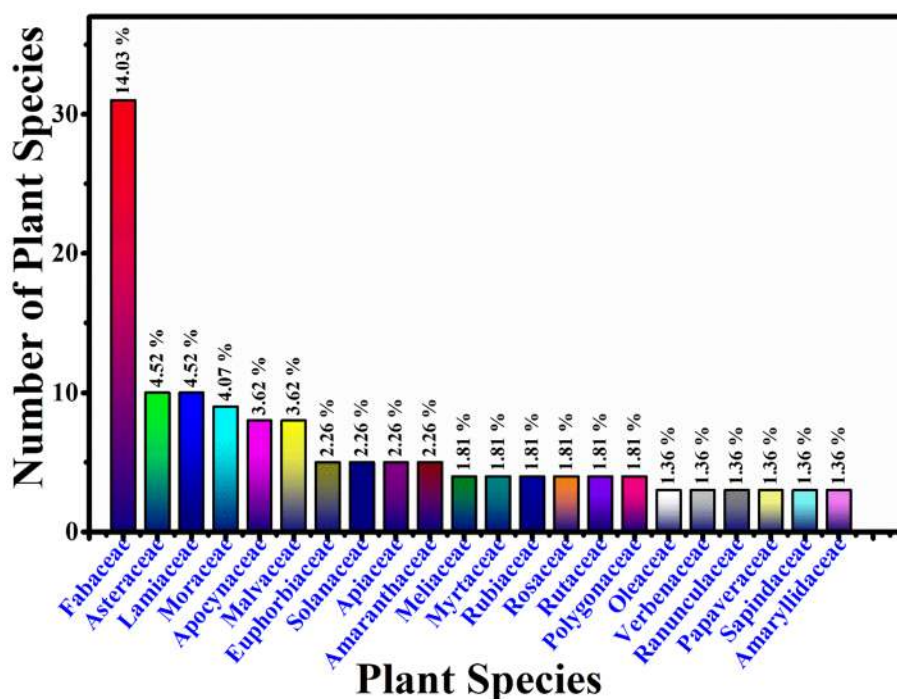
### Statistics of plant-mediated AgNP synthesis

Plants are widely distributed throughout the world including both the hydrosphere and lithosphere. Christenhusz and Byng (2016) identified 452 vascular plant families which contain about 308,312 plant species (Angiosperms: 295,383, Gymnosperms: 1079, Lycopods: 1290 and Ferns: 10,560), where the number of the plant species in the largest families (including Asteraceae, Fabaceae, and Orchidaceae) is increasing daily. The global distribution of these newly

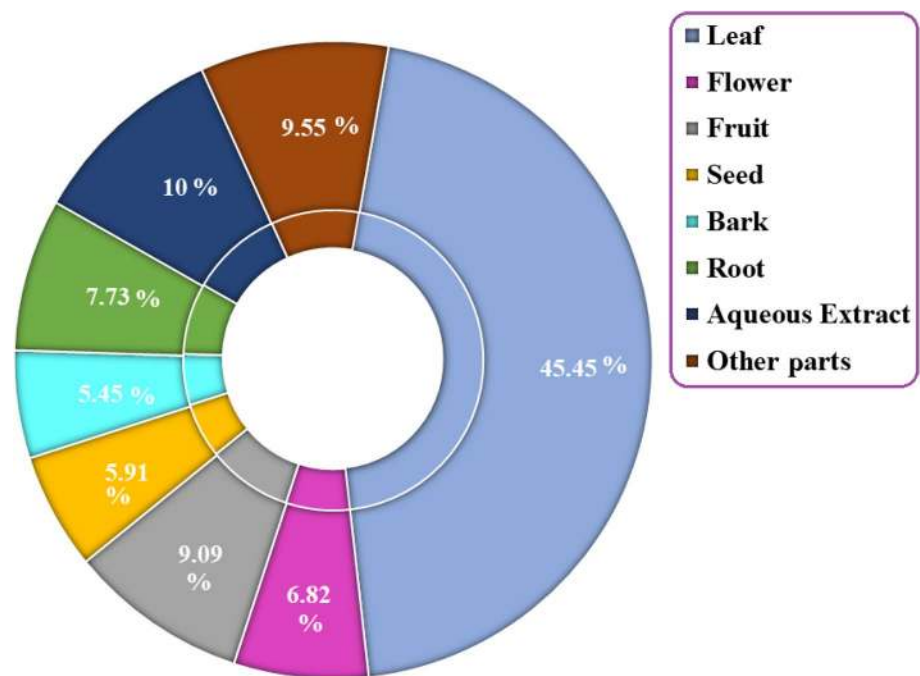
discovered species is clustered mainly in tropical countries like Australia, Brazil, China, and New Guinea (Christenhusz and Byng 2016). Unfortunately, Joppa et al. (2011) reported that many biodiversity hotspots for these newly discovered plant species were also the most vulnerable. Moreover, the global distribution and economic value of these plant families are quite different. It has been estimated that 17 plant families contribute ~80% of plant foods. Orchidaceae as the largest vascular plant family (~736 genera and 28,000 plant species) followed by Asteraceae (~1623 genera and 24,700 plant species) and Fabaceae (~751 genera and 19,500 plant species). Though, Orchidaceae is the largest plant family, in terms of AgNP biosynthesis, the highest number of plant species (31) used was in the Fabaceae family followed by Asteraceae (10) and Lamiaceae (10). Overall our literature review suggests that 221 plant species belonging to 85 families (18.8% of total plant families found worldwide) were used for the plant mediated AgNP synthesis, might be due to their abundance and presence of the flavonoid compounds (Fig. 4). This might be due to the global distribution, abundance, and most importantly the presence of high quantity of phytochemicals, which reduce  $Ag^+$  to  $Ag^0$  and stabilize AgNP in the colloidal medium.

Among the 221 plant species, there are several plant parts which were successfully used in plant-mediated biosynthesis of AgNP. More than 45% of the plant-mediated biosynthesis of AgNP was conducted using leaves alone. However, some other plant parts including aqueous extract (10%), fruit (9.09%), and root (7.73%) were also found to significantly contribute in several studies (Fig. 5). Leaves are the most

**Fig. 4** Distribution of the number of plant species within each family used for AgNP biosynthesis



**Fig. 5** Distribution of plant parts used for biosynthesis of AgNP



active part of the plant because they generally contain greater numbers and quantities of phytochemicals including flavonoids, phenolic compounds, and reducing sugars (Altemimi et al. 2017).

### Factors affecting plant-mediated biosynthesis of AgNP

The main challenges in AgNP biosynthesis are the control of crystallinity, shape, size, and dispersity, where the main factors directly influencing these parameters are discussed below.

#### Effect of precursor concentration (AgNO<sub>3</sub>)

In majority of the plant-mediated biosynthesis of AgNP, AgNO<sub>3</sub> has been used as a precursor, and its concentration exhibited a significant impact on the particle size of the resultant NPs. For example, Shaikh et al. (2020) found maximum yield of AgNP with minimum size was achieved at 1.25 mM AgNO<sub>3</sub>. Zhang et al. (2013) reported optimum biosynthesis of AgNP at a AgNO<sub>3</sub> concentration of 1.0 mM, showing that at lower AgNO<sub>3</sub> concentrations a wider Surface Plasmon Resonance (SPR) band was formed and upon increasing the AgNO<sub>3</sub> concentrations, the product peak become narrower indicating decreasing particle size till the optimum AgNO<sub>3</sub> concentration. A similar result was reported by Muthu and Priya (2017) using *Cassia auriculata* flower extract for spherical and triangular AgNP ranging

from 10 to 35 nm with a 1.0 mM AgNO<sub>3</sub> precursor solution also being optimal. Moreover, the investigations of Zhang et al. (2013) also suggested that by increasing the AgNO<sub>3</sub> concentration better AgNP (position and shape) were formed and the SPR peak of AgNP trended toward red-shift, indicating larger particle size.

#### Effect of precursor and phytoextractant ratio (V/V)

Several earlier works have suggested that the ratio of the precursor to phytoextractant solution on a volume basis could also affect AgNP biosynthesis. This seems reasonable because the biomolecules within the phytoextractants are the key components responsible for both reduction of the Ag salt as well as stabilization of the produced AgNP. It is well established that increasing the phytoextractant dose can not only enhance AgNP yield but also increase the size increase and alter shape up to an optimum ratio (Vijayaraghavan and Ashokkumar 2017).

#### Effect of reaction time

The size, shape, and properties of biosynthesized AgNP are significantly influenced by the reaction (or incubation) time for specific plant extractant. Though interspecific variation is evident, incubation time for a specific plant part normally shows an optimum value for effective bio-reduction. Vijayaraghavan and Ashokkumar (2017) reported that the yield and size of AgNP were both positively correlated with incubation time up to an optimum duration. While



Shaikh et al. (2020) reported complete bio-reduction within 20 min using Flavans, Flavanonol, and Flavonol present in the *Shorea robusta* leaf extract, Muthu and Priya (2017) reported 99% bio-reduction of silver ions within 23 h using a *Cassia auriculata* flower extract where the main phytochemicals were carbohydrates, glycosides, and polyphenolic compounds. In both the cases, deviation from the optimum incubation period led to decreased yield and size variation. Li et al. (2020) reported the size of AgNP increased with increasing incubation time (with 1 mM AgNO<sub>3</sub>) from 10 ± 2 nm at 5 h, to 25 ± 3 nm at 9 h and 40 ± 5 nm at 13 h, where the increasing size might be due to the agglomeration of colloidal AgNP.

### Effect of pH

The pH of the solution medium is an important parameter that influences both the rate, shape, and size of plant-mediated biosynthesised AgNP. For example, Sathishkumar et al. (2009) found that when using a *Cinnamom zeylanicum* bark extract for bio-reduction over a wide pH range (1–11) large ellipsoidal AgNP formed at acidic pH (pH < 7), whereas smaller spherical AgNP formed at alkaline pH (pH > 7). This was attributed to the presence of a larger numbers of functional groups at higher pH, leading to nucleation at higher pH while at lower pH aggregation was favoured over nucleation. Nucleation increases with increasing solution pH indicating the formation of Ag<sup>0</sup> from Ag<sup>+</sup> due to bio-reduction. At the same time, the solution pH also influences the rate of bio-reduction by influencing the activity of the phytochemicals (Veerasamy et al. 2011). For example, it was suggested that better AgNP formation occurred under basic conditions with 0.1–2.0 mM AgNO<sub>3</sub> and efficiency decreased with decreasing pH of the reaction medium (Yazdi et al. 2019). Furthermore, AgNP synthesized in acidic medium (pH 4) yielded larger particle size, whereas highly dispersed and small-sized AgNPs were observed at pH 8 (Khandan Nasab et al. 2020). This phenomenon revealed enhancement of nucleation \*centres with increases in solution pH. The result also suggested that at lower pH (< 7) large number of functional groups of phytochemicals bind with AgNP increasing the chance of agglomeration, resulting in larger sized AgNP (Veerasamy et al. 2011). However, Ondari Nyakundi and Padmanabhan (2015) suggested that as a soft metal, Ag binds with soft ligands like amino and sulphhydryl groups, where these positive charged groups then reduce Ag<sup>+</sup> to Ag<sup>0</sup> at low pH. The bio-reduction was thus mainly through ionic binding and phytochemicals which was favoured at low pH due to the presence of positively charged functional groups. The study also suggested the involvement of some hard ligand-like carboxylic groups which became protonated at low pH and also helped to in the formation of AgNP. It is also evidential from examination of the literature that the

zeta potential of acidic colloidal AgNP is generally lower than that of alkaline colloidal AgNP. Rapid bio-reduction with highly dispersed AgNP and negative zeta potential was observed at higher pH (Akhtar et al. 2013).

### Effect of temperature

Temperature is yet another key factor that significantly affects the shape and size of biosynthesised AgNP with a positive correlation between temperature and AgNP yield up to an optimum temperature, normally around 27 °C for 0.5–3.0 mM AgNO<sub>3</sub> (Akhtar et al. 2013; Kumar et al. 2021a). Verma and Mehata (2016), when investigated AgNP biosynthesis using a *Azadirachta indica* leaf extract at different temperatures ranging from 10 to 50 °C, found that AgNP size decreased with increasing temperature upto 27 °C, which might be due to an increased bio-reduction rate with increasing temperature. Most studies agree that smaller and more uniformly distributed AgNP are produced at room temperatures (around 27 °C). Increasing temperature (beyond room temperature; 27 °C) resulted in larger sized AgNP, potentially due to the denaturation of phytochemicals and increasing agglomeration of AgNP (Ahmed et al. 2016). However, as with many studies, this article failed to identify the specific phytochemicals responsible for changes in the size of AgNP with increasing temperature.

### Mechanism of the biosynthesis process

The biochemical composition of plant extracts may vary considerably with the plant species or even within the same plant when different plant parts are extracted. While a wide range of phytochemicals, commonly implicated in either bio-reduction and/or stabilization are known and listed briefly in the Sect. 4.1, the exact mechanistic role this chemical play is not always clear. None of these articles clearly indicated or identified the specific active biomolecules responsible for the bio-reduction process of Ag<sup>+</sup> to Ag<sup>0</sup>. Jha and Prasad (2010) reported that some metabolites can trigger the bio-reduction process via transformation of silver ions to AgNP due to redox activity of dehydroascorbic acid/ascorbic acid or amenthi/hinoki flavones or some other metabolite. Some researchers reported that the carbonyl and hydroxyl groups of flavonoids, terpenoids, carbohydrates, and phenolic compounds as reducing agents, played a key role in the reduction of Ag<sup>+</sup> ions to metallic Ag<sup>0</sup> nanoparticles (Ajitha et al. 2015). Proteins, peptides and the carbonyl groups of amino acid have all been shown to have strong affinity to bind with metallic Ag<sup>0</sup> to form a coating layer around the AgNP that assists colloidal stabilization in solution (Fig. 6) (Karthik et al. 2017). These authors also claimed that the quantity

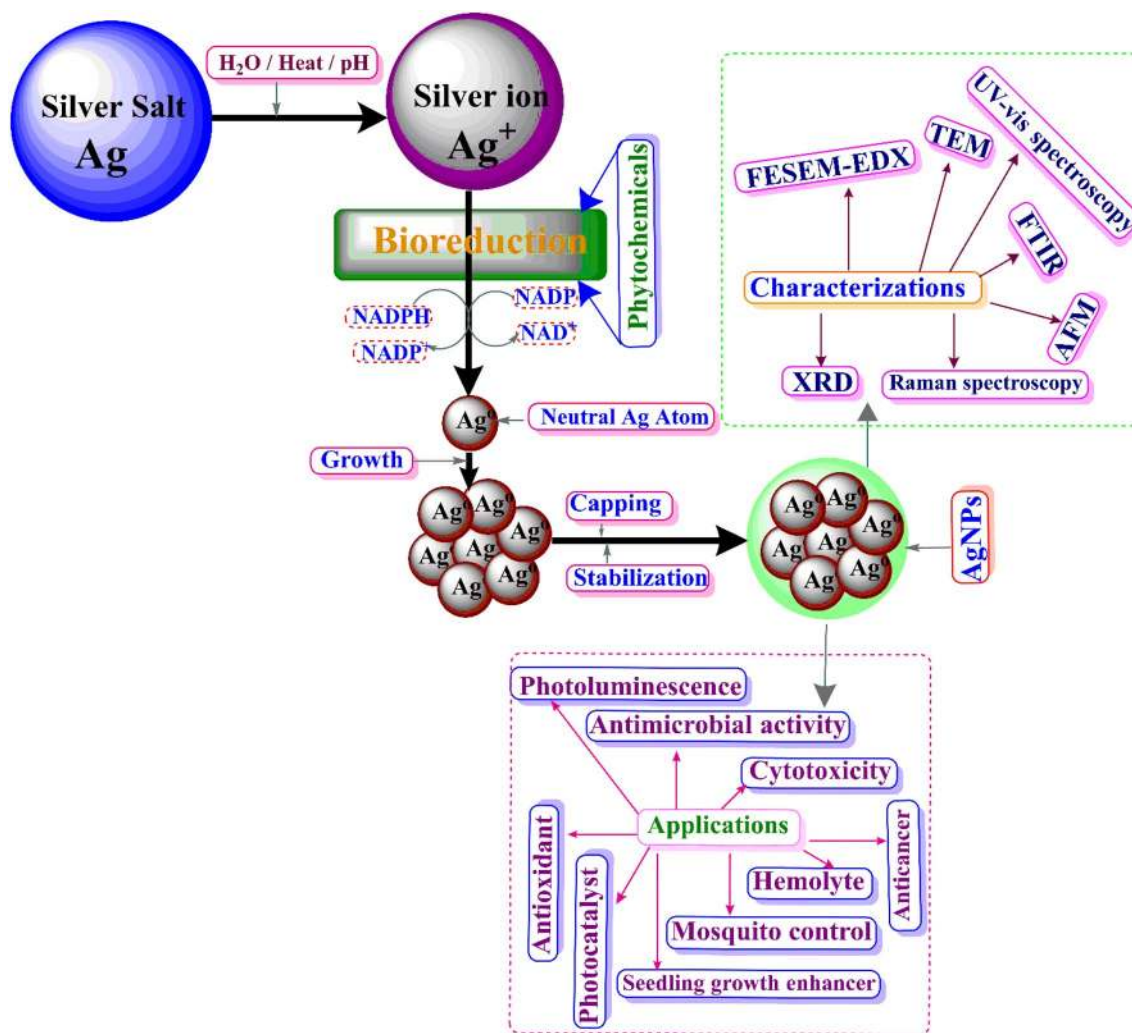


Fig. 6 Probable bio-reduction mechanism of silver salts ( $\text{AgNO}_3$ ) leading to the formation of AgNP

of leaf extract used in the experiment has an important role in regulating the size of the nanoparticles and inhibited the oxidation of the produced AgNP (Ajitha et al. 2015).

Extracts of *Desmodium trifolium* were successfully used to biosynthesise AgNP via reduction of silver ions where the presence of ascorbic acid in the extract played a significant role (Ahmad et al. 2011). Previously, Kesharwani et al. (2009) had also reported a bio-reduction mechanism when using *Datura metel* to produce stable AgNP havin a particle size between 16 and 40 nm. However, rather than ascorbic acid, this study had shown that the *D. metel* leaf extract contained a wide variety of enzymes, amino acids, alkaloids, proteins, and polysaccharides these were responsible for bio-reduction.

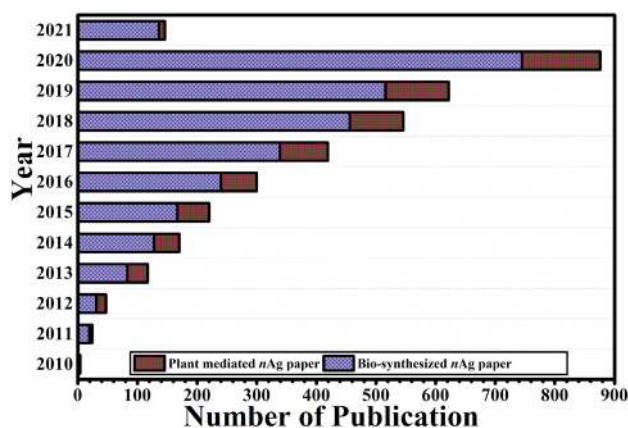
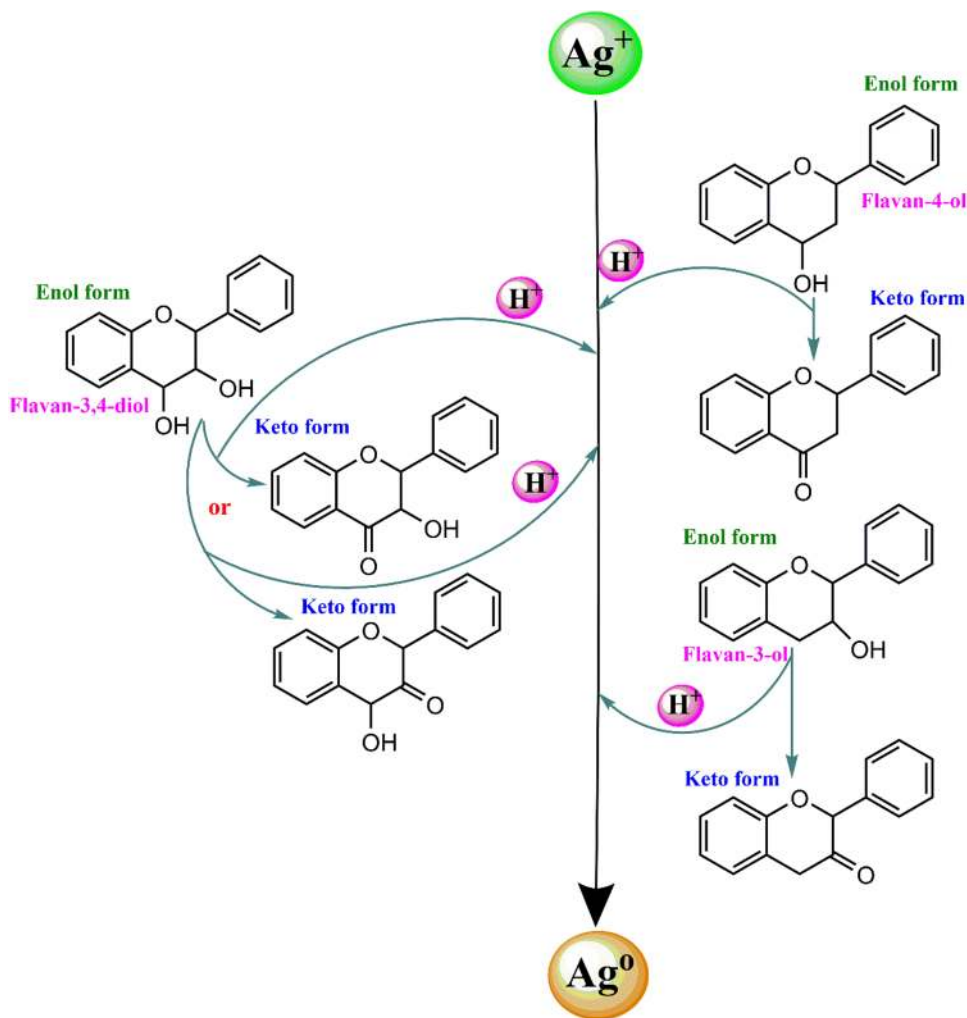
However, in all of these studies, while a mechanism was prosed involving simultaneous reducing and/or capping biomolecules, none of the studies could unambiguously identify the specific phytochemicas involved in each role.

### Role of phytochemicals in the biosynthesis of AgNP

The bio-inspired routes for AgNP synthesis are attractive because they not only produce nontoxic and inexpensive nanoparticle in a one-step synthesis but, also depending on the interaction between AgNP and the phytochemical capping agents present, the AgNPs so produced are often produced with controllable size and morphology. Therefore, it is important to identify and understand the specific interaction(s) between the phytochemicals present in the extracts and the silver salts in solution which react to produce AgNP. While a vast myriad of phytochemicals including amides, flavonoids and peptides for example (details listed in Sect. 2) have been identified as being involved in AgNP biosythesis, the specific interaction of all phytochemicals is yet to be conclusively established.

Trouillas et al. (2006) employed a density-functional theory (DFT) method to investigate the interactions during bio-reduction of a silver salt which showed that the O–H bond dissociation energies of the hydroxyl group of the catechol moiety of flavonoids was less than that of other –OH groups present in most phytochemicals. Similar result was reported by Bose and Chatterjee (2016) for the biosynthesis of AgNP using a *Psidium guajava* leaf extract. These results indicated that the carbonyl and hydroxyl groups of flavonoids play a significant role in the reduction of Ag ions through the metal chelation with the catechol moiety of flavonoids, where the electrostatic interaction and charge transfer between the OH group of flavonoids and Ag<sup>+</sup> ion are responsible for biochemical interaction leading to bio-reduction. Shaikh et al. (2020) also reported three specific flavonoids (Flavan-3-ol, Flavan-3,4-diol, Flavan-4-ol) acted as reducing and/or capping agent for the reduction Ag<sup>+</sup> to Ag<sup>0</sup> (Fig. 7), during the biosynthesis of AgNP using a *Shorea robusta* leaf extract. This bio-reduction mechanism might be due to the tautomeric transformations of flavonoids to flavone and/or flavonol (from enol to keto), where the reactive hydrogen was

**Fig. 7** Role of phytochemicals in the biosynthesis of AgNP



**Fig. 8** Year wise number of publications of bio-synthesized AgNP and plant mediated bio-synthesized AgNP

released by some hydroxyl (–OH) containing groups including Flavans, flavanonol and flavonol (Shaikh et al. 2020).

The review was conducted to identify the trends emerging in nanoparticle research using an open-access database

search engine (Dimensions), and VOSviewer software (version 1.6.16) for data analysis to acquire a holistic view of the current trends in AgNP synthesis. Research articles published during 2008–2021 (till 31st January 2021) were considered in this statistical analysis (Fig. 8a). Among the 115 million publications searched, 2877 research articles were found for the search term ‘bio-synthesized silver nanoparticles’, where only 629 research articles were also related to ‘plant-mediated bio-synthesized silver nanoparticles’. For this search, we used two keywords, “bio-synthesized silver nanoparticles” and “plant-mediated silver nanoparticle synthesis” under the “research article” section only.

The refined search criteria yielded 2877 research articles published in the 65 most common journals in the fields of science, technology, and engineering, where ~50% of articles were published in the 14 major analytical and environmental chemistry journals (Fig. S1). Most studies (> 65%) were published between 2013 and 2020, indicating a growing interest in the advancement of analytical techniques in the field of nanotechnology. It was observed from the analysis that though initially, these studies were only focused on the AgNP biosynthesis, from 2015 onwards, focus shifted to environmental pollution control domain from only biosynthesis of AgNP (Fig. 8). Moreover, an analysis on the research articles based on ‘plant-mediated silver nanoparticle synthesis’ in the past decade (2010–2020) showed that the number of publications in the 1st and 2nd quarter (2010–2016) was gradual. However, a significant increment was observed at the end of the 2nd quarter (after 2013–2014).

The first research article on plant-mediated bio-synthesized silver nanoparticles was published by Rajasekharreddy et al. (2010), which reported biosynthesis of both AgNP and gold nanoparticle (*n*Au) using the leaf extracts of *Calotropis gigantea* L. (*Calotropis*), *Carica papaya* L. (*Papaya*), *Citrus aurantium* L. (*Bitter orange*), *Datura metel* L. (*Datura*), *Jatropha curcas* L. (*Barbados nut*), *Solanum melongena* L. (*Eggplant*), and *Tridax procumbens* L. (*Coat buttons*) using the sunlight exposure method. Moreover, the network map of 2792 authors with a citation weightage, represented in Fig. 9a, showed that the research group of Prof. Giovanni Benelli of University of Pisa, Italy, Prof. Kadarkarai Murugan of Bharathiar University, India, Dr. Chellasamy Panneerselvam of University of Tabuk, Saudi Arabia and Prof. Zia Ul Haq Khan of University of Engineering and Technology Peshawar, Pakistan were the major contributor in the field of ‘plant-mediated bio-synthesized silver nanoparticles’. It was also seen from Fig. 9b that Prof. Giovanni Benelli had the highest number of articles (10) of plant-mediated AgNP synthesis, owing to the highest total link strength (344), followed by Prof. Kadarkarai Murugan (number of articles 9; total link strength 229). The highest citations and the highest

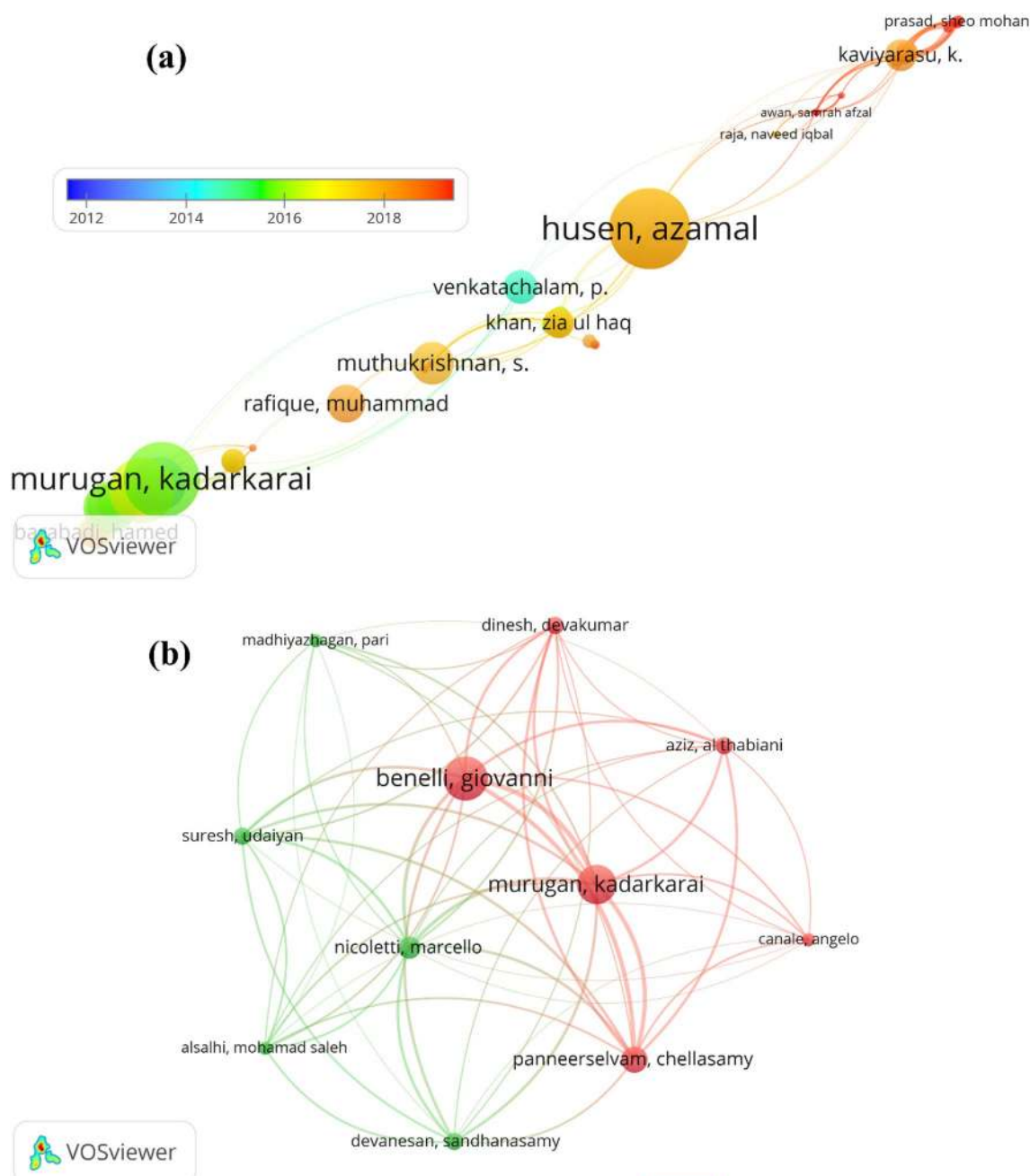
total link strength indicated the novelty, reliability of the phenomenon, and acceptability in the scientific community which swiftly triggered the progress and advancement of the field of nanoparticle research, more precisely in the field of plant-mediated AgNP research. Some of the researchers (Prof. Zia Ul Haq Khan; Number of articles 7 of plant-mediated AgNP synthesis; Total link strength 90) have a high number of papers but lower total link strength indicating the research articles are not cited in this field. However, Dr. Chellasamy Panneerselvam has only 6 articles related to plant-mediated AgNP synthesis owing total link strength of 200 indicating the higher citation rate and acceptability of his articles.

From the 629 published research articles on AgNP across 67 countries, it was found that the research was predominantly conducted in Asia compared to Europe, America, and Africa. The result showed most of the research articles were published by India (258), followed thereafter by China (58), and least among among European countries, where Italy (12) had the highest number of published on AgNP papers, and Nigeria (11) had the highest AgNP papers in Africa (Fig. S2), while Australia and the USA contributed 5 and 30 papers of AgNP, respectively. The incremental sequence in the greatest number of published AgNP papers (> 10) was India > China > Pakistan > Saudi Arabia > Iran > USA > South Korea > Malaysia > Egypt > South Africa > Mexico > Italy > Nigeria. Nineteen per cent of the countries in the database, including India, China, Pakistan, Iran, Malaysia, Mexico, Egypt, and Nigeria, had ten or more research articles contributing > 95% plant-mediated AgNP articles (Fig. 10).

Similarly, the data were also examined for citation analysis considering a minimum threshold limit of three published research articles from a country, which showed India to be the largest contributor with 258 articles, followed by China with 58 (Fig. 10). Moreover, India has the highest total link strength (1218), indicating Indian studies were cited by most other countries as a reliable source of information in this field. The spread of research mainly clustered in Asia revealed strong bias at the continental scale, having > 60% of the total citation between 2008 and 2021. In North America, South America, Europe, and Africa, the cluster centred in the USA, Mexico, Italy, and South Africa, respectively.

This bibliometric study showed good knowledge of plant-mediated AgNP synthesis in Asian countries like India, China Pakistan and some European countries. Among these, India is one of the pioneers and most experienced country in the field of plant-mediated AgNP research. The enhanced research in this country might be due to the following: (1) availability of numerous plants due to geographical location and wide ranges of climatic conditions, (2) significant development in the field of nanoscience and nanotechnology, and (3) directionless research,





**Fig. 9** **a** Citation of authors for the publication of plant-mediated AgNP papers and **b** Bibliometric network of authors for plant-mediated biosynthesized AgNP

which generally followed the previous one (changing the plants and applications) to publish a paper without realizing the significance and feasibility for real application. However, this study also showed that AgNP research was deficient or even absent in a considerable part of the globe, mostly in poor and developing countries of South America and Africa. The major reason behind this could be less nanotechnology development and lower funding in these regions resulting in the observed publication gap.

Moreover, some research articles were focused on AgNP biosynthesis and detailed sampling techniques and preservations in the 2nd and 3rd quarters of the decade (Ahmed et al. 2016). Among the different sampling technique, non-repeated grab sampling was a commonly adopted technique for the collection of most of the plant samples (Mohammadi et al. 2016). However, some repeated grab sampling and composite sampling were also observed in some of the studies (Raja et al. 2017).



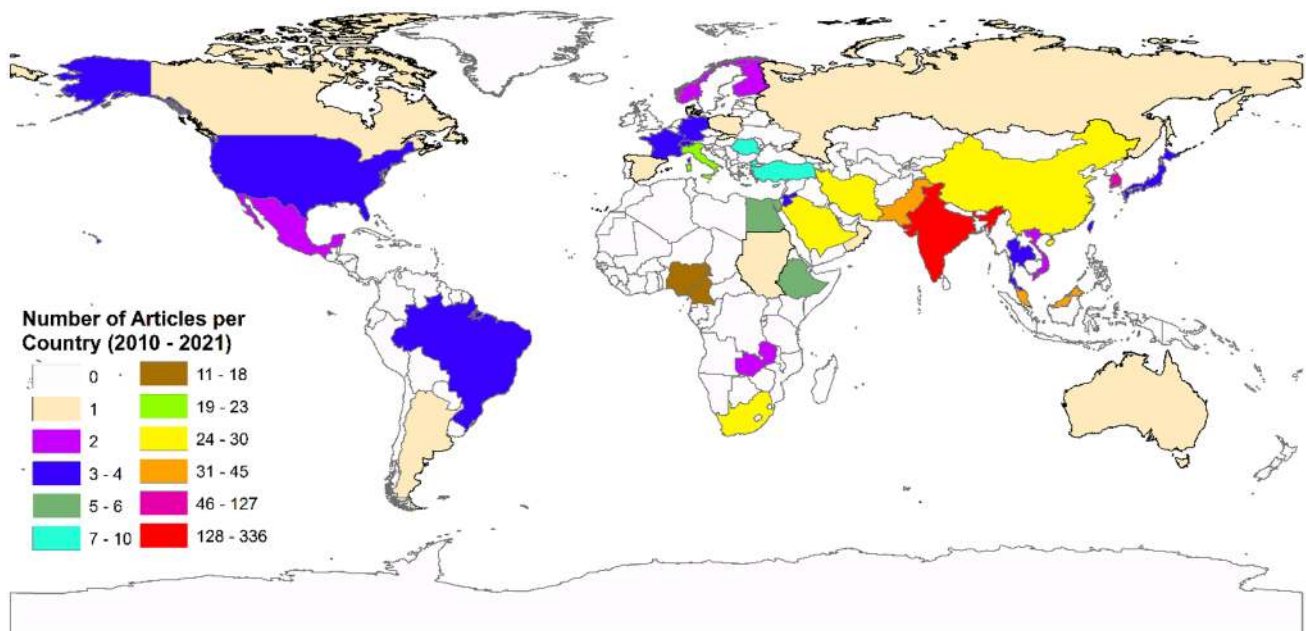


Fig. 10 Country-wise publication of plant-mediated AgNP papers

## Limitations of plant-mediated AgNP biosynthesis

While most of the review articles on plant-mediated biosynthesis of AgNPs focussed simply on the success of the process, very few articles addressed the potential challenges and shortcomings of this approach. Therefore, in this review, an attempt has been made to discuss the major factors limiting the yield, and operational scalability of AgNP production, like concentration of the plant extracts, the source/type of phytochemicals, the stoichiometric ratios of the reagents and differing optimal experimental conditions. These limitations have been broadly categorized below under the following sections: technical limitations, engineering and economical.

### Variability in synthesis parameters

The precursor concentration, the stoichiometric ratio of precursor to plant extract, reaction incubation time, reaction temperature and pH are all key factors that can affect the morphological characteristics (size and shape) of AgNP as well as yield. Khalil et al. (2014) reported the formation of smaller particles (8–15 nm) was favoured by a higher ratio of the bio-extract: precursor (5 mL in a 10 mL Ag<sup>+</sup> solution), and particle size increased (up to 30 ± 6 nm) with a decreasing ratio of the bio-extract: precursor solution (1 mL in a 10 mL Ag<sup>+</sup> solution), whereas Johnson and Prabu (2015) reported increasing phytochemical concentration increased AgNP size. However, Shaikh et al. (2020)

reported increasing AgNP size with decreasing precursor (AgNO<sub>3</sub>) concentration due to the occurrence of a narrow SPR band. Suresh et al. (2014) had suggested that during the biosynthesis of AgNP using *Delphinium denudatum* root extracts the reaction incubation time affected the bio-reduction. In this work, while an initially sharp UV absorption spectra was observed, increasing the incubation time caused the UV absorption spectra to become wider indicating larger AgNP. Most studies support a positive correlation between AgNP yield and temperature increase (up to an optimum temperature, generally 300 K). Karthik et al. (2017) found that pH was a most important factor influencing variation in size and shape when using a *Camellia japonica* leaf extract. Moreover, the literature of Karthik et al. (2017) also suggests that not only do different plant extracts have different pH, but also that even the extracts of different plant parts from the same plant may have different pH, which also affects morphology, shape, and size.

The main limitation of all of these different studies was together the observations tended to be contradictory and no similar trends were observed between disparate studies. Generally, this occurs because to a large extent the results vary significantly depending both on the different plant extracts used and thus the differences in the presence of various phytochemicals. Another limitation is purity and yield of the final compounds because separation of AgNP from the colloidal phase is challenging. One of the most significant concerns of plant-mediated AgNP synthesis is obtaining high yield. Increasing yield by changing the biosynthesis parameter(s) often leads to the higher-sized AgNP. Moreover, specific

phytochemicals and background chemistry control the size and morphology of AgNP shape and size. Thus, identification of the specific phytochemicals involved in AgNP biosynthesis is required to understand the mechanism. However, the lack of good extract characterization in terms of the common phytochemicals present is a major issue when trying to understand relationships between yield, morphology, and plant extract chemicals. Though research has claimed that biosynthesis of AgNP is more environment friendly than either chemical or physical approaches, failed to be prove it due to a significant lack of clear scientific evidence (Mittal et al. 2013). The assertion of environmental friendliness is based mainly on consideration of how much toxic chemicals are substituted by non-toxic alternatives during biosynthesis or how much energy is saved, and the perceived ecological and economic impacts (Kumar et al. 2020; Kumar and Bhattacharya 2021).

### Engineering limitations

To maintain quality, in terms of uniformity of size and surface composition, which are both essential for assuring enhanced performance, commercial AgNP is synthesized under rigorous standards. However, most biosynthesized AgNP particle's shape and size cannot be well controlled and this often affects physicochemical properties like electrical conductivity. Furthermore, most studies indicate that biosynthesized AgNP are polydispersed due to the diversity in the phytochemicals originating from different plant materials. Nouri et al. (2020) reported that the presence of alkene, amide, alcohol, alkaloids, flavonoids, phenol, proteins, saccharides, steroids, saponins, sugar, and tannins in *Mentha aquatica* leaf extract induced the biosynthesis of spherical AgNP. However, Khoshnamvand et al. (2019) reported formation of spherical, ellipsoidal, and hexagonal AgNP using an *Allium ampeloprasum* leaf extract, while cubic (size ~ 68.06 nm), flower shaped (size 25 nm), hexagonal (size 20–80 nm), rectangular flakes (size 7–24 nm), and truncated triangular shaped (size 10–30 nm) AgNP were biosynthesized when using *Andrographis echioides* leaf extract (Elangovan et al. 2015), *Chrysophyllum oliviforme* leaf extract (Anju Varghese et al. 2015), *Alpinia officinarum* Rhizome extract (Li et al. 2020), *Waltheria americana* Root extract (Deshi et al. 2016), and *Platanus orientalis* leaf extract (Al-Thabaiti et al. 2015), respectively.

Some researchers used additional chemicals including cetyltrimethylammonium bromide (CTAB), amphiphilic molecules, surfactants, anionic, cationic and Gemini for shape-controlled AgNP biosynthesis (Al-Thabaiti et al. 2015). However, the particular factor or interaction governing the shapes of the AgNP is still unknown and no scientific evidence or justification was provided to indicate how the

parameters or the phytochemicals determined the shape of biosynthesized AgNP.

### Economic limitations

Currently the main economical uncertainty associated with the biosynthesis approach is that most studies till date have been performed at the laboratory scale and there is little information on the feasibility of the process on an industrial scale. The large amounts of plant extract required to scale up production, may be an important limiting factor for large scale production. In addition to this, continuous supply of plant materials for extraction of active biomolecules is also uncertain. Johnson and Prabu (2015) reported AgNP synthesis using *Commelina benghalensis*, *Cycas circinalis*, *Ficus amplissima*, and *Lippia nodiflora*, where the leaf extracts were concentrated by centrifugation at 10,000 rpm for 30 min, exhibiting significant bio-reduction within 15 min with larger spherical sized AgNP formed with a small enhancement in yield. Moreover, to produce large quantities of phytoextractant a significant amount of biomass waste is generated from the process.

Other important factors to consider include maintaining of high yield and stability, where the chemistry and/or mechanism associated with these factors is not well understood. In addition, the metrics used in calculating AgNP yield is poorly characterized between different studies (i.e., mass intensity, effective mass yield, and the stoichiometric factor). Likewise, economic feasibility analysis of the biosynthesis of AgNP compared to traditional synthetic methods has not been considered.

However, the identification and separation of phytochemical particularly involved in the bio-reduction is quite impossible but the biosynthesis of continuous, long term industrial scale AgNP may be maintained by using some common native plant species. Plant species having no economic value (i.e., *Eichhornia* sp., *Parthenium hysterophorus*) which creates adverse effects due to overgrowth may be a good alternative.

### Conclusions

In recent years, the bioinspired synthesis of AgNP has attracted significant attention, and plant-mediated AgNP synthesis has been the most sought method due to the wide availability of plant sources, environment friendliness of the procedure (without the use of toxic chemicals), high stability of the produced nanoparticles and suitability of the method for large-scale synthesis. This nontoxic size-controlled biosynthesis of AgNP has become inexpensive nanotechnology suitable for a wide range of applications. A wide range of applications like

biomedical, environmental, agricultural, biosensing, to name a few, have been successful with biosynthesized AgNP particles. Numerous plant species across the globe showed the capability to biosynthesize AgNP particles, as evident from the extensive list summarised in the review due to their presence of a wide array of phytochemicals, though specific biochemical for each function is yet to be elucidated. However, these bio-reduced nanoparticles still pose limitations in large-scale applications, owing to their yield and purity. The limitations and disadvantages have been discussed under engineering, environmental and economic bottlenecks. Finally, the bibliometric analysis of the trend of this plant-mediated AgNP synthesis research revealed that initially, the biosynthesis of AgNP was the main objective, and maximum work on this was done in Asia, with India producing the highest number of scientific articles and citations, while the USA or Europe worked more on applications of the AgNP particles later. Recently instead of using isolated AgNP particles, the technique has shifted to target specific applications with hybrid systems. Finally, it can be concluded that plant-mediated synthesis of silver and other nanoparticles is a lucrative option but requires more research into improving its applicability for a sustainable result.

## Research gaps and future recommendations

In recent years, the biological synthesis of NP (like AgNP) has emerged as an important scientific field. Among the wide number of natural materials, plant extract/biomass has gained significant importance due to the simple one-step inexpensive process, more environment friendly process, and safe to handle chemicals. However, several researchers hypothesized the involvement of specific potential chemical agents/functional groups of the plant species during the synthesis of AgNP. However, considering the diversity of plants and the phytochemicals composition, none of the papers describes the particular phytochemical(s) responsible for the bio-reduction of  $Ag^+$  (reduce  $Ag^+$  into  $Ag^0$ ) or stabilization rather than the hypothetical bio-reduction mechanism. This area still presents a lacuna in the research of Phyto mediated synthesis of nano particles and need to be addressed. This will help in controlling and achieving the desired size and morphology of the nanoparticles for various applications. This will also help to assess the toxicity of the specific phytochemicals involved in the process on biological organisms once they come in contact in nature.

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## Declarations

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