

Selection of Suitable Biological Method for the Synthesis of Silver Nanoparticles

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Abstract

This paper aims to present a brief overview of different biosynthesis routes of silver nanoparticles (NPs), their applications and influence of the method used on the size and morphology of these nanoparticles. A detailed and comprehensive study of available biological methods, also referred to as a bottom-up approach, as well as techniques reported, have been provided with an eye for details and comparison between the techniques involving fungi, bacteria, algae and plant extracts. Plant-derived bioreductants such as leaf, stem or root extracts of various plants are seen as suitable solutions to green synthesis of silver NPs, implementing an easy, non-toxic, clean and environmentally friendly approach. Furthermore, reports on the antimicrobial activities with the zone of inhibition for various pathogens have also been included.

Keywords Nanotechnology, Silver Nanoparticles, Biological Methods, Antimicrobial Activity

1. Introduction

The sphere of nanotechnology has been in the spotlight in recent years, as the remarkable growth of many important

industries, such as chemicals, electronics, agriculture, medicine and the space industry, has been revolutionized due to its influence on the above-stated industries [1-6].

The production of metallic nanoparticles is an active area for researchers for academic purposes as well as in the development of nanotechnology. Metallic nanoparticles have attracted significant attention as they are observed to have unusual physical and chemical properties, which significantly differ from their properties when taken in bulk amounts [7, 8]. Any change in their size would cause a direct change in the catalytic, electronic and optical properties of the nanoparticles [9-12]. For instance, metallic silver in the form of silver nanoparticles has enhanced chemical and physical properties as compared with normal silver metal [13, 14]. Moreover, they demonstrate better antibacterial [15-19], antifungal [20-24] and antiviral [25-29] properties in comparison with metallic silver and various silver compounds. Applications of silver nanoparticles (AgNPs) include, but are not limited to, electronics [30-33], biosensing [34], photonics, optoelectronics, sensing, pharmaceuticals [35, 36], textiles, water treatment [37], DNA sequencing [38] and surface-enhanced Raman scattering (SERS) [39]. Ag nanoparticles act as an antimicrobial agent [40] and are being used for the treatment as well as the prevention of HIV [41]. AgNPs have assorted application, such as pigments, photographics, wound

treatment and conductive/autostatic composites [42]. Such a wide variety of applications has led researchers to design better and more economical ways for the production of AgNPs on a large scale. The design of experimental methods for the production of nanoparticles with different chemical composition, sizes, shapes and dispersity is an important facet of nanotechnology [3, 43, 44].

Over the last few years, the vital significance of manufacturing clean, non-toxic and environmentally friendly solvents and chemicals [45, 46] has catalysed the biosynthesis of nanoparticles. Biological processes centred on bacteria, fungus, bio-derived chemicals and plant extracts are keenly studied due to their eco-friendly nature and morphological control [47- 49]. Biological sources available in nature, including bacteria, algae, yeast, fungi, lower plants and higher angiosperm plant products, can be used for the synthesis of nanoparticles. These ambient biological systems provide excellent examples of nano-phasic materials with highly optimized characteristics [50]. The manufacturing of inorganic materials may occur in two ways, either extracellular or intracellular [51, 52]. In current research areas of nanotechnology, developing reliable experimental regulations for the synthesis of nanoparticles over a range of chemical composition, size, and synchronized, non-toxic, clean and eco-friendly monodispersity is a big challenge. Although many papers have been reported in the last few years [53-55], a greater number of comprehensive publications are needed so that the world may discover the applications of the biosynthesis of various metal nanoparticles. The use of environmentally friendly materials such as plant extract [56], bacteria [57], fungi [58] and enzymes [59] for the synthesis of silver nanoparticles offers many benefits of compatibility with pharmaceutical and other biomedical applications, owing to the use of non-toxic chemicals for the synthesis procedures. Chemical syntheses of nanoparticles involve the presence of some toxic chemicals absorbed on the surface that may have a disastrous effect if used in the field of pharmaceuticals. In contrast, green synthesis has an edge over chemical and physical methods of synthesis as it is cheap, eco-friendly and can be scaled up to larger-scale synthesis with ease. This method does not even require the use of high pressure, energy, temperature and toxic chemicals as compared with chemical synthesis [60]. Synthesis of nanoparticles using biological means, especially plants, is biocompatible as they secrete functional biomolecules which actively reduce metal ions [26, 61]. Moreover, biological agents such as plants involved in the reducing process also act as capping agents and are eco-friendly [62, 63]. Herein, we provide blueprints of various reports on the biological means of nanoparticle synthesis with desired characteristics, with an eye for details to allow effective comparison and valuable selection.

1.1 Synthesis of silver nanoparticles

The synthesis of silver NPs can be carried out by several methods including chemical (e.g., chemical reduction, microemulsion techniques, pyrolysis, UV-initiated photo-

reduction, photoinduced reduction, electrochemical synthetic method, irradiation methods, microwave-assisted synthesis, polymers and polysaccharides, Tollens method), physical (e.g., evaporation-condensation, laser ablation, arc discharge method, direct metal sputtering into the liquid medium) and biological methods (e.g., use of algae, fungi, bacteria and plants as bioreductant) [64-66]. The chemical and physical processes mostly involve hazardous chemicals, high energy requirements and other strict conditions. The sizes and morphologies of silver nanoparticles synthesized from these two methods are quite variable depending on the conditions and methods applied. In contrast to the chemical and physical methods, the biological method, also known as the bottom-up approach, has been able to biosynthesize silver nanoparticles with better sizes and morphologies. Most of the NPs produced were reported to have a predominantly spherical shape. Other benefits of the use of the green approach are the use of biological reductants, low to zero energy requirements and better characteristics of the metallic silver nanoparticles, with the advantage of elimination of the need for toxic chemicals to be used as surfactants or stabilizers since various proteins present in the plant extracts act as reducing as well as capping agents for silver NPs [67]. Below is a comparison between various bio-based methods in order to analyse and practise the most suitable biological approach for the biosynthesis of silver NPs to meet the future challenges of demand and supply of metallic silver NPs.

2. Biological Methods

2.1 Fungi

Several researchers, including Ahmad et al., Macdonald et al., Ahmad et al., Kumar et al. and Korbekandi et al., have shown great interest in the potential of *Fusarium oxysporum* to synthesize silver NPs in order to establish new ways to produce them in an environmentally friendly and cost-effective way [68-72]. Ahmad A et al. examined the given strain to produce 5 – 50 nm silver NPs extracellularly and mentioned the high stability of these silver NPs due to proteins in the strain [68]. Macdonald IDG et al. showed keen interest in this topic and worked to understand the interaction of these proteins including cytochrome c (Cc) with silver NPs [69]. The works of Ahmad A et al. and Kumar SA et al. provide further insight into the bioreduction of silver ions by using bioreductant *F. oxysporum* and describe the enzymatic process and the resulting stability of silver NPs [70, 71]. The morphology of the biosynthesized NPs and the effects of pH on the capping proteins were illustrated by Kumar SA et al. [71] Korbekandi H et al. reported the morphology of silver NPs prepared using *Fusarium oxysporum* to be almost spherical, with a size range of 25 – 50 nm and 100 nm in the case of individual and agglomerates respectively, by Scanning Electron Microscope (SEM) micrographs [72]. The authors state the

biosynthesis of silver NPs by *Fusarium oxysporum* to be intracellular instead of extracellular [72]. The bioreduction of silver ions and its stability was further explained to be the result of an enzymatic process [72].

The potentials of *Fusarium acuminatum* Ell. and Ev. (USM-3793) cell extracts were exploited to obtain metallic silver NPs with an average diameter of 13 nm [73]. The NPs were synthesized quite rapidly, i.e., within 15 – 20 minutes of reaction, by the cell extracts of the mentioned algae and remained within the size range of 5 – 40 nm [73].

Vigneshwaran N et al. reported the use of *Phanerochaete chrysosporium* to reduce silver ions acquiring predominantly pyramidal-shaped silver NPs [74].

Aspergillus flavus and *Aspergillus fumigatus* were exploited for biosynthesis of silver NPs [75, 76]. The *Aspergillus flavus* was claimed to be highly stable in water [75]. The morphology of the extracellularly biosynthesized silver particles, size 5 – 25 nm, was reported to be predominantly spherical with few triangular shapes; such exceptions or few changes thereof are expected to be present in bio-based synthesis of silver NPs [76].

Balaji DS et al. studied the extracellular biosynthesis of silver NPs by filtrate of *Cladosporium cladosporioides* fungus. The chemical compounds released by the strains of *Cladosporium cladosporioides* were considered to be responsible for the stability and shape of the silver NPs [77].

Penicillium sp. J3, *Penicillium fellutanum* and *Penicillium* genus were successfully treated for the reduction of silver ions into silver NPs [78-80]. *Penicillium fellutanum* was able to reduce silver ions into silver NPs successfully using incubation under dark conditions [79].

Monodisperse spherical silver NPs were reported to be produced by reduction of silver nitrate solution by *Coriolus versicolor* [81]. The characteristics of these silver NPs were recorded through UV-visible absorption spectrophotometry, Transmission Electron Microscope (TEM), Atomic Force Microscopy (AFM) and Fourier Transform Infrared spectroscopy (FT-IR) [81]. Sanghi R et al. reported the

influence of parameters such as pH and temperature on the reaction time and characteristics of the NPs [81]. Proteins were reported to be the main cause for the stability and were suggested to be performing as a capping agent as well [81]. A list of organisms used for the biosynthesis of silver NPs and the characteristics of these silver NPs have been summarized in Table 1.

2.2 Bacteria

Highly stable silver NPs with an average size of 40 nm were prepared by reduction of silver ions using culture supernatant of *Bacillus licheniformis* [94]. Similar bacteria were reported to be able to synthesize well dispersed silver NPs with an average diameter of 50 nm [95].

Microwave irradiation was used to support uniform heating in the case of extracellular biosynthesis of silver NPs by bioreductant culture supernatant of *B. subtilis*. The silver metal NPs produced by this method were reported to be monodispersed, within the size range of 5 – 20 nm [96]. Various researchers reported the ability of *Pseudomonas stutzeri* AG259 to biosynthesize intracellularly silver NPs of varying compositions [97], with a size range of 35 – 46 nm [98], or up to 200 nm in the case of high concentrations of silver ions [99, 97] of varying geometrical structures [97].

Shahverdi AR et al. successfully demonstrated the rapid bioreduction abilities of culture supernatants of *Enterobacter cloacae*, *Escherichia coli* and *Klebsiella pneumonia* to reduce silver ions into metal silver NPs within five minutes of exposure [100].

The effects of visible-light irradiation on the biosynthesis of silver NPs using culture supernatant of *Klebsiella pneumonia* were studied by Mokhtari N et al. The size range of such NPs was calculated to be 1 – 6 nm [101].

The mechanism of bioreduction of Diamminesilver to biosynthesize metallic silver NPs using *Aeromonas* sp. SH10 and *Corynebacterium* sp. SH09 was suggested by Mouxing FU et al. [102]

Organism	Mode	Characteristics of Silver NPs			Characterization Instrument	Microbial activity against / Applications*	Ref.
		Size (nm)	Shape	Others			
Verticillium	intracellular	25 ± 12	–	Uniformly distributed bound to the surface of cells	UV-vis spectroscopy, X-ray diffraction (XRD), scanning electron microscopy (SEM), energy dispersive analyses of X-ray (EDX)	Bionanocomposites in catalysis and other electronic applications	[92]
Verticillium sp.	–	25	–	–	UV-vis spectroscopy, XRD, SEM, EDX	Bionanocomposites in catalysis and other electronic applications	[93, 94]

Organism	Mode	Characteristics of Silver NPs			Characterization Instrument	Microbial activity against / Applications*	Ref.
		Size (nm)	Shape	Others			
Phoma sp.	–	71.06 ± 3.46	–	–	Atomic absorption spectrometer (AAS), Transmission electron microscopy (TEM), X-ray photoelectron spectroscopy (XPS)	Can be used as catalyst and as bactericide	[95]
F. oxysporum	extracellular	20 – 50	Spherical	Few agglomerations	UV-vis spectra, fluorescence emission spectra, SEM, energy dispersive spectroscopy (EDS), TLC (Chromatography of Thin Layer) analysis	Antibacterial in non-linear optics	[96]
Fusarium oxysporum	–	2 – 5	Spherical	Few agglomerations	UV-vis spectroscopy, XRD, SEM, EDX	–	[97]
Fusarium oxysporum	–	5 – 15	Spherical	Few agglomerations	UV-vis spectroscopy, XRD, SEM, EDX	–	[98]
Fusarium oxysporum	extracellular	5 – 50	Spherical Occasionally triangular shapes	Highly variable, spherical and well dispersed	UV-vis absorption spectrum, Fluorescence emission spectra (luminescence spectrophotometer), FT-IR, XRD, TEM, Debye-Scherrer formula	Non-linear optics Optoelectronic applications	[78]
F. oxysporum	–	10 – 25	–	Crystalline	UV-vis absorption, XRD, TEM, X-ray photoelectron spectrometer, Debye-Scherrer formula	Non-linear optics Optoelectronic applications	[81]
Fusarium oxysporum	intracellularly	25 – 50	Spherical	–	UV-vis absorption spectrophotometer, SEM, TEM	–	[82]
Phanerochaete chrysosporium	extracellular	100	Pyramidal shape	Hexagonal structures	UV-vis spectroscopy, XRD, SEM, TEM, photoluminescence spectroscopy	–	[84]
Aspergillus fumigatus	extracellular	5 – 25	Spherical, triangular shapes	Crystalline, fairly monodispersed	UV-vis spectrophotometer, TEM, XRD	–	[86]
Fusarium semitectum	extracellular	10 – 60	Mostly spherical	Crystalline, polydisperse	UV-vis spectrophotometer, XRD, TEM, Fourier transform infrared (FT-IR) spectroscopy	–	[99]
Aspergillus flavus	extracellular	7 – 10	–	Monodisperse FCC	UV-visible spectra, XRD, SEM, TEM, FT-IR, Photoluminescence spectra	–	[100]
Coriolus versicolor	extracellular intracellular	10 few formed	Spherical Spherical	Symmetrical, monodisperse	AFM, TEM, XRD, FT-IR, UV-visible absorption spectrophotometry	–	[91]

Organism	Mode	Characteristics of Silver NPs			Characterization Instrument	Microbial activity against / Applications*	Ref.
		Size (nm)	Shape	Others			
<i>Fusarium solani</i>	extracellular	5 – 35	Spherical	Large distribution range, polydisperse	UV-vis spectrophotometer, FT-IR, TEM	–	[101]
<i>Aspergillus clavatus</i>	extracellular	10 – 25	Spherical few polyhedral	Highly variable, polydisperse	XRD, TEM, atomic force microscopy (AFM)	<i>Candida albicans</i> , <i>Pseudomonas fluorescens</i> and <i>Escherichia coli</i>	[102]
<i>Cladosporium cladosporioides</i>	extracellular	10 – 100	Mostly spherical	Different crystallite shapes, polydisperse	UV-vis spectrophotometer, XRD, TEM, FT-IR, Scherrer's equation	–	[87]
<i>Penicillium fellutanus</i>	–	5 – 25	Mostly spherical	Variable Well dispersed	UV-vis absorption spectra, TEM	–	[103]
<i>Fusarium acuminatum</i> Ell. and Ev. (USM-3793)	extracellular	5 – 40	Spherical	Spherical with a broad size	UV-vis spectrophotometer, TEM	<i>Staphylococcus aureus</i> , <i>Salmonella typhi</i> , <i>Staphylococcus epidermidis</i> and <i>Escherichia coli</i>	[83]
<i>Penicillium fellutanum</i>	–	5 – 25	Mostly spherical	Well dispersed	UV-vis absorption spectra, TEM	–	[89]

*as mentioned by author

Table 1. Biosynthesis of Silver NPs using Fungi

Organism	Mode	Characteristics of Silver NPs			Characterization Instrument	Microbial activity against / Applications*	Ref.
		Size (nm)	Shape	Others			
<i>Pseudomonas stutzeri</i> AG259	–	Up to 200	Equilateral triangles, hexagons	Agglomerations	TEM, quantitative EDX, electron diffraction	–	[107]
<i>Plectonema boryanum</i> (strain UTEX 485)	extracellular	1 – 15	Spherical	25°C	TEM, TEM-SAED, TEM-EDS, XPS	–	[116]
	intracellular	1 – 40	Spherical	60°C			
		5 – 200	Spherical, octahedral crystal platelets	100°C			
<i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> , <i>Enterobacter cloacae</i>	extracellular	50 – 100	Predominantly spherical	–	SEM, UV-visible spectroscopy	–	[117]
<i>Klebsiella pneumoniae</i> , <i>Escherichia coli</i> and <i>Enterobacter cloacae</i> (<i>Enterobacteriaceae</i>)	extracellular	28.2 – 122	Predominantly spherical	–	UV-visible spectroscopy, TEM, EDS	–	[118]
<i>Escherichia coli</i> ATCC 8739, <i>Bacillus subtilis</i> ATCC 6633 and <i>Streptococcus thermophilus</i> ESh1	extracellular	5 – 25	Spherical	Dispersed with few agglomerations, reasonably monodispersed	UV-visible spectrum, TEM, XRD	<i>Escherichia coli</i> , <i>Bacillus subtilis</i> , <i>Salmonella typhimurium</i> , <i>Klebsiella pneumoniae</i> , <i>Staphylococcus aureus</i> , <i>Pseudomonas aeruginosa</i> , <i>Candida albicans</i>	[119]

Organism	Mode	Characteristics of Silver NPs			Characterization Instrument	Microbial activity against / Applications*	Ref.
		Size (nm)	Shape	Others			
Bacillus licheniformis	extracellular	<50	Spherical	–	UV-visible spectroscopy, SEM, XRD, EDX, Debye–Scherrer equation	–	[104]
Bacillus licheniformis	extracellular	40 – 50	–	Well dispersed	UV-vis spectroscopy, SEM, EDX, XRD	–	[105]
Lactobacillus fermentum	extracellular intracellular	5 – 15 15 – 40	Nearly spherical	–	Atomic absorption spectrophotometer, XRD, TEM, UV-vis absorption spectra	–	[120]
L. farciminis	Mostly	15.7 –		–			
L. rhamnosus	intracellular	21.7					
L. plantarum							
Klebsiella pneumonia (using Liquid mixing's effects under visible light density)	extracellular	1 – 6	–	Uniformly dispersed, uniform size and shape	UV-vis spectroscopy, visible light irradiation, XRD	–	[111]
Klebsiella pneumonia (reducing silver nitrate)	extracellular	5 – 100	–				
Klebsiella pneumonia (reducing synthesized silver chloride)	extracellular	Up to 55	Variable				
Brevibacterium casei	–	10 – 50	Spherical	Relatively uniform	UV-vis spectra, surface plasmon absorbance, TEM, XRD, FT-IR, fluorescence spectra	Showed anti-coagulative activity	[121]
Staphylococcus aureus	extracellular	160 – 180	Irregular	Agglomerations	UV-vis spectrophotometer, AFM	Antimicrobial	[122]

*as mentioned by author

Table 2. Biosynthesis of Silver NPs using Bacteria

Organism	Mode	Characteristics of Silver NPs			Characterization Instrument	Microbial activity against / Applications*	Ref.
		Size (nm)	Shape	Others			
Spirulina platensis	extracellular	7 – 16	Predominantly spherical	Relatively uniform	HRTEM, FT-IR, UV-vis spectrophotometry, XRD	Showed anti-coagulative activity	[124]
Oscillatoria willei NTDM01	extracellular	100 – 200	–	Agglomerations	HRTEM, FT-IR, UV-vis spectrophotometry, XRD	Antimicrobial	[74]
C. vulgaris	extracellular	Mean length of 44 and width of 16 – 24	Rod-like particles	–	TEM, FT-IR, UV-vis spectrophotometry, XRD, field emission scanning electron microscopy (FESEM)	Antimicrobial	[123]

*as mentioned by author

Table 3. Biosynthesis of Silver NPs using Algae

Spherical silver particles were observed when strains of Lactobacillus were used to reduce silver ions [104, 105] with an average size of between 25 – 50 nm [104]. In the case of agglomeration of silver NPs, the average size of the agglomerated metal particles was observed to be 100 nm [104]. Enzymatic process was attributed as the reason for the stability of the biosynthesized silver NPs [104]. Table

2 provides sizes of silver NPs' biosynthesis by reducing silver ions by bioreductant bacteria.

2.3 Algae

Yellowish brown colour indicating the formation of silver NPs was observed when Spirulina platensis biomass was

challenged with 0.001 M aqueous AgNO₃ solution. Surface plasmon absorbance, X-ray powder diffraction (XRD), High-resolution transmission electron microscopy (HRTEM) and Fourier transform infrared spectroscopic (FT-IR) measurements were utilized for recording the characteristic dispersions of nanometallic particles, confirmation of formation of silver NPs, crystalline nature, predominantly spherical shape, size range of silver NPs 7 – 16 nm and the possible action of proteins for reduction and capping of silver NPs respectively [114].

Iravani S et al. mentioned in their review the ability of *C. Vulgaris* and *Oscillatoria willei* to synthesize silver NPs [64]. *C. Vulgaris* biosynthesized silver nanoparticles in a rod-like shape with a mean length of 44 nm and width of 16 – 24 nm, while *Oscillatoria willei* biosynthesized silver NPs with a diameter range of 100 – 200 nm [64]. The efforts of a few researchers to biosynthesize silver NPs using algae have been presented in Table 3.

2.4 Plants

Silver nitrate was reduced using grape extract (*Vitis vinifera*), resulting in nearly spherical-shaped particles with an average size of between 18 – 20 nm. The characteristics of these silver NPs were established using UV-vis Spectroscopy, Dynamic Light Scattering (DLS), Energy Dispersive X-ray Spectroscopy (EDX) and Transmission Electron Microscopy (TEM). Antibacterial activity of these silver NPs was studied against *Bacillus subtilis* and *Escherichia coli*, showing inhibition in growth rate of both bacteria [115].

Gavhane AJ et al. demonstrated the ability of plant leaf extract of *Azadirachta indica*, commonly referred to as Neem, and *Triphala* to synthesize silver NPs. Predominantly spherical and polydispersed silver NPs of mean size 43 nm with concentration 3.6×10^{10} particles/ml and 59 nm with concentration 5.15×10^6 particles/ml were obtained from Neem and *Triphala* respectively. The respective inhibition zones are represented by 15, 14, 13, 11 and 16, 14, 13, 10 for *C. albicans*, *K. pneumoniae*, *S. typhi* and *E. coli* MDR in the case of Neem and *Triphala* respectively, showing their antimicrobial activities [116].

Nanoparticle biosynthesis was further carried out by Lalitha A et al., using the *Azadirachta indica* aqueous leaf extract. UV-vis spectroscopy, Size Analyser and FT-IR analysis confirmed the synthesis of silver NPs with a mean size of 21.07 nm [117]. These particles when tested against Gram positive (*Salmonella typhi*) and Gram negative (*Klebsiella pneumoniae*) bacteria showed the zone of inhibition of 1 mm [117], in contrast to those calculated by Gavhane AJ et al. [116]. The antioxidant properties of these silver NPs were established through 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay and hydrogen peroxide assay [117].

Similar work to establish the antibacterial activity of silver NPs biosynthesized by using Neem extracts was carried

out by Sharma S et al. The zones of inhibition for *S. aureus* were 10 mm and 13 mm, while for *E. coli* they were 12 mm and 15 mm in the case of 50 µg/ml and 100 µg/ml concentration of the silver NPs in chloroform [118].

Non-linear optical studies for NPs synthesized by reducing 1 mM of AgNO₃ (AgNO₃, 99.99%, Sigma-Aldrich) by *Coriandrum Sativum* leaf extract (reducing agent) was carried out by Sathyavathi R, Balamurali Krishna M, Venugopal Rao S, Saritha R and Narayana Rao S. Non-linear optical properties of these silver NPs were determined by z-scan technique with 6 ns pulse duration at 532 nm. UV-visible, XRD, FT-IR and TEM were applied for the characterization of these silver NPs, showing mostly spherical shape, ranging in size from 8 nm to 75 nm with a mean size of 26 nm [119].

The works of Phanjom P and his colleagues reported that the pale yellow coloured solution turned to yellowish brown coloured solution when 60 ml of *Elaeagnus latifolia* leaf extract was allowed to react with 10 ml of 1 mM aqueous solution of silver nitrate at room temperature. This colour change is attributed to the formation of silver NPs. UV-vis absorption spectroscopy and X-ray diffractometer (XRD) verified the formation of silver NPs. The shape (mostly spherical) and size (30 nm to 50 nm) of silver NPs prepared by this technique was determined by TEM [120].

Eco-friendly green synthesis of silver NPs when carried out by the reduction of aqueous silver nitrate with ascorbic acid, in the presence of gelatin acting as stabilizer, resulted in the formation of silver NPs having an average size of 20 nm. The characteristics and morphologies of the obtained silver NPs were examined using XRD, TEM and EDX. Electroactivity and electrocatalytic properties of these nanoparticles and AgNPs–CPE were determined using cyclic voltammetry respectively, revealing excellent electrocatalytic potential towards H₂O₂, representing the suitability of these NPs as an effective catalyst for H₂O₂ reduction. The results were quite favourable in terms of high sensitivity and excellent in terms of reproducibility and operational stability of AgNPs – CPE [121].

A study of the change in the time required for the formation of silver NPs by varying the volume of the 3 mM silver nitrate solution added to 1 ml of the aqueous extract of *Portulaca oleracea* was carried out by Jannathul Firdhouse M and Lalitha P under three different experimental conditions, i.e., reaction at room temperature, reaction at elevated temperature and sonication. The change in colour, from yellow to reddish brown, was observed after 60 min, 50 min, 40 min, 35 min and 30 min for each 1 ml addition of 3 mM silver nitrate to 1 ml of aqueous extract of *Portulaca oleracea* respectively. Similar trends but with less time in each case were observed for reactions at 75°C. The minimum time in each case was observed for sonication, in which the time required was 27 min, 24 min, 20 min, 20 min and 18 min for reaction of 6 ml, 7 ml, 8 ml, 9 ml and 10 ml of 3 mM silver nitrate respectively with 1 ml of aqueous

extract of *Portulaca oleracea*. The size of NPs in each case was less than 60 nm and the characteristics of these silver NPs were assessed by UV-visible spectroscopy, XRD, Scherrer's formula and SEM analysis. It was further recorded by Jannathul Firdhouse M and Lalitha P that the carbonyl and CN triple bond stretching in proteins act as capping as well as reducing agents and are the reasons for the stabilization of synthesized NPs [122].

Effective reduction of silver ions to metal silver nanoparticles as a function of ratio of available silver ions to the polyphenols, such as catechins and stilbenes, present in *R. hymenosepalus* extract, was demonstrated by Rodríguez-León et al. in 2013. The silver ions were reduced to silver nanoparticles with face-centred cubic and hexagonal geometry and ranged from 2 – 40 nm characterized by UV-visible spectroscopy, TEM and fast Fourier transform. It was observed that an increase in concentration of the reacting silver nitrate solution resulted in relatively larger silver NPs and increased NPs' formation. The results show potential for industrial applications of biological systems owing to kinetics and simple conditions such as room temperature and single step procedure [123].

Silver NPs characterized as highly dispersed with an average diameter of 20 nm were synthesized using pomegranate peel extract. The formation of metal NPs were confirmed by UV-visible Power wave microplate reader spectroscopy and TEM. Although the biosynthesis of silver NPs using *Punica granatum* L. only occurred at alkaline media, yet the stability of these NPs for several weeks without the addition of any surfactants make it a strong candidate for biosynthesis of silver NPs and its implementation on a larger scale [124].

Awwad et al. established that the rapid synthesis of mostly spherical silver nanoparticles can be achieved by challenging silver ions with carob leaf extract at ambient temperature. The UV-vis absorbance study and the colour change suggested the rapid synthesis of silver NPs and the completion of the reaction in almost two minutes after the start of the reaction. Stability and capping agent properties were attributed to the Carbonyl group of amino acid residues as suggested from FT-IR spectroscopic study. Further characterization of the obtained NPs was done by scanning electron microscopy, atomic absorption spectroscopy and X-ray diffraction (XRD). The resulting NPs were polydispersed and crystalline with face-centred cubic geometry. The size of these NPs was within 5 to 40 nm with an average diameter of 18 nm, as confirmed by SEM analysis. Strong antimicrobial effects were observed using these NPs against *E. coli* [125].

Fairly uniform, polydispersed nanocrystalline silver particles with FCC geometry having a size range of 10 – 25 nm and tendency to agglomerate, as confirmed by UV-visible spectrophotometry, TEM, AFM and XRD analysis, were biosynthesized when silver nitrate solution was

allowed to react with curry leaf extract. Christensen et al. studied the effects of increase in broth concentration on the decrease in reaction time as well as size of the biosynthesized NPs. The optimized ratio for leaf extract to 0.001 M silver nitrate solution was determined to be 1:20 by the authors for the reduction process [126].

Elizondo N et al. documented the morphology of Ag NPs obtained by reducing silver nitrate (0.001 M) against different concentrations of extracts of *Aloe Barbadensis* at 60°C as pure crystalline with face-centred cubic nanocrystals. Using different concentrations for plant extract, the silver particles obtained were predominantly spherical and quasi-spherical with few exceptions of oval or elliptical at high concentrations [127]. A pH-based novel method for the control of size and crystallinity of silver NPs using a complexing agent was described by Ghorashi SAA et al. [128].

Relatively spherical silver nanoparticles of size 40 – 50 nm, with polydispersed nature, were obtained by the reduction of silver ions using leaf extract of *Tulashi*, *Ocimum sanctum*. These NPs exhibit strong antimicrobial property against *S. saprophyticus*, *S. Aureus*, *C. albicans*, *C. kefyr* and *A. niger*. Apart from these two bacteria and three fungal species, these particles showed intermediate inhibitory effects in the case of *Candida tropicalis*, *Candida krusei*, *Aspergillus flavus* and *Aspergillus fumigates* [129].

A novel method of the preparation of silver NPs for medical purposes was proposed by Lalitha P et al. under various experimental conditions. In this context, leaf ethanol extract of *Pisonia grandis* (R. Br) was chosen to reduce silver nitrate solution to silver metal particles due to its extensive use in common medical treatments and pharmacologically important compounds present in its leaf extracts. The mechanism of the reduction of silver ions to silver metal particles by compounds such as pinitol and allantoin has been suggested by Lalitha P et al. UV-vis spectrophotometer, XRD, Scherrer's equation and SEM predicted the characteristics of these silver NPs, having a spherical shape with a diameter in the range of 20 – 150 nm in all experimental conditions, with an average size of 56.86 nm, 29.14 nm and 39.62 nm obtained for room temperature, higher temperature and sonication respectively. The authors compared the three conditions with the conclusion that sonication was the most effective, although the size attained from sonication was relatively larger than that attained from elevated temperature; however, the uniformity in shape and the decrease in reaction time were appreciably higher for the sonication than for any of the other two conditions [130].

Ahmad N and Sharma S suggested the use of extracts of *Ananas Comosus* (pineapple juice) for the production of silver NPs by reducing aqueous silver nitrate solution. They suggested that a variety of antioxidants in pineapple

juice can be responsible for reduction and that further research is required; however, phenols were mainly attributed as the reason for the reduction and stability, even after up to four weeks of incubation, of silver particles. The morphology of the *Ananas comosus* biosynthesized silver NPs was observed to be predominantly spherical, with a few exceptions of oval or elliptical, crystalline, face-centred cubic shape with an average diameter of 12 nm [131].

Leaf broth of *Rauvolfia Tetraphylla*, a small evergreen woody species, was exploited in order to synthesize silver NPs economically and in an environmentally friendly way. The silver particles were formed by reducing aqueous silver nitrate solution using *Rauvolfia Tetraphylla* leaf extract as reducing agent at room temperature, representing a simple, unconventional approach compared with the usual methods that involve materials toxic to the environment and human life. These highly crystalline silver particles with a size range of 26 – 37 nm confirmed through UV-visible spectroscopy, SEM, XRD, EDX and FT-IR were tested against *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Bacillus subtilis* for their antibacterial activity [132].

In order to investigate the rapid synthesis of silver nanoparticles for applications such as antibacterial and antifungal medicines, the use of fresh extracts of stem and leaves of *Tridax procumbens* Linn in aqueous medium to reduce silver ions provided results sufficient enough to be explored further. These silver NPs influenced/caused strong inhibitory effects in the case of two bacteria *Escherichia coli*, *Vibrio cholera* and two fungal species *Aspergillus niger* and *Aspergillus flavus*. The characteristics of silver NPs produced by this method were verified using UV-visible spectroscopy, SEM, XRD and FT-IR, which included spherical shape, 13.51 – 17.24 nm size range, elemental crystal geometry and polydiverse nature of the particles [133].

Yuet Ying Loo et al. reported the use of tea leaf extract from *Camellia Sinensis* for the synthesis of spherical, highly crystalline, FFC-structured, well-dispersed silver nanoparticles, having an average diameter of 4 nm with the size range of 2 – 10 nm. The reduction was carried out at room temperature. The crystallinity and phases, function and composition, and morphology of the silver NPs were illustrated by X-ray diffractometer, FT-IR and TEM respectively [134].

Aqueous bamboo leaves (*Phyllostachys* genus) extract consisting of phenolic acids and flavonoids [136-139] were used as bioreductant to yield nearly spherical crystalline silver NPs from 3 mM aqueous AgNO_3 at 65°C [135]. The size of these silver NPs was 13 ± 3.5 nm, while a few particles with triangular or hexagonal shape were also achieved [135]. Apart from the excellent characteristics defined by UV-vis spectroscopy, EDX, XRD, TEM and Debye-Scherrer equation, highly appreciable results of

antibacterial and antimicrobial activities of these NPs were established through the disc diffusion method and minimum inhibitory concentration (MIC) / minimum bactericidal concentration (MBC) study, respectively [135].

Plant extract in water and ethanol of Bryophytes (Thalli of *Anthoceros*) after treatment with various concentrations of silver nitrate solution at room temperature gave cuboidal and triangular-shaped silver NPs with size ranges of 20 - 50 nm. These NPs were tested for their antibacterial properties against *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Bacillus subtilis* by Kulkarni AP et al. [140].

Green synthesis of silver NPs was carried out using leaf extract of *Cochlospermum Religiosum* (medicinal plant) at 50 – 95°C by Sasikala A and Savithramma N. Reduction of silver ions to silver NPs took place highly rapidly, with an appreciable change in colour from yellow to brownish within two minutes. UV-vis spectrum and AFM techniques were used to verify the presence and characteristics of silver NPs. The results for antibacterial activity depict high toxicity of these biosynthesized silver NPs in the case of *E. coli* and *Staphylococcus*, with moderate toxicity for *Bacillus*, *Pseudomonas* and *Klebsiella* strains [141].

In order to synthesize NPs for use in antimicrobial systems, Zain NM et al. followed a green and eco-friendly method by reducing silver nitrate using ascorbic acid as reducing agent in chitosan solutions. The presence of the respective nanoparticles was confirmed using spectrophotometry technique. Zain NM et al. reported the use of Zetasizer to measure the size and zeta potential of the corresponding NPs. Antibacterial activity of these NPs against *Bacillus subtilis* and *E. coli* were evaluated by MIC and MBC tests [142].

Forough M and Farhadi K utilized aqueous extracts of the manna of *hedysarum* plant silver NPs as bioreductant for 3 mM aqueous silver nitrate solution, resulting in formation of spherical NPs with almost 90% conversion at 86°C in 13 minutes, thus providing a rapid route for the synthesis of silver NPs utilizing the biological and eco-friendly route [143].

Banerjee et al. demonstrated the use of three bioreductants, namely leaf extracts of Banana (*Musa balbisiana*), Neem (*Azadirachta indica*) and Black Tulsi (*Ocimum tenuiflorum*) to carry out the reduction of silver ions to metallic silver utilizing a microwave oven (300 W). UV-visible (vis) spectrophotometer, particle size analyser (DLS), SEM, TEM, EDS and FT-IR analysis depicted the characteristics and morphologies of the biosynthesized silver NPs, which showed inhibition effects on the growth of both *Escherichia coli* (*E. coli*) and *Bacillus* sp. While the biosynthesized silver NPs were predominantly spherical in all cases, the average diameter of the biosilver particles was 50 nm, 20 nm and 50 nm for leaf extracts of Banana, Neem and Black Tulsi

respectively [144]. Ahmad N et al. reported that the reduction of silver ions to silver nanoparticles using *Catharanthus roseus* Leaf Extract produced irregular, monodispersed particles with an average diameter of 11 nm [145].

Table 4 lists the characteristics, the characterization equipment used and the antimicrobial activity of silver NPs biosynthesized by different researchers using different plant extracts (leaf, root, stem and juice).

Plant Extracts (Leaf / Root / Stem / Juice)	Characteristics of Silver NPs			Characterization Instrument	Microbial Activity against	Ref.
	Size (nm)	Shape	Others			
Grape Extract	18 – 20	Nearly spherical		UV-vis spectroscopy, DLS, EDX, TEM	<i>Bacillus subtilis</i> , <i>Escherichia coli</i>	[125]
Neem Leaf Extract	43	Predominantly spherical	Polydispersed	UV-vis spectroscopy, NTA, TEM, EDX	<i>K. pneumoniae</i> , <i>S. typhi</i> , <i>E.coli</i> , <i>C. albicans</i>	[126]
Triphala	59	Predominantly spherical	Polydispersed	UV-vis spectroscopy, NTA, TEM, EDX	<i>K. pneumoniae</i> , <i>S. typhi</i> , <i>E.coli</i> , <i>C. albicans</i>	[126]
Coriandrum Sativum Leaf Extract	26 8 – 47	Mostly spherical	Few agglomerated	UV-visible, XRD, FT-IR, TEM, Z-scan	–	[129]
Elaeagnus Latifolia Leaf Extract	30 – 50	Almost spherical	–	UV-vis absorption spectroscopy, XRD, TEM	–	[130]
Aqueous Extract of <i>Portulaca Oleracea</i> (L.)	< 60	–	–	UV-vis spectroscopy, XRD, Scherrer's formula, SEM	–	[132]
<i>Rumex Hymenosepalus</i> Extracts	2 – 40	–	Face-centred and hexagonal	UV-vis Spectroscopy, TEM, fast Fourier transform	–	[133]
Pomegranate Peel Extract	20	–	Highly monodisperse	UV-visible Power wave microplate reader spectroscopy, TEM	–	[134]
Carob Leaf Extract	50 – 40	Mostly spherical	Face-centred cubic geometry, polydispersed	UV-vis spectroscopy, SEM, Atomic absorption spectroscopy, XRD, FT-IR	<i>Escherichia coli</i>	[135]
Curry Leaf	10 – 25	Spherical	FCC crystal, fairly uniform	UV-vis spectroscopy, TEM, AFM, XRD	–	[136]
<i>Rosa Berberifolia</i>	variable	Spherical	–	UV-vis spectroscopy, TEM, SEM, XRD	–	[137]
<i>Azadirachta Indica</i> Aqueous Leaf Extract	21.07	–	–	UV-vis spectroscopy, Size Analyser, FT-IR	<i>Salmonella typhi</i> , <i>Klebsiella pneumonia</i>	[127]
Leaf Extract of <i>Tulashi</i>	40 – 50	Relatively spherical	Polydispersed	UV-visible, SEM, XRD	<i>S. saprophyticus</i> , <i>S. Aureus</i> , <i>C. albicans</i> , <i>C. kefyr</i> , <i>A. niger</i> , <i>Candida tropicalis</i> , <i>Candida krusei</i> , <i>Aspergillus flavus</i> , <i>Aspergillus fumigatus</i>	[139]
Leaf Ethanol Extract of <i>Pisonia Grandis</i> (R. Br)	20 – 150	Spherical	–	UV-vis spectroscopy, XRD, Scherrer's equation, SEM	–	[140]
Extracts of <i>Ananas Comosus</i>	12	Predominantly spherical	FCC crystalline	UV-vis spectroscopy, EDAX, HRTEM, XRD, TEM	–	[141]
Leaf Broth of <i>Rauvolfia Tetraphylla</i> (Leaf Extract Aq.)	26 – 37	Spherical, triangle and square	–	UV-vis spectroscopy, SEM, XRD, FT-IR	<i>Staphylococcus aureus</i> , <i>Escherichia coli</i> and <i>Pseudomonas aeruginosa</i>	[142]
Fresh Extracts of <i>Tridax Procumbens</i> Linn	13.51 – 17.24	Spherical	Elemental crystal, polydiverse	UV-vis spectroscopy, SEM, XRD, FT-IR	<i>Escherichia Coli</i> , <i>Vibrio cholerae</i> , <i>Aspergillus niger</i> and <i>Aspergillus flavus</i>	[143]

Plant Extracts (Leaf / Root / Stem / Juice)	Characteristics of Silver NPs			Characterization Instrument	Microbial Activity against	Ref.
	Size (nm)	Shape	Others		Organism	
Tea Leaf Extract From Camellia Sinensis	2 – 10	Spherical	FCC, well dispersed	UV-vis spectroscopy, TEM, XRD, FT-IR	–	[144]
Aqueous Bamboo Leaves Extract	13 ± 3.5	Nearly spherical	–	UV-vis spectroscopy, TEM, XRD, EDX, Debye-Scherrer equation	E. coli and S. aureus	[145]
Bryophytes	20 – 50	Cuboidal and triangular	–	UV-vis spectroscopy, SEM, EDS,	Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Bacillus subtilis	150
Leaf Extracts of Cochlospermum Religiosum	40 – 100	Spherical	–	UV-vis spectroscopy, AFM	E.coli, Staphylococcus, Bacillus, Pseudomonas and Klebsiella	[151]
Aqueous Extracts of the Manna of Hedysarum Plant	29 – 68 40	Spherical	–	UV-vis spectroscopy, XRD, EDX, SEM	–	[153]
Banana Leaf Extract	50	Predominantly spherical	–	UV-visible (vis) spectrophotometer, DLS, SEM, TEM, EDS, FT-IR	Escherichia coli (E. coli) and Bacillus sp.	[154]
Neem Leaf Extract	20	Predominantly spherical	–	UV-visible (vis) spectrophotometer, DLS, SEM, TEM, EDS, FT-IR	Escherichia coli (E. coli) and Bacillus sp.	[154]
Black Tulsi Leaf Extract	50	Predominantly spherical	–	UV-visible (vis) spectrophotometer, DLS, SEM, TEM, EDS, FT-IR	Escherichia coli (E. coli) and Bacillus sp.	[154]
Catharanthus roseus Leaf Extract	11	Irregular	Monodispersed	UV-vis spectroscopy, TEM	–	[155]
Lemon Extract	75	–	–	UV-vis spectroscopy, AFM, SEM	E. coli and Bacillus subtilis	[156]
Extracts of Securinega Leucopyrus	11 – 20	Spherical to oval	–	UV-vis absorption spectroscopy, FT-IR, SEM, TEM	Pseudomonas aeruginosa, Bacillus subtilis, Klebsiella pneumonia, Staphylococcus aureus, Pseudomonas putida, Escherichia coli	[157]
Green Carambola Extract	Variable according to pH	Spherical	–	UV-vis absorption, XRD, FT-IR, TEM	–	[158]
Alfalfa Sprouts	2 – 20	Spherical	FCC crystals, display coalescence characteristics	X-ray absorption spectroscopy, TEM, EDS, scanning Transmission electron microscopy (STEM), HRTEM	–	[159]
Pelargonium Graveolens Leaf Extract	16 – 40	Spherical, small percentage elongated	Crystalline	UV-vis spectroscopy, TEM, XRD, energy dispersive analysis of X-rays (EDAX), FT-IR	–	[160]
Jatropha curcas (Latex)	20 – 40	Spherical, few larger with uneven shapes	Crystalline	HRTEM, XRD, UV-vis absorption spectroscopy, EDX	–	[161]
Piper Betle (Broth)	3 – 37	Spherical	Few agglomerations	UV-vis absorption spectroscopy, XRD, FT-IR, TEM	–	[162]
Carica Papaya Fruit	25 – 50	Hexagonal	Cubic structure	UV-vis absorption spectroscopy, FT-IR, XRD and SEM	Escherichia coli and Pseudomonas aeruginosa	[163]

Plant Extracts (Leaf / Root / Stem / Juice)	Characteristics of Silver NPs			Characterization Instrument	Microbial Activity against Organism	Ref.
	Size (nm)	Shape	Others			
Apiin (from Henna Leaves)	39 (average)	Quasi-spherical	Crystalline	UV-vis-near-infrared (NIR), TEM, FT-IR, XRD, Thermogravimetric analysis (TGA)	–	[164]
Desmodium triflorum	5 – 20	Predominantly spherical, few elliptical	Crystalline	UV-vis spectroscopy, TEM, XRD	Staphylococcus spp (gram-positive bacteria), Escherichia coli (gram-negative bacteria) and Bacillus subtilis	[165]
Ocimum Leaf Extract	3 – 20	Spherical	Crystalline, few agglomerations	UV-vis spectrophotometer, XRD, TEM, FT-IR	–	[166]
Coriandrum Sativum Leaf Extract	8 – 75	Spherical	Crystalline	UV-visible spectroscopy, XRD, FT-IR, TEM	–	[167]
Cassia Auriculata Leaf Extract	20 – 40	Spherical, few agglomerations with irregular shapes	Polydispersed	UV-visible spectroscopy, XRD, Scherrer's formula, SEM, FT-IR	–	[168]
Cynamon Zeylanicum Bark Powder	31	Roughly circular, smooth edges	Cubic	UV-vis spectroscopy, TEM, XRD, EDX	Escherichia coli BL-21	[169]
Cynamon Zeylanicum Bark Extract	40 few exceeded 100	Roughly circular, few ellipsoidal	Hexagonal	UV-vis spectroscopy, TEM, XRD, EDX	Escherichia coli BL-21	[169]

Table 4. Biosynthesis of Silver NPs using Plants

3. Discussion

The biosynthesis of silver NPs using biological techniques – fungi, algae, bacteria, yeast and plants – has proved to be environmentally friendly and an economical approach. Although microbial species have been able to biosynthesize predominantly spherical metallic silver NPs within the range of 1 – 70 nm and fungi able to produce SNPs with an average size range of 13 nm, yet the lack of knowledge of the mechanism of the reduction process represents a barrier still to be overcome [160-168].

The suggested mechanism for the biosynthesis of intracellular and extracellular silver NPs by bacteria involves reduction of silver by sulphur-containing proteins [169] or deoxyribonucleic acid (DNA) [161], while in the case of fungi the mechanism is thought to occur with the involvement of carboxylic group [162, 164, 168] or through nitrate-dependent reductase [167]. In the case of plants, the reduction is suggested to be carried out by a wide variety of compounds such as terpenoids [163], flavonoids [135], phenols [131], pinito and allantoin [130], present in different parts of the plants including leaves, roots, bark and latex.

In the case of intracellular synthesis, the downstream processing is difficult and expensive due to the separation

and purifying steps involved, thus making extracellular synthesis preferable, owing to its easier and simpler downstream processing.

Compared with bacteria or algae, fungi provide a more rational and economical approach for biosynthesis of silver NPs due to the fact that not only is the downstream processing and biomass handling much simpler and easier in the case of fungi, but the amounts of proteins known to reduce silver are also secreted in much higher amounts, thus increasing the biosynthesis productivity severalfold [68, 97, 103, 170-173]. In the case of microorganisms, not only is the strain preparation and growth intricate, but the isolation of strain is also difficult and requires too many precautions. The difficulty of maintaining the culture medium and respective conditions such as (but not limited to) pH, temperature, salinity of the culture and reaction mixture points towards the complexity of these techniques to be applied on a large scale [73, 75, 76, 81, 96-99, 101]. Plant broths or extracts, on the other hand, are quite simple and easy to handle and eliminate the complicated procedures of cell culture maintenance. Furthermore, the clear filtrate production from bacterial broths necessitates the use of complicated equipment in process technology, thus increasing investment costs to a considerable extent, which

is yet another major drawback in the case of the bacterial biosynthetic approach [174]. Conversely, in the case of fungi and plants, simple equipment such as a filter press can be utilized to obtain clear filtrates, thus promising economic feasibility [68, 170-173].

The synthesis of silver NPs using microorganisms [97, 103, 175] or their leached cell components [68] generates silver NPs at rates much slower than the rates at which plants can biosynthesize silver NPs [125, 141]. The time required for the complete reduction of silver ions is known to range from 24 hours to 120 hours in the case of microorganisms, while the reaction completion time is much less in the case of plants, ranging from a few hours to a maximum of 48 hours, as described in the studies mentioned earlier in the plants section [68, 97, 103, 125, 141, 175]. This represents another drawback in terms of feasibility of microorganisms to be used for large-scale biosynthesis of silver NPs in comparison with plants, which require less time for completion of reaction. The reduction rate by plants is fast enough to draw attention to the possibility of developing a rational biosynthesis methodology with reduction rates proportionate to those of physical and chemical techniques [125, 141]. Recent studies were conducted with the objective of finding a suitable procedure to acquire desired sizes and morphological characteristics of biosynthesized silver NPs along with an increased production rate. As clearly represented in the properties of silver NPs discussed in their respective categories, only plants in comparison with other biological techniques are able to demonstrate better control over morphological characteristics, sizes and rates of production of silver NPs by exploiting simple reaction conditions such as broth concentrations, silver nitrate solution concentration, salinity, ratio of plant (leaf, stem, bark or latex) extract to silver nitrate solution, pH, temperature, mixing time, time of extraction, sonication and light [122, 123, 126, 130, 141, 143]. The reduction of silver nitrate can be carried out at normal temperature, although elevated temperatures are preferable due to the increased rate of reaction and less time required for the formation of silver NPs. Sonication has proved to be the most effective, although the size attained from sonication was reported to be relatively larger than that attained from elevated temperature, but the uniformity in shape and decrease in reaction time were appreciably higher for sonication than any of the other two conditions [122, 141, 130].

Thus, in accordance with the need for an economically viable, green approach for the synthesis of silver NPs, the plant-based technique represents an optimistic alternative not only to biological methods, but also to other methods including physical techniques, and is also appreciable for large-scale production. However, there is still a great need to exploit the plant-based biotechnique to achieve even better control over dispersity, morphology, particle size and production rates if it is to replace chemical methods for production of silver NPs on an industrial scale.

4. Conclusions

Biosynthesis of silver nanoparticles can be carried out by biological methods in which the biological species range from bacteria, fungi and algae to plants capable of reducing silver ions to metallic silver nanoparticles. Biological methods have been proved to be more environmentally friendly than chemical and physical methods due to several reasons including, but not limited to, formation of hazardous/toxic bioproducts, utilization of biological species as reductants and lower energy requirements. Although microbial species have shown effective potential for the biosynthesis of metallic silver NPs, nevertheless the lack of expertise to fully understand and control the mechanism of the reduction process represents a barrier yet to be overcome. In addition to this, the complexity of maintaining the stabilized culture medium and respective conditions such as, but not limited to, optimum pH, temperature feasibility, or salinity of the culture and reaction mixture points towards the intricacy of these techniques to be applied on an industrial scale. Furthermore, in the case of plants and a few other biological species such as algae, some natural chemical compounds present in the extract act as reducing as well as capping agents, thus eliminating the need for toxic chemicals to be used as capping agents. The approach to synthesize metallic silver NPs using plant-derived extracts (leaf, root, and stem) represents the beginning of an eco-friendly, easy and simple approach with no economic and environmental barriers. Further advancements in the selection of plant-derived extract bioreductant and an adequate knowledge of the reduction process mechanism will also be helpful in determining an industrial, cost-effective way to synthesize silver NPs with excellent characteristics, morphologies and properties such as, but not limited to, antimicrobial, optical and electrical.

5. References

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