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Green Synthesis of Silver Nanoparticles: A Review

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Abstract

The bio-molecules from various plant components and microbial species have been used as potential agents for the synthesis of silver nanoparticles (AgNPs). In spite of a wide range of bio-molecules assisting in the process, synthesizing stable and widely applicable AgNPs by many researchers still poses a considerable challenge to the researchers. The biological agents for synthesizing AgNPs cover compounds produced naturally in microbes and plants. More than 100 different biological sources for synthesizing AgNPs are reported in the past decade by various authors. Reaction parameters under which the AgNPs were being synthesized hold prominent impact on their size, shape and application. Available published information on AgNPs synthesis, effects of various parameters, characterization techniques, properties and their application are summarised and critically discussed in this review.

Keywords

AgNPs, Green Synthesis, Silver Nano, Plant Extract, Microbe

1. Introduction

Materials in the nano dimensions (1 - 100 nm) have remarkable difference in the properties compared to the same material in the bulk. These differences lie in the physical and structural properties of atoms, molecules and

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bulk materials of the element due to difference in physiochemical properties and surface to volume ratio [1]. With advancement in nanotechnology, a large number of nanomaterials are appearing with unique properties, opening spectrum of applications and research opportunities [2].

About 5000 years ago, many Greeks, Romans, Persians and Egyptians used silver in one form or other to store food products [3]. Use of silver ware during ancient period by various dynasties was common across the globe utensils for drinking and eating and storing various drinkable and eatable items probably due to the knowledge of antimicrobial action [4]. There are records regarding therapeutic application of silver in literature as earlier as 300 BC. In the Hindu religion, till date silver utensils are preferred for the "panchamrit" preparation using curd, *Ocimum sanctum* and other ingredients. The therapeutic potentials of various metals are mentioned in ancient Indian Aurvedic medicine book medicinal literature named "Charak Samhita" [5]. Until the discovery of antibiotics by Alexzander Flemming, silver was commonly used as antimicrobial agent.

In the recent past, silver nano particles (AgNps) have received enormous attention of the researchers due to their extraordinary defense against wide range of microorganisms and also due to the appearance of drug resistance against commonly used antibiotics [2]. The exceptional characteristics of AgNPs have made them applicable in various fields like biomedical [6], drug delivery [7], water treatment [8], agricultural etc. [9]. AgNps are applied in inks, adhesives, electronic devises, pastes etc. due to high conductivity [10]. AgNps have been synthesized by physio-chemical techniques such as chemical reduction [11], gamma ray radiation [12], micro emulsion [13], electrochemical method [14], laser ablation [15], autoclave [16], microwave [17] and photochemical reduction [18]. These methods have effective yield, but they are associated with the limitations like use of toxic chemicals and high operational cost and energy needs. Considering the drawbacks of physio-chemical methods, cost-effective and energy efficient new alternative for AgNP synthesis using microorganisms [2], plant extracts [19] and natural polymers [20] as reducing and capping agents are emerging very fast. The association of nanotechnology and green chemistry will unfold the range of biologically and cytologically compatible metallic nanoparticles [21] [22].

Over the past decade, few reviews focusing on green synthesis of AgNPs were published [23]-[27]. Most of these reviews focused on several plant and microbial sources for synthesis, several characterization techniques for analysis, certain tabular data representing source, shape and size and information regarding various applications. The present review, unlike the earlier ones, summarizes the synthesis procedure, parameters, characterizations, applications and predicted antibacterial mechanism in a systematic manner, focusing on various green routes for AgNPs synthesis.

2. Green Synthesis

The primary requirement of green synthesis of AgNPs is silver metal ion solution and a reducing biological agent. In most of the cases reducing agents or other constituents present in the cells acts as stabilizing and capping agents, so there is no need of adding capping and stabilizing agents from outside.

2.1. Metal Ion Solution

The Ag^+ ions are primary requirement for the synthesis of AgNPs which can be obtained from various water soluble salts of silver. However, the aqueous $AgNO_3$ solution with Ag^+ ion concentration range between 0.1 - 10 mm (most commonly 1 mm) has been used by the majority of researchers.

2.2. Biological Reducing Agents

The reducing agents are widely distributed in the biological systems. The AgNPs have been synthesized using different organisms belonging to four kingdom out of five kingdom of living organisms *i.e*. Monera (prokaryotic organisms without true nucleus) Protista (unicellular organisms with true nucleus), fungi (eukaryotic, saprophyte/parasite), plantae (eukaryotic, autotrophs) and animalia (eukaryotic, heterotrophs). Data are not available regarding use of animal materials for the synthesis of AgNP' till date to the best of our knowledge. Due to this limitation, green synthesis of AgNPs has been discussed under headings microorganisms, plants, and bio-polymers.

Green syntheses of AgNPs have been performed using plant extracts, microbial cell biomass or cell free growth medium and biopolymers. The plants used for AgNps synthesis range from algae to angiosperms; however, limited reports are available for lower plants and the most suitable choice are the angiosperm plants. Parts

like leaf, bark, root, and stem have been used for the AgNP synthesis. The medicinally important plants like *Boerhaavia diffusa* [28], *Tinospora cordifolia* [29], *Aloe vera* [30], *Terminalia chebula* [31] *Catharanthus roseus* [32], *Ocimum tenuiflorum* [33], *Azadirachta indica* [34], *Emblica officinalis* [35], *Cocos nucifera* [36], common spices *Piper nigrum* [37]), *Cinnamon zeylanicum* [38]. Some exotic weeds like *Parthenium hysterophorus* [39] growing in uncontrolled manner due to lack of natural enemies and causing health problems have also been used for AgNP's synthesis. The other group includes alkaloids (*Papaver somniferum*) and essential oils (*Mentha piperita*) producing plants. All the plant extracts played dual role of potential reducing and stabilizing agents with an exception in few cases where external chemical agents like sodium-do-decyl sulphate were used for stabilization the AgNPs [40]). Metabolites, proteins [41] and chlorophyll [42] present in the plant extracts were found to be acting as capping agents for synthesized AgNPs.

The preferred solvent for extracting reducing agents from the plant is water in most of the cases however, there are few reports regarding the use of organic solvents like methanol [43]-[46], ethanol [47] [48] and ethyl acetate [49]. Some researchers pre-treated the plants materials in saline [39] or acetone [50] atmospheres before extraction. On the whole, even though the extracting solvents differed, the nanoparticle suspensions have made in aqueous medium only. Synthesis using plant extracts generate nanoparticles of well-defined shape, structure and morphology in compared to those obtained through the utilization of bark, tissue and whole plant [51].

The AgNPs synthesis by microbes is strenuous compared to the use of plant extracts and biopolymers as reducing and capping agents mainly due to the difficulty in growth, culture maintenance, and inoculums size standardization. Several fungal and bacterial species have been successfully used in the synthesis. The AgNPs synthesis mainly followed one of the two distinct routes, one utilizing extracellular materials secreted in the growth medium whereas the other utilizing microbial cell biomass directly. The microbes synthesize AgNP intracellularly as well as extracellularly. The Intracellular synthesis of AgNPs was observed by few researchers [52].

AgNPs synthesis supports better control on size and shape of AgNPs, due to easy down streaming and larger adaptability to nano systems. However, extracellular AgNP synthesis is been widely reported [53] [54]. One of the commonly used fungal genera for synthesizing AgNPs is *Fusarium* [53] [55]-[57]. No special capping agent was used in the work of many researchers for stabilizing synthesized AgNPs, except Perni *et al*. [58] and Shahverdi *et al*. [59] who used L-cystine and piperitone as stabilizing agents, respectively. Among the wide varieties off bio-polymers used for AgNP synthesis, almost all played the dual role of reducing and stabilizing agents with an exception of using starch as a capping agent [60].

3. Separation of AgNPs

Centrifugation technique is mostly used by researchers to obtain the pellet or powder form of synthesized silver nanoparticles. The AgNPs suspensions were also oven dried to obtain the product in powder form [44].

Some common characterizations of AgNPs include UV-Vis Spectra, SEM, TEM, FTIR, XRD and EDAX or EDX/EDS. DLS study is mostly used for AgNPs synthesized from bio-polymers rather than plant extracts and microorganisms. Zeta potential values indicate the stability of synthesized AgNPs. Thermo-Gravimetric Analysis (TGA) is used to find the effect of $AgNO_3$ and L-cystine on the organic composition of AgNPs [58] to find out the amount of organic material in synthesized AgNPs [61] and predict the thermal stability of AgNPs [62]. Inductive Coupled Plasma (ICP) analysis was performed to analyze the concentration and conversion of AgNPs [19].

4. Monitoring of AgNPs

The appearance of yellow to slight brownish-yellow color in the colorless solution has been taken as indicative of AgNPs synthesis by almost all the researchers. The SPR peak of the synthesized AgNPs was witnessed in the range of 400 - 450 nm, the significant range for AgNPs [63]. The UV-Vis spectral analyses have been used to analyze the dependency of pH, metal ion concentration, extract content on the formation of AgNPs and reveal the size-stability of synthesized AgNPs by exhibiting red shift in the SPR peak with increase in size of nanoparticles and blue shift for decrease in size. The SEM morphological analysis in most of the studies revealed spherical AgNPs, whereas few authors reported irregular [64], triangular [65], hexagonal [66], isotropic [67], polyhedral [60], flake [68], flower [69], pentagonal [70], anisotropic [71] and rod like structures [72]. A pictorial representation of SEM/TEM images of AgNPs with different shapes is shown in **Figure 1**. Using XRD studies of almost all the researchers reported the formation of face centered cubic (FCC) crystalline structured AgNPs.

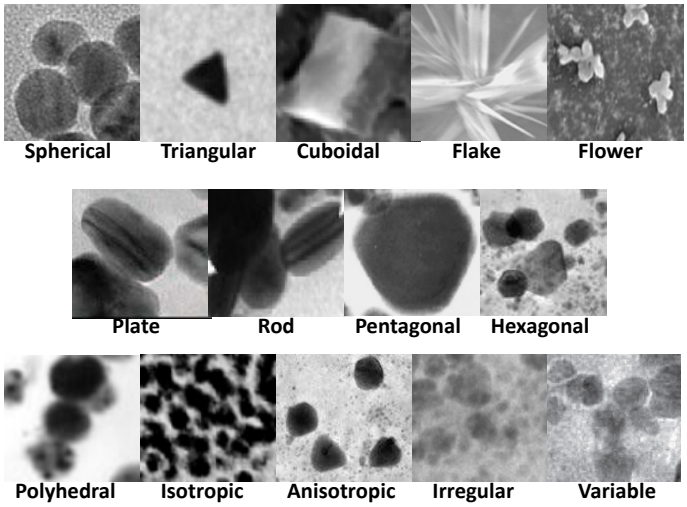

Figure 1. Various shapes of AgNPs synthesized (from various sources).

However, cubic and hexagonal structures were also reported in some cases. EDS or EDAX, for analyzing elemental composition in the nanomaterials, exhibited a characteristic optical absorption band peak around 3 KeV with silver weight percentage ranging from 45% to 80%. The reported stability of synthesized AgNPs has varied from 1 day to 1 year depending upon reducing agents and other operating conditions.

5. Mechanism of AgNPs Synthesis

The synthesis of AgNP by biological entities is due to the presence of large number of organic chemical like carbohydrate, fat, proteins, enzymes& coenzymes, phenols flavanoids, terpenoids, alkaloids, gum, etc capable of donating electron for the reduction of Ag^+ ions to Ag^0. The active ingredient responsible for reduction of Ag^+ ions varies depending upon organism/extract used. For nano-transformation of AgNPs, electrons are supposed to be derived from dehydrogenation of acids (ascorbic acid) and alcohols (catechol) in hydrophytes, keto to enol conversions (cyperaquinone, dietchequinone, remirin) in mesophytes or both mechanisms in xerophytes plants [73]. The microbial cellular and extracellular oxidoreductase enzymes can perform similar reduction processes. A schematic diagram showing the silver ion reduction, agglomeration and stabilization to form a particle of nano size is shown in **Figure 2**.

6. Factors Affecting AgNPs Synthesis

The major physical and chemical parameters that affect the synthesis of AgNP are reaction temperature, metal ion concentration, extract contents, pH of the reaction mixture, duration of reaction and agitation. Parameters like metal ion concentration, extract composition and reaction period largely affect the size, shape and morphology of the AgNPs [62]. Most of the authors have reported suitability of basic medium for AgNPs synthesis due to better stability of the synthesized nanoparticles in basic medium [36] [44] [45] [74]. Some other advantages reported under basic pH are rapid growth rate [31] [75] [76] good yield and mono dispersity [77] and enhanced reduction process. Small and uniform sized nanoparticles were synthesized by increasing pH of the reaction mixture [60] [72] [77]-[79]. The nearly spherical AgNPs were converted to spherical AgNP by altering pH [22], However, very high pH (pH > 11) was associated with the drawback of formation of agglomerated and unstable AgNPs [80].

The Reaction conditions like time of stirring and reaction temperature are important parameters. Temperatures up to 100˚C were used by many researchers for AgNP synthesis using bio-polymers and plant extracts, whereas the use of mesophilic microorganism restricted the reaction temperature to 40˚C. At higher temperatures the mesophilic microorganism dies due to the inactivation of their vital enzymes. The temperature increase (30˚C - 90˚C) resulted in increased rate of AgNPs synthesis [81] and also promoted the synthesis of smaller size AgNPs [82]. On the whole, most of workers have synthesized AgNPs at room temperature (25˚C to 37˚C) range. A plot representing the size range of AgNPs synthesized in the room temperature range is elucidated in **Figure 3**.

Figure 2. Synthesis mechanism of AgNPs.

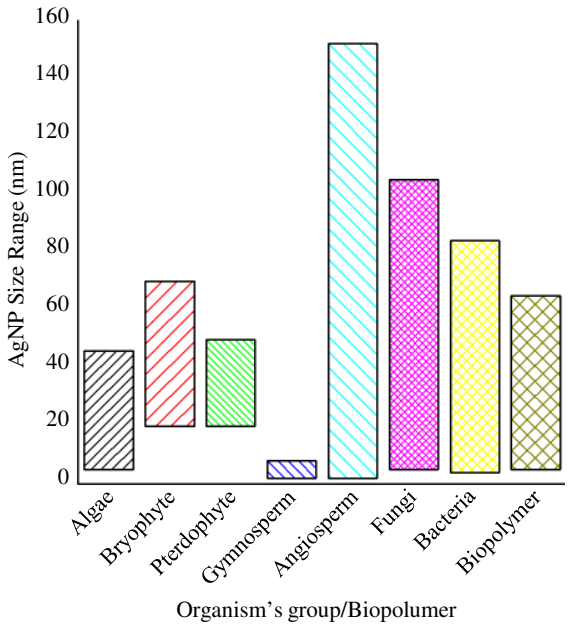

Figure 3. Size range of AgNPs synthesized at room temperature range (from various sources).

It has been found that the size range of AgNPs synthesized from algae, bryophytes, pteridophytes, gymnosperms and bio-polymer sources lie below 50 nm and that of AgNPs synthesized using from angiosperms, algae and bacterial sources ranged between 100 nm and more. The reaction mixture synthesizing AgNP using microorganisms and bio-polymers were continuously agitated to protect agglomeration compared to plant extracts without any suitable reason by the authors. Reaction mixture agitation achieved by applying external mechanical force might accelerate the formation of nanoparticles. Aging of the synthesized AgNP solution changed spherical nanoparticles into flower like structure [83] (**Table 1**).

7. Applications of AgNPs

The recent research results have shown that the AgNPs, due to their special characteristics, have immense potential for applications as anti-microbial, anti-parasitic and anti-fouling agents; as agents for site-specific medication, water purification systems, etc. The essential features of some of these applications are discussed in the following sections.

7.1. Anti-Microbial Activity

The AgNPs have been found to exhibit promising anti-micribial activity. Researchers have used several novel techniques to confirm and quantify the anti-micribial activity of AgNPs.

7.1.1. Disc/Well Diffusion Methods

The disc diffusion method, a most commonly used technique to access the antimicrobial activity of a liquid, has been employed by many researchers to confirm antimicrobial action of the AgNPs solution. In this method, uniform sized disc of adsorbent material are dipped in the increasing concentration of AgNP and placed over surface of the targeted microbe inoculated on the nutrient medium plates. An inhibition zone formation around the disc reflects antimicrobial action of the nanomaterials [72] [94] [95] [101] [104] [111] and well diffusion [29]

Table 1. Summary of the work related AgNPs synthesis using green route.

S. No.	Author	Reducing Agent	Operating Conditions	Characterization	Particle Characteristics	Remarks
			Algae			
1	Kathiraven *et al.* (2014) [84]	Filtered aqueous extract of *Caulerpa racemosa* marine algae	$AgNO_3$ concentration—1 mM, Reaction period—3 hr, Reaction temp—room temp. Extract: 10 ml/90 ml ($AgNO_3$), Motion: static	UV-Vis FTIR TEM XRD	Size—5 - 25 nm Shape—sph, tri. Structure—FCC	Antibacterial action against *P. mirabilis* and *S. aureus*
2	Rajesh *et al.* (2012) [49]	Ethyl acetate extract of *Ulva fasciata*	1 mM, 2 min, room temp. 3 ml/100 ml, Static	UV-Vis FTIR SEM XRD EDX	Size—28 - 41 nm Shape—sph Structure—cryst Nature—PD	Antibacterial action against *X. campestrispv malvacearum* pathogen
3	Vivek *et al.* (2011) [85]	Aqueous filtrate of *Gelidiella acerosa*	1 mM, room temp, 10 ml/90 ml, Agitated	UV-Vis FTIR SEM TEM XRD	Size—~22 nm Shape—sph. Structure—FCC Nature—PD	Antifungal against *Mucor inicus* and *Trichoderma reesei*
4	Govindaraju *et al.* (2009) [86]	Aqueous filtrate of *Sargassum wightii*	1 mM, 1 hr, room temp, 10 ml/90 ml, Static	UV-Vis FTIR TEM XRD	Size—8 - 27 nm Shape—sph/variable Structure—cryst	Antibacterial against *S. aureus*, *B. rhizoids*, *E. coli* and *P. aeruginosa*
			Bryophyte			
5	Kulkarni *et al.* (2012) [47]	Ethanol filtrate of *Riccia*	1 mM, 25˚C, dark, 5 ml/1 ml, Agitated	UV-Vis SEM EDS	Shape—cub/triang	Antibacterial against *p. aeruginosa*
6	Kulkarni *et al.* (2012) [47]	Ethanol filtrate of *Anthoceras*	0.5 mM, 10 min, room temp. 5ml/1 ml, Static	UV-Vis SEM EDS	Size—20 - 50 nm Shape—cub/triang	Antibacterial activity after incorporation into gauze cloth
7	Srivastava *et al.* (2011) [87]	Aqueous and ethanol filtrate of *Fissidens minutes*	0.5 mM, 1 hr, room temp. 10 ml/1 ml, Shaken	UV-Vis SEM EDS	Shape—nearly sph	Antibacterial action against *E. coli*, *B. cereus*, *K. pneumoniae*, *P. aeruginosa*
8	Kulkarni *et al.* (2011) [88]	Aqueous filtrate of *Anthoceras*	1 mM, 25˚C, dark, 5 ml/1 ml, Agitated	UV-Vis SEM EDS	Size—20 - 50 nm Shape—cub/triang	Antibacterial action against *E. coli*, *B. subtilis*, *K. pneumoniae*, *P. aeruginosa*
			Pteridophyte			
9	John De Britto *et al.* (2014) [89]	Aqueous filtrate of *Pteris argyreae*, *Pteris confuse* and *Pteris blaurita*	1 mM, 28 hr, room temp. 5 ml/100 ml, Static, Centrifugation: 25 min at 10000 rpm.			Antibacterial action against *Shigella boydii*, *Shigella dysenteriae*, *S. aureus*, *Klebsiella vulgaris* and *Salmonalla typhi*
10	Bhor *et al.* (2014) [90]	Aqueous filtrate of *Nephrolepis sexaltata* L. fern	1mM, 4h, 10 ml/90 ml	UV-Vis SEM XRD	Size—avg 24.76 nm Shape—sph. Structure—FCC	Antibacterial against many human and plant pathogens
11	Sant *et al.* (2013) [71]	Aqueous filtrate of *Adiantum philippense* L.	1 - 10 mM, 30˚C Extract: $AgNO_3$ Ratio-1: 10; 1:100; 1:1000, Agitated	UV-Vis FTIR EDS TEM DLS XRD	Size—10 - 18 nm Shape—anisotropic Structure—FCC Nature—MD	AgNps from medicinally important plants opens spectrum of medical applications.
12	Nalwade *et al.* (2013) [91]	Aqueous filtrate of *Cheilanthes forinosa* Forsk leaf	1 mM, 4 hr, room temp. 10 ml/90 ml	UV-Vis SEM XRD	Size—~26.58 nm Shape—sph. Structure—FCC	Antibacterial action against *S. aureus* and *Proteus morgani*
13	Kang *et al.* (2008) [92]	Aqueous filtrate of *Pteridophyta*	1 mM, 12 hr, room temp. 10 ml/100 ml, Static	UV-Vis TEM EDX	Size—20 - 30 nm Shape—sph.	AgNps are stable for 12 months.
			Gymnosperms			
14	Jha and Prasad. (2010) [93]	Filtered aqueous-ethanol extract of *Cycas* leaf	0.25 M, 10min, room temp. 80 ml/20 ml, Static	UV-Vis TEM XRD	Size—2 - 6 nm Shape—sph. Structure—FCC	The extraction of *cycas* leaf is done in 50% EtOH as solvent.
15	Song and Kim. (2009) [19]	Decanted aqueous extract of *Pinus desiflora* and *Ginko biloba* leaf	0.1 - 2 mm, 30 min, 25˚C - 95˚C, 10 ml/190 ml, Static, 20 min (15k rpm)	UV-Vis SEM TEM EDS ICP	Size—15 - 500 nm Shape—sph. Structure—cryst	AgNps were stable for 4 weeks.
			Angiosperms			
16	Ashokkumar *et al.* (2015) [94]	Filtered aqueous extract of *Abutilon indicum* leaf	10 mm, 15 min, room temp. 2 to 3.5 ml/30 ml, Static	UV-Vis FTIR FE-SEM TEM XRD FS	Size—7 - 17 nm Shape—sph. Structure—FCC	Antimicrobial action against *S. typhi*, *E. coli*, *S. aureus*, *B. substilus*
17	Sadeghi *et al.* (2015) [45]	Filtered aqueous-methanol extract of *Pistacia atlantica* seed powder.	1 mM, 35 min, room temp. 1 ml/10 ml, Shaken,15 min at10 k rpm	UV-Vis FTIR SEM TEM XRD EDAX ZP	Size—10 - 50 nm Shape—sph. Structure—FCC	Stability: 7 - 11 pH range. Antibacterial affect against *S. aureus.*

Continued

18	Sadeghi and Gholamhoseinpoor (2015) [44]	Methanol extracted aqueous filtrate of *Ziziphora tenuior* leaf	0.1 mM, 35 min, room temp. Static, Oven dried	UV-Vis FTIR SEM-EDAX TEM XRD ZP	Size—8 - 40 nm. Shape—sph. Structure—FCC	Stability: 6 - 12 pH range
19	Ajitha *et al*. (2014) [95]	Filtered aqueous extract of *Tephrosia purpurea* leaf powder	1 mM, 5 min, 37˚C, 10 ml/50 ml, Stirred,10 min at 10000 rpm	UV-Vis FTIR FESEM TEM XRD EDAX FS RS	Size—~20 nm Shape—sph. Structure—FCC	Antimicrobial agents against *Pseudomonas* spp. and *Penicillium* spp.
20	Suresh *et al*. (2014) [96]	Filtered aqueous extract of *Delphinium denudatum* root powder	1 mM, 2hr, room temp. 1.5 ml/30 ml, Static, 20 min at 12000 rpm	UV-Vis FTIR FESEM XRD	Size—<85 nm Shape—sph. Structure—FCC Nature—PD	Anti-bacterial against *S. aureus, B. cereus*, *E. coli and P. aeruginos* Larvicidal to *Aedes aegypti*
21	Rahimi-Nasrabadi *et al*. (2014) [45]	Methanol extract and essential oil of *Eucalyptus leucoxylon* leaf	120 min, room temp. Static	UV-Vis SEM TEM XRD	Size—~50 nm Shape—sph. Structure—FCC	AgNP with biomedical potential
22	Zuas *et al*. (2014) [97]	Filtered aqueous extract of *Myrmecodia pendan* plant.	2.5 mM, room temp. 0.3 ml/60 ml, Static	UV-Vis FTIR SEM TEM XRD	Size—10 - 20 nm Shape—sph. Structure—FCC	Promising therapeutic value
23	Mondal *et al*. (2014) [39]	Saline washed, filtered aqueous extract of *Parthenium hysterophorous* root	10 mM, 24 hr, room temp. 1:3 to 1:9, Static	UV-Vis FTIR SEM	Shape—spherical	Potential larvacidal for *Culex quinquefasciatus*
24	Raut *et al*. (2014) [98]	Filtered aqueous extract of *Withania somnifera* leaf powder.	100 mM, sunlight: 5min, dark room: 12hr, room temp. 100 ml/1 ml, Static	UV-Vis FTIR TEM XRD EDAX	Size—5 - 30 nm Shape—sph. Structure—FCC	AgNPs with quasi-reversible redox behavior Anti-bacterial to *E. coli and S. aureus* Anti-fungal to *A. niger*, *A. flavus and C. albican*
25	Vijaykumar *et al*. (2014) [28]	Aqueous extract of *Boerhaavia diffusa* plant powder.	0.1 mm, 24 hr, 100˚C, 10 ml/90 ml, Stirred	UV-Vis FTIR SEM-EDAX XRD TEM	Size—~25 nm Shape—sph. Structure—FCC, Cub	Antibacterial to fish pathogens *A. hydrophilia*, *F. branchiophilum*, *P. fluorescens*
26	Ajitha *et al*. (2014) [95]	Aqueous extract of *Plectranthus amboinicus* leaf	1 mM, 5 min, room temp. 20 ml/50 ml, Stirred, 10 min at 10000 rpm	UV-Vis FTIR FESEM TEM XRD EDAX RS	Size—~20 nm Shape—sph. Structure—FCC	Antimicrobial agents against *E. coli and Penicillium* spp.
27	Singh *et al*. (2015) [99]	*Lantana camara*		UV-Vis FTIR FESEM	48.1 nm	Anti microbial to E coli and *S. aureus* Leakage due to cell wall rupturing
28	Rao *et al*. (2014) [40]	Decanted aqueous filtrate of lemon	1 - 5 mM, room temp and 40˚C, dark 10 ml/50 ml, pH—3 - 10, Stirred	UV-Vis SEM AFM	Size—~75 nm Shape—small grains	SDS is added for stability. Antibacterial action to *E. coli* and *B. subtilis*
29	Vimala *et al*. (2015) [100]	Leaf and fruit of *Couroupita guianensis*		FTIR XRD TEM	Cubic size 10-45 nm 5—15 nm	water soluble phenolic compounds as reducing and stabilizing agent larvicidal to *A. aegypti*extensive mortality rate (LC90~5.65 ppm)
30	Shafaghat (2014) [46]	Vacuo evaporated methanol extract of *Viburnum lantana* leaf	500 mM, 4 hr, 25˚C, 5 g/100 ml, Stirred, 30 min at 3000 rpm	UV-Vis XRD TEM FTIR SEM	Size—20 - 80 nm Shape—sph Structure—FCC Nature—uniform	Antibacterial to variousgram positive and gram negative species
31	Elumalai *et al*. (2014) [41]	Filtered coconut water	1 mM, 15 min, 80˚C, 10 ml/90 ml, Static, 20 min at 18000 rpm	UV-Vis XRD SEM EDAX FTIR	Size—70 - 80 nm Structure—FCC Nature—PD	Metabolites and proteins served as capping agents.
32	Roopan *et al*. (2013) [36]	Filtered aqueous extract of mesocrap layer of *Cocos nucifera*	1 mM, 1 hr, 60˚C, 20 ml/80 ml, Stirring, pH—2 - 11	UV-Vis TEM XRD	Size—24 nm. Shape—sph. Structure—FCC	Larvicidal nature
33	Anuj and Ishnava (2013) [29]	Filtered aqueous extract of *Tinospora cordifolia* stem powder.	1 mM, 30 min, room temp. 40 ml/200 ml, 15 min at 10000 rpm, Stirring	UV-Vis FTIR TEM XRD EDAX	Size—60 nm. Shape—sph. Structure—cryst	Antibacterial nature
34	Zhang *et al*. (2013) [101]	Filtered aqueous extract of *Aloe* leaf	0.1 - 1.5 mM, 20 min, 20˚C - 40˚C, 0 to 15 ml/1 ml, Hydrazine hydrate content: 1 to 15 ml, Static	UV-Vis TEM XRD	Size—~20 nm. Shape—sph. Structure—FCC	Antibacterial to *E. coli* and *S. aureus*

Continued

35	Yang *et al.* (2013) [74]	Filtered aqueous extract of *Mangifera indica linn* peel	0.5 to 4 mM, 15 to 90 min, 25 to 100˚C, 0.1 to3 ml/27 ml, pH: 2 - 11. Static	UV-Vis TEM XRD	Size—7 - 27 nm Shape—sph. Structure—FCC	Stable for 3 months, AgNPs loaded on fabrics exhibited antimicrobial property.
36	Jagtap and Bapat. (2013) [64]	Filtered aqueous extract of *Artocarpus heterophyllus lam.* Seed powder	2 to 10 mM, 5 min, 121˚C, 15 psi. 2 to 10 w/v%, 1:4, Static, 15min at 10000 rpm	UV-Vis FTIR SEM-EDAX TEM	Size—3 - 25 nm Shape—irregular	Anti-bacterial to *B. cereus, B. subtilis, S. aureus and P. aeruginosa.* AgNP-lectin hybrid has promising use in glycol nanosensors for disease diagnosis.
37	Khalil *et al.* (2013) [75]	Filtered aqueous extract of olive leaf	1 mM, 2 min, 30˚C to 90˚C, 0.5 to 5 ml/10 ml, pH: 2 – 11, Stirred	UV-Vis FTIR SEM TEM XRD TGA	Size—20 - 25 nm Shape—sph. Structure—FCC	Stability: 1 week, AgNPs inhibited growth of *E. coli, S. aureus* and *P. aeruginosa*
38	Karuppiah and Rajmohan (2013) [102]	Filtered aqueous extract of *Lxora coccinea L.* leaf	1 mM, dark and room temp. 0.5 ml/10 ml, 15 min at 10000 rpm, Static	UV-Vis FTIR. FE-SEM XRD	Size—13 - 57 nm Shape—sph. Structure—FCC	
39	Logeswari *et al.* (2013) [103]	Filtered ethanolic extracts of *Solanum tricobactum, syzygium cumini, centella asiatica and citrus sinensis* plant powders	1 mM, 24 - 48 hr, 37˚C, 10 ml/5 ml, Additive: ammonium solution= 2.5 ml, agitated	UV-Vis FTIR XRD AFM	Size—41 - 53 nm. Shape—irregular Structure—FCC	Antibacterial against pathogenic *P. aeruginosa*
40	Geetha lakshmi and Sarada (2013) [104]	Sponin extracted from *Trianthema decendra* L.	1 mM, dark and incubated, 1 ml/5 ml, Static, 15min at 10000 rpm	UV-Vis FTIR FE-SEM EDAX	Size—17.9 - 59.6 nm. Shape—sph.	Antibacterial to *P. aeruginosa*, *E. faecalis*, *S. typhi*, *K. pneumonia*, *E. coli and C. albicans*
41	Yasin *et al.* (2013) [105]	Filtered aqueous extract of Bamboo leaf	3 mM, 65˚C, 5 ml/5 ml, Stirring	UV-Vis TEM XRD EDX	Size—13 ± 3.5 nm Shape—nearly sph. Structure—cryst	Antibacterial to *E. coli* and *S. aureus*
42	Rodriguez-Leon *et al.* (2013) [106]	Ethanol/aqueous extract of *Rumex hymenosepalus* root	2.5 - 15 mM, 24 - 96 hr, room temp. 5% v/v, Static	UV-Vis TEM EDS	Size—2 - 40 nm cub and hex Structure—FCC	AgNPs are synthesized in ethanol medium.
43	Rajathi and Sridhar (2013) [107]	Decanted aqueous filtrate of *Wrightia tinctoria* leaf	1 mM, 2 hr, room temp. 0.5 ml/10 ml, Static, 10 min at 10000 rpm	UV-Vis FTIR XRD	Size—5 - 20.5 nm Structure—cryst	Antibacterial to *S. aureus*, *V. cholerae*, *M. luteus* and *K. pneumonia*
44	Kannan *et al.* (2013) [108]	Filtered aqueous extract of *codium captium* sea weed powder.	1 mM, 48 hr, room temp, dark, 12ml/1 ml, Static, 20 min at 12000 rpm	UV-Vis FTIR SEM-EDAX TEM	Size—3 - 44 nm Nature—nano-clusters	Fresh extract was more potent for AgNP synthesis.
45	Natarajan *et al.* (2013) [109]	Powdered *Elaeagnus indica* leaves	0.5 - 2 mM, 20 - 60 min, 40˚C - 100˚C, 10 g/3 ml, Static, 10 min at 12000 rpm	UV-Vis FTIR TEM DLS	Size—avg 30 nm Shape—sph. Nature—MD	Antimicrobial against *E.coli*, *P. putida*, *B. subtilis*, *S. aureus*, *A. flavus* and *F. oxysporum*
46	Kirubaharan *et al.* (2012) [110]	Filtered aqueous extract of *Azadirchata indica(neem)* leaves	1 mM, 90 min, room to 90˚C, 1.25 ml/50 ml, pH: 6 - 8. Stirred	UV-Vis TEM XRD	Size—15 - 20 nm Shape—sph. Structure—FCC Nature—MD, PD	Stability: 4 months, Heavy metal ion sensors in aqueous media
47	Satishkumar *et al.* (2012) [72]	Filtered aqueous extract of *Morinda citifolia L.* leaf powder	1 mM, 0 - 60 min, 37˚C - 100˚C, 5 ml/95 ml, 5 min at 5000 rpm, Static	UV-Vis FTIR SEM HR-TEM	Size—10 - 60 nm Shape—sph. Structure—FCC	Stability 1 month, Inhibitory to human pathogens like *E. coli*, *P. aeroginosa*, *K. pneumoniae*, *B. cereus*, *Enterococci* spp. *and Enterobacter aerogenes*
48	Edison and Setturaman. (2012) [31]	Filtered aqueous extract of *Terminalia chebula* fruit powder.	10 mM, room temp. 1 ml/25 ml, pH: 4 – 9, Static	UV-Vis FTIR HR-TEM XRD EDS DLS ZP	Size—25 nm Structure—FCC Nature—phyto capped	Stabile for 10 days, AgNps showed catalytic activity on the reduction of methylene blue.
49	Kaviya *et al.* (2012) [68]	Aqueous filtrate of *Crossandra infundibuliformis* leaf	1 mM, 1 hr, room temp, 3 ml/40 ml, Stirring, 20 min at 4000 rpm	UV-Vis. FTIR FESEM-EDAX XRD	Size—~38 nm Shape—flake Structure—-FCC	
50	Gopinath *et al.* (2012) [111]	Filtered aqueous extract of *Tribulus terrestris L* dried fruit	1 mM, room temp, dark, 100 ml/150 ml, Static	UV-Vis FTIR TEM XRD AFM	Size-16-28 nm Shape-sph. Structure-FCC.	Stability-6 months. Antibacterial to *S. pyogens*, *P. aeruginosa*, *E. coli*, *S. aureus and B. subtilis*
51	Vijayaraghavan *et al.* (2012) [65]	Filtered aqueous extract of *Trachyspermum ammi and Papavera somniferum* plant powders	1 mM, *Trachyspermum ammi*: 15 min, *Papavera somniferum*: 35 min, 28˚C, 1 ml/50 ml, Shaking	UV-Vis SEM-EDAX	*Trachyspermum ammi:* Size—87 - 998 nm Shape—tri *Papavera somniferum:* Size—3.2 - 7.6 µm Shape—sph.	Essential oil in *T. ammi* was found to be good reducing agent when compared to alkaloids in *P. somniferum.*

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52	Sreekanth *et al.* (2012) [69]	*Dioscorea batatas* rhizome powder	1 mM, 25 and 80˚C Static, 20 min at 5000 rpm	UV-Vis FTIR SEM XRD	Shape—circular and flower Structure—FCC Nature—MD	
53	Chaudhary *et al.* (2012) [112]	Aqueous filtrate of *Vitis viniera* fruit	1 mM, 10 hr, room temp. 10 ml/90 ml, Static.15 min at 2000 rpm	UV-Vis SEM XRD	Size—10 - 880 nm Shape—sph Structure—FCC, cubic and hexl	Antibacterial to *B. subtilis, E. coli*, *P. aeruginosa* and *S. pnemoniae*
54	Ashok kumar (2012) [113]	Aqueous filtrate of *Prathemium hysterophorus* plant	1 mM, 24 hr, room temp. 1 ml/9 ml, Static, 20 min at 5000 rpm	UV-Vis FTIR SEM XRD	Size—avg 10 nm Shape—nearly sph Structure—FCC	
55	Patil *et al.* (2012) [33]	Filtered aqueous extract of *Ocimum tenuiflorum* leaf	1 mM, 10 min, room temp. 2 ml/20 ml, Static	UV-Vis TEM PS ZP	Size—15-25 nm Shape-sph Structure—FCC	Antibacterial against *E. coli*, *C. bacterium*, *B. subtilis*
	Arunachalam *et al.* (2013) [114]	Indigofera aspalathoides, aqueos leaf t extracts		UV Vis SEM EDAX FTIR	Size—20 - 50 nm	Water-soluble organics leaf extract responsible to reduction. Wound healing applications
56	Mubarakali *et al.* (2011) [115]	Filtered aqueous extract of *Mentha piperita* plant powder	1 mM, 24 hr, 28˚C, 1.5 ml/30 ml, Static, 10 min at 6000 rpm	UV-Vis FTIR SEM EDS	Size—90 nm Shape—sph.	Active against clinically isolated human pathogens like *E. coli* and *S. aureus.*
57	Mukunthan *et al.* (2011) [32]	Aqueous extract of *Catharanthus roseus* leaf	1 mM, 15 min, 80˚C, 10 ml/90 ml, Static	UV-Vis SEM XRD EDAX	Size—48 - 67 nm Structure—FCC Nature—uniform	Antibacterial activity against *S. aureus*, *E. coli*, *K. pneumoniae*, *B. aureus and P. aeruginosa*
58	Rajakumar and Abdul Rahuman (2011) [70]	Filtered aqueous extract of *Eclipta prostrate* leaf	1 mM, 1 hr, room temp. 12 ml/88 ml, 45 min at 10000 rpm, Static	UV-Vis FTIR SEM TEM XRD	Size—35 - 60 nm Shape—TEM: sph. SEM: triang, hex and pentagon Structure—crystalline Nature—biphasic	Stabile for 6 hr Larvicidal to filariasis vector *C. quinquefasciatus* and malarial vector *A. subpictus*
59	Kumar and Yadav. (2011) [116]	Filtered aqueous extract of *Lonicera japonica L* leaf.	1 to 9 mM, 24 hr, 40˚C - 80˚C, 5% to 40% (v/v), Static, 5 min at 10000 rpm	UV-Vis FTIR SEM TEM AFM ZP	Size—36 - 72 nm Shape—sph, plate, and other shaped	Stability: zeta potential—41mV
60	Gnanadesigan *et al.* (2011) [117]	Filtered aqueous extract of *Rizophora mucronata* leaf	1 M, 10 min, room temp. 10 ml/90 ml, 20 min at 12000 rpm, Static	UV-Vis FTIR XRD AFM	Size—60 - 95 nm Shape—sph. Structure—cryst	Larvicidal to *Ae. aegypti and Cx. quinquefasciatus*
61	Rani and Reddy (2011) [118]	Decanted aqueous extract of *Piper betel L.* leaf	1 mM, 1 min to 2 hr, room temp, sunlight, 10 ml/190 ml, Static, 15min at 6000 rpm.	UV-Vis FTIR TEM XRD	Sunlight: 5min. Size—~120 nm Shape—irregular Structure—FCC Nature—agglomerated Sunlight: 10 - 80 min Size—28 - 17 nm Shape—sph. Structure—FCC Nature—shelled AgNP	AgNP toxic to aquatic plant *D. magna.* Biosynthesized AgNP less toxic compared to chemically synthesized ones
62	Veerasamy *et al.* (2011) [119]	Aqueous filtrate of *Garcinia mangostana* leaf	0.25 - 5 mM, 0 - 70 min, 37˚C - 90˚C, 5 ml/95 ml, Static, pH—4, 7, 8 30 min (5k rpm)	UV-Vis FTIR TEM	Size—avg 35 nm Shape—sph	Stable for 30 days, Antibacterial against *E. coli* and *S. aureus*
63	Santoshkumar *et al.* (2011) [120]	Decanted aqueous filtrate of *Nelumbo nucifera* leaf	1 mM, 10 min, room temp. 12 ml/8 ml, Static	UV-Vis FTIR TEM XRD	Size—25 - 80 nm Shape—sph, tri and dec Structure—FCC	Larvicidal against *A. subpictus* and *C. quinquefasciatus*
64	Ahmad *et al.* (2011) [121]	Aqueous extract of *Desmodium triflorum*	0.025 M, 1 hr	UV-Vis TEM XRD	Size—5 - 20 nm Structure—cryst	Antibacterial against *S. spp, E. coli*, *B. subtilis*
65	Prathna *et al.* (2011) [122]	Filtered and centrifuged juice of *Citruslimon* fruit	0.1 - 10 mM, 4 hr, 30˚C, 1:4 to 4:1, Shaken, 10 min at 10000 rpm	UV-Vis XRD TEM FTIR AFM DLS ZP	Size—~50 nm Shape—nearly sph. Structure—cryst Nature—PD	AgNPs were stable for 14 days. Size-XRD-18.306 nm AFM—<100 nm TEM—25 - 50 nm DLS—153.68 nm
66	Bankar *et al* (2010) [50]	Acetone treated, aqueous extracted, filtered and precipitated powder of Banana peel	0.125 to 1mM, 3 min, 40˚C to 100˚C, 0.5 to 10 mg/2 ml, pH: 2 – 5, Static	UV-Vis FTIR. SEM-EDS XRD	Size—< 100 nm Structure—FCC	Antifungal and antibacterial action
67	Njagi *et al.* (2010) [123]	Filtered aqueous extract of Sorghum bran	0.1 M, 1min, room temp. 2:1 volume ratio, Shaken	UV-Vis FE-SEM HR-TEM-EDS XRD	Size—10 nm Shape—sph. Structure—FCC Nature—uniform nano clusters	AgNP of smaller size at 50˚C of extraction temperature compared to 25˚C and 80˚C

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68	Kumar *et al.* (2010) [124]	Filtered aqueous extract of *Syzygium cumini* leaf (LE) and seed (SE) powder	1 mM, 24 hr, room temp. 10% (v/v), Static, 20 min, 12k rpm	UV-Vis FTIR SEM AFM	Size—LE: 30nm, Water content of LE: 29 nm, SE: 92 nm, Water content of LE: 73nm.	SE have higher synthesis rates and larger size AgNP compared to LE.
69	Dubey *et al.* (2010) [79]	Filtered aqueous extract of *Tanaetum vulgare* fruit.	1 - 3 mM, 10 min - 5hr, 25˚C - 150˚C, 0.5 - 4.8 ml/50 ml, pH: 2 - 10, Static	UV-Vis FTIR TEM XRD EDAX	Size—10 - 40 nm Shape—sph. Structure—FCC	AgNP more stable in basic compared to acidic medium
70	Shukla *et al.* (2010) [37]	Filtered aqueous extract of *Piper nigrum* (black pepper)	10 mM, room temp. 1 ml/100 ml, Stirred, 10 min at 3000 rpm	UV-Vis TEM XRD	Size—20 - 50 nm Shape—sph. Structure—FCC Nature—large grain, WD, uniform and polycrystalline	
71	Krishnaraj *et al.* (2010) [125]	Aqueous filtrate of *Acalypha indica* leaf	1 mM, 30 min, 37˚C, dark 12 ml/100 ml, Static, 30 min at 75000 g	UV-Vis SEM TEM EDS XRD	Size—20 - 30 nm Structure—cub	Antimicrobial against water borne pathogens *E. coli* and *Vibrio cholera*
72	Satish kumar *et al.* (2009) [38]	Aqueous bark and powder extracts of *Cinnamon zeylanicum* plant	1 mM, 25˚C, 1 to 5 ml/50 ml, Powder content: 0.1 to 1 g/50 ml, pH: 1 - 11, Shaken	UV-Vis TEM XRD EDX	Size—powder: 31 nm, Extract: 40 nm Shape—quasi sph and R, Structure—cub and hex Nature—bi-phasic	Stable for 3 months, Served as antimicrobial agents
73	Tripathi *et al.* (2009) [34]	Aqueous filtrate of *Azadirachta indica* leaves	10 mM, 24 hr, 28˚C, 1:4. 15 min at 10,000 rpm Shaken	UV-Vis TEM SEM FTIR	Size—50 - 100 nm Shape—irregular Nature—PD	AgNPs loaded on cotton disks shown antibacterial activity.
74	Leela and Vivekanandan. (2008) [126]	Aqueous extract of *Helianthus annus* plant		UV-Vis XRD SEM	Structure-cryst	
75	Chandran *et al.* (2006) [30]	Aqueous extract of *Aloe vera* leaf	1 mM, 24 hr, room temp. 5 ml/5 ml, Static	UV-Vis XRD TEM	Size—15.2 ± 4.2 nm Shape—sph. Structure—FCC	
76	Ankamwar *et al.* (2005) [35]	*Emblica Officinalis* fruit extract		UV-Vis TEM	Size—10 - 20 nm	Transmetallation reaction promoted the AgNPs synthesis
77	Shankar *et al.* (2004) [127]	Decanted aqueous extract of *Azadirachta indica* leaf	1 mM, 24 hr 5 ml/45 ml, 15 min at 10000 rpm. Static	UV-Vis XRD TEM FTIR	Size—5 - 35 nm Shape—Sph Structure—cryst Nature—PD	AgNPs stable for 4 weeks
78	Shankar *et al.* (2003) [42]	Decanted aqueous broth of *Pelargonium graveolens* leaf	1 mM, 24 hr 5 ml/100 ml, 15 min at 10000 rpm. Static	UV-Vis XRD FTIR TEM EDAX	Size—16 - 20 nm. Shape—nearly sph Structure—FCC Nature—PD	Chlorophyll of leaf extract formed 5 nm capping around the AgNP.
			Fungi			
79	Das *et al.* (2012) [76]	Mycelia of *Rhizopus oryzae*	1 to 5 mM, 72 hr, 30˚C, 0.2 g/25 ml. pH—2 to 8, Shaken	UV-Vis FTIR HRTEM EDAX	Size—~15 nm Shape—sph. Structure—FCC	Stable for 3 months, Antimicrobial to *E. coli* and *B. subtilis*, Used for treating contaminated water and adsorption of pesticides
80	Naveen *et al* (2010) [128]	Aqueous cell filtrate of *Penicillium Sp.* fungi	1 mM, 24 hr, room temp, dark 50 ml/50 ml, Agitated, Lyophilized	UV-Vis FTIR AFM	Size—52 - 104 nm	
81	Balaji *et al* (2009) [129]	*Cladosporium clado sporioides* fungal aqueous filtrate	78 h, 27˚C. 10 ml, Shaken	UV-Vis TEM XRD FTIR	Size—Avg: 35 nm Shape—Sph. Structure—FCC Nature—PD	
82	Shaligram *et al* (2009) [130]	*Penicillium brevicompatum* WA 2315 fungal aqueous filtrate	1 mM, 72 hr, 25˚C, Shaken	UV-Vis FTIR TEM XRD	Size—58.35 ± 17.8 nm Structure—FCC	
83	Fayaz *et al.* (2009) [82]	Harvested cell aqueous filtrate of *Trichoderma viride* fungus	1 mM, dark, 10˚C - 40˚C. Shaken.	UV-Vis XRD TEM FTIR	10˚C: 2 - 4 nm, sph. 27˚C: 10 - 40 nm, sph. 40˚C: 80 - 100 nm, Plate like, Structure: Cryst, Nature: MD	Increase in temperature led to blue shift in UV-Vis peak, decreased size and increased dispersity
84	Kathiresan *et al.* (2009) [131]	Aqueous Cell filtrate of *Penicillum fellutanum* fungus	0.5 - 2.5 mM, 0 - 48 hr, 0˚C - 40˚C, dark, pH: 5 - 7.5. Salinity-1% - 5% NaCl, Shaken	UV-Vis TEM	Size—5 -2 5 nm Shape—Sph.	$(NH_4)_2SO_4$ solid used for precipitation and phosphate buffer (pH-8) for dissolution of nanoparticles
85	Ingle *et al* (2009) [57]	Aqueous cell filtrate of *Fusarium solani* fungus	1mM, room temp. Static, 10 min, 10000 g	UV-Vis FTIR TEM	Size—5 - 35 nm Shape—Sph.	
86	Basavaraja *et al* (2008) [132]	Aqueous filtrate of *Fusarium semitetum* fungus	1 mM, 48 hr, 27˚C, Shaken	UV-Vis XRD TEM FTIR	Size—10 - 60 nm Shape—Sph. Structure—cryst Nature—PD	AgNP stable for 6 - 8 weeks

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87	Vigneswaran *et al*. [66]	*Asphergillus flavus* fungal cells	1 mM, 24 hr, 37˚C, Dark. 5 g/100 ml, Shaken	UV-Vis TEM XRD FTIR FS	Size—8.92 ± 1.61 nm Shape—Isotropic Structure—FCC Nature—MD	AgNP stable for 3 months
88	Bhainsa and D'souza (2006) [54]	*Aspherillus fumigates* aqueous cell filtrate	1 mM, 1 hr, 25˚C, Dark, Shaken	UV—Vis TEM XRD	Size—5 - 25 nm Shape—Sph and Tri. Structure—Crystal Nature—WD	No precipitation of AgNP observed upto 72 hrs
89	Vigneswaran *et al*. [67]	*Phaenerochaete chrysosporium* mycelium	1 mM, 24 hr, 37˚C, Dark, Shaken	UV-Vis XRD SEM TEM FS	Size—50 - 200 nm Shape—sph. and hex. Structure—FCC Nature—non uniform	AgNP formed on the surface of mycelium
90	Duran *et al* (2005) [56]	Aqueous filtrate and biomass of *Fusarium oxysporum* species.	1 mM, 28 hr, 28˚C. 10 g/100 ml Static.	UV-Vis SEM	Size—20 - 50 nm Shape—sph.	Nitrate based reductase promoted the AgNP synthesis
91	Senapati *et al*. (2004) [133]	*Verticillium* and *F. oxysporum*		UV-Vis SEM/TEM	Size—*Verticillium* 25 ± 8 nm, *F. oxysporum*—5 - 50 nm	*Verticillium* (intracellular) and *F. oxysporum*—extracellular synthesis.
92	Ahmad *et al*. (2003) [55]	*Fusarium oxysporum* biomass	1 mM, 72 hr, room temp, dark 10 g/100 ml, Static	UV-Vis XRD TEM FTIR FS	Size—5 - 50 nm Shape—sph/tri. Structure—FCC	
93	Mukherjee *et al*. (2001) [52]	Harvested mycelia of *Verticillium sp.* fungi	0.2 mM, 72 hr, 28˚C, 10 g/100 ml, Shaken, pH: 5.5 - 6	UV-Vis SEM TEM EDAX	Size—25 ± 12 nm Shape—nearly sph, Nature—monodispersed	AgNPs were synthesized on intracellular bases.
			Gram positive Bacteria			
94	Zhang *et al*. (2014) [134]	*Lactobacillus fermentum*.LMG 8900 cells	10 g/L, 24 hr, 30˚C, 10 g/L, Shaken, 6 min at 5000 rpm and 10 min at 6000 rpm	UV-Vis TEM XRD ZP	Size—~6 nm Shape—sph. Structure—FCC	Stable for 3 months. Resist growth of *E. coli*, *S. aureus* and *P. aeruginosa* Act as promising anti-biofouling agent
95	Zonnoz and Salouti (2011) [83]	Aqueous cell filtrate of *Streptomyces sp. ERI*-3	1 mM, 48 hr, 28˚C. Dark. Shaken.	UV-Vis XRD TEM SEM	Size—10 - 100 nm Shape—Spherical	After 3 months, nanoparticles developed floret shape
96	Deepak *et al*. (2011) [135]	Fibrinolytic URAK enzyme produced by *Bacillus cereus* NK1	1 mM, 24 hr without NaOH and 5 min with NaOH, 37˚C, URAK content: 1 mg, additives: 10 ml of Tris-Hcl buffer of pH 9	UV-Vis TEM XRD AFM	Size—50 - 80 nm Shape—sph. Structure—FCC Nature—WD	AgNP with mmobilized enzyme
97	Kalishwarlal *et al* (2010) [136]	*Brevibacterium casei* harvested cells	1 mM, 24 hr, 37˚C, 1 g, Shaken, 30 min at 16000 g	UV-Vis TEM XRD FTIR FS	Size—10 - 50 nm. Shape—Sph. Structure—FCC	AgNP act as stable anti-coagulant
98	Ganeshbabu and Gunasekaran (2009) [137]	Isolated and harvested *Bacillus cereus* PGN1 cells.	1 mM, 120 hr, 37˚C. 10 g/100 ml. 15 min at 15000 rpm. Shaken.	UV-Vis FTIR XRD TEM	Size-4-5 nm Shape-Sph. Structure-FCC. Nature-MD.	Tris Buffer (pH-7) as suspension media for nanoparticles
99	Nanda *et al* (2009) [138]	*Staphylococcus aureus* supernatant	1 mM, 5 min	UV-Vis AFM	Size—160 - 180 nm Nature—PD.	AgNP antibacterial action against human pathogenic bacteria MRSA, MRSE, *S. pyogenes*
100	Kalimuthu *et al* (2008) [139]	*Bacillus icheniormis* cells	1 mM, 24 hr, 37˚C, 30 min at 15000 rpm. Shaken	UV-Vis SEM EDX XRD	Size—50 nm Structure—Crystal Nature—WD	
			Gram negative bacteria			
101	Perni *et al* (2013) [58]	*Escherichia coli* cells	1 or 5 mM, 24 hr, 30˚C, Ratio of $AgNO_3$: L-cysteine = 1:5, Shaken, 10 min at 1851 g	UV-Vis FTIR TEM TGA	Size—~5 nm	Capping agent: L-cysteine, Antimicrobial against *E. coli*and *S. aureus*
102	Juibari *et al*. (2011) [140]	*Ureibacillus thermo sphaerius* supernatant	1 - 100 mM, 24 hr, 60˚C - 80˚C, Dark, 15 min (13 k, rpm Static	UV-Vis DLS XRD FTIR TEM	Size—10 - 100 nm Shape—Sph. Structure—FCC Nature—PD	Temperature around 80˚C stands possible because of thermophilic nature of bacteria
103	Gurunathan *et al*. (2009) [141]	*E. coli* supernatant	1 - 10 mM, 24 hr, 20˚C - 90˚C, pH: 5 - 12, 10 min at 10k rpm Static	UV-Vis DLS TEM FTIR	Size—10 - 90 nm Shape—Sph. Structure—Crystal Nature—Uniform	Nitrate medium (pH-8) is used for culture.
104	Shahverdi *et al* (2007) [59]	*K. pneumonia* (*Enterobacteria*) supernatant	1 mM, 5 min, Room temp.	UV-Vis TEM EDS	Size—Avg: 52.25 nm Shape—Sph.	Nanoparticles are unstable after 5 min. Addition of *piperitone* resisted the nanoparticle growth.
			Bio-polymers			

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105	Cheng *et al.* (2014) [142]	Chondrotin sulfate	1 and 6.25 mM, 3 - 120 hr, 25˚C and 80˚C, 0.8 to 20 mg/l, 10 min at 5000 g, Stirred	UV-Vis FTIR TEM DLS	Size—<20 nm Shape—sph.	Stable for 2 months, Served as nano carrier for drug delivery
106	Chen *et al.* (2014) [143]	*Chitosan* biopolymer		UV-Vis FTIR TEM DLS	Size—~218.4 nm Shape—oval and sph. Nature—Ag/ chitosan nano hybrids	Antimicrobial to *E. coli*, *S. choleraesuis*, *S. aureus and B. subtilis*
107	Tagad *et al.* (2013) [80]	Locust bean gum polysaccharide.	1 - 5 mM, 6 hr, 60˚C, 0.1 to 0.4 (w/v)/25 ml, pH: 4 to 12, Static	UV-Vis AFM	Size—18 - 51 nm	Stability: 7 months, AgNP served in development of H_2O_2 sensor
108	El-Rafie *et al.* (2013) [144]	Crude hot water soluble polysaccharide extracted from different marine algae	0.1 mM, 20 min, 70˚C, 0.3 (mg/ml)/1 ml, pH: 10.10 min at 5000 rpm, Stirring	UV-Vis FTIR TEM	Size—7 - 20 nm Shape—sph	Stability: 6 months, AgNP treated cotton fibers antibacterial to *E. coli* and *S. aureus*
109	Ashraf *et al.* (2013) [77]	Casein milk protein	1 mM, 5 - 10 min, 50˚C - 60˚C, 1-c10 ml/25 ml, pH: 10 - 14, vigorous stirring	UV-Vis FTIR SEM TEM DLS ZP	Size—pH > 7: 3 - 18 nm, pH < 6: 60 - 80 nm. Shape—sph.	Cytotoxocity and cellular uptake of AgNP was studied.
110	Dehnavi *et al.* (2013) [78]	Fructose	10 - 100 ppm, 11 - 100 min, 55˚C - 95˚C, 1(g/L)/9.35 ml, Other contents: Diammonium hydrogen citrate, 1 M ammonium solution, pH: 8.5 to 11.5, stirring	UV-Vis FE-SEM TEM XRD DLS	Size—36 nm Shape—sph. Structure—crystalline Nature—WD and homogenous	Stability for 1 month, Antibacterial to *E. coli and S. aureus*
111	Ortega-arroyo *et al.* (2013) [60]	D-glucose	0.13 to 0.97 M, 1 min, 26˚C - 94˚C, 150 µL (0.1 M)/100 µL, Capping agent-6ml of 1.7 wt%, pH: 7 to 13, Stirred	UV-Vis TEM XRD RS	Size—2 - 24 nm Shape—sph and polyhedral Structure—FCC Nature—homogenous WD	Smaller particle range of silver nanoparticles are observed at 0.55M D-glucose, pH-11 and temperature > 70˚C.
112	Lu *et al.* (2012) [145]	Egg white extract	10 mM, 72 hr, room temp. 1 ml/2 ml, Vigorous stirring, 15 min, 15k rpm	UV-Vis FTIR TEM DLS	Size—~20 nm Shape—sph Structure—Cryst	Silver nanoparticle conjugate is used in cancer radiation therapy.
113	Guidelli *et al.* (2012) [146]	DL-Alanine	Ag/alanine ratio (%): 0.045 to 0.36, 40 min, 100˚C. vigorous stirring.	UV-Vis FTIR TEM XRD	Size-~7.5 nm Shape-sph. Structure-FCC	Nanoparticle stands applicable for ESR-Dosimetry.
114	Tanvir *et al.* (2012) [147]	Co-enzyme (β-NADPH)	0.31 - 10 mM, 20˚C. 1:1 to 3:1. Stirring, 30 min at 15000 rpm	UV-Vis TEM XRD DLS ZP EDAX	Size—20.77 ± 0.67 nm Shape—sph. Structure—FCC Nature—narrow and MD	Stabile for 2 months, The reagent used for the synthesis of nanoparticles can be regenerated.
115	Bankura *et al.* (2012) [148]	Dextran	0.01 M, room temp. 5%, Additive: 0.4 ml of 0.001 M NaOH, static	UV-Vis TEM XRD EDAX AFM	Size—5 - 60 nm Shape—sph. Structure—FCC Nature—WD	Stable for 1 months, Antimicrobial to *B. subtilis*, *B. cereus*, *E. coli*, *S. aureus, P. aeruginosa*
116	Sasikala *et al.* (2012) [149]	Soyabean protein	1 mM, 24 hr, room temp. 1 g/100 ml, 10 min at 10000 rpm, Static	UV-Vis FTIR HR-SEM HRTEM XRD EDAX	Size—7 - 29 nm Shape—sph. Structure—FCC Nature—WD	Protein of 51 kDa was responsible for the formation of AgNP formation.
117	Morales-Sanchez *et al.* [61]	Albumin	30 mM, 24 min, room temp. Additive: Ammonium hydroxide (pH: 11), Stirred	UV-Vis TEM TGA DLS	Size—~26 nm Shape—sph.	Stable for 6 months
118	El-rafie *et al.* (2011) [81]	Hydropropyl starch	100 - 750 ppm, 15 - 90 min, 30˚C - 90˚C, 9 g/l with 0.84 molar substitutions, pH: 2 - 12, Stirring	UV-Vis TEM	Size-6-8 nm	Stable for 6 months, More reduction at higher pH, rate increased rate with temp; particle aggregation with time
119	Philip (2010) [21]	Honey	1 mM, 1 min, 15 ml/20 ml, pH: 6.5 - 8.5, Stirred	UV-Vis FTIR HR-TEM XRD	Size—4 nm Shape—sph. Structure—FCC Nature—MD	Stabile for 6 months, NaOH is added for pH adjustment
120	Kora *et al.* (2010) [62]	Gum kondagogu (*Cochlospermum gossypium*)	1 - 5 mM, 10 - 60 min, 121˚C, 15 psi, 0.1 - 0.5(w/v), gum mean particle size: 30 - 300µm, Static	UV-Vis TEM XRD TGA	1 mM $AgNO_3$, (0.1) and (0.5) w/v% gum: Size—30 min—(55) and (11.2) nm; 60 min—(18.9) and (4.5) nm Shape—(R, hex) and (sph). Structure—FCC Nature—PD, WD	Anti-bacterial to *S. aureus*, *E. coli*, and *P. aeruginosa*

Note: DLS—Dynamic light scattering, EDAX/EDS Energy Dispersive X-ray Analysis/Energy Dispersive Spectroscopy; FTIR—Fourier transform infrared spectroscopy, HRTEM—High Resolution Transmission Electron Microscopy; SEM—Scanning Electron Microscopy, TGA—Thermogravimetric analysis, UV-Vis—Ultra violet-visible spectroscopy; XRD—X Ray Diffraction, DEC—decahedral, sph—spherical, Tri—Triangular, R—Rod, Hex—Hexagonal, PD—Polydispersed, MD—monodispersed, WD—Well Dispersed, Cryst—Crystalline.

[32] [62] [75] [104] [115] [148]. In the Well diffusion method instead of using discs, small disc shaped pits are created on the agar plate for filling the test solution. In both the techniques, the microbe inoculated plates are incubated under standard condition for the formation of clear inhibition zone. The inhibition zone diameter around the disc or well, directly relates the effects of AgNPs on the chosen microbe.

7.1.2. Minimum Inhibitory Concentration (MIC)/Minimum Bactericidal Concentration (MBC)

The MIC is defined as the minimum concentration of the analyte which inhibit 100% visible growth of the targeted microbe after 24 hours. The MIC is determined by monitoring growth of bacteria in culture tubes inoculated with the same amount of bacterial culture but increasing concentration of AgNPs in the growth medium. The minimum concentration of AgNP which checks growth of bacteria is called the minimum inhibitory concentration. For the determination of MBC, fixed AgNP concentration greater than MIC value is added to the nutrient mediums containing increasing bacterial inoculum and bacterial growth is monitored, using UV-Vis spectroscopy or plate analyzer, for change in the optical density of the samples [58] [134] [142]. The broth dilution test is also used to conduct MIC and MBC analysis, in which the results after experimentation are compared with a standard data [96] [98].

7.1.3. Analysis of SEM and TEM Micrographs

The SEM and TEM analyses have been used to monitor changes in the morphology of the bacterial cell before and after treatment with "AgNPs"; The visible alterations in the cell shape and perforations in the cell wall have been reported and used as indicator of the antimicrobial action of AgNPs by several workers [45] [134] [142].

7.2. Antibacterial Action

The AgNPs have potent antibacterial action against gram positive bacteria, *Lactobacillus fermentum* [134], *Streptomyces* sp. [83]. *Bacillus cereus* [135] *Brevibacterium casei* [136], *S. aureus* [138] *B. licheniromis* [139], and gram negative bacteria, *E. coli* [58] *Entrobacteria* [59] and *Ureibacillus thermo sphaerius* [140]. The antibacterial action of AgNPs on gram positive and gram negative bacterial strains is not the same but competes one over the other. There are contradictory reports regarding antibacterial action against gram positive and gram negative bacteria. According to some researchers the gram negative bacteria are reported to be more sensitive to AgNPs compared to gram positive bacteria [32] [78] [111] [134] whereas reverse results were observed by other researchers [62] [75] [76] [98]. The reported differential sensitivity of both the bacterial species could be attributed to the difference in structural characteristics of the bacterial species [62] [111] as well as shape and size of AgNP, bacterial inoculum size, exposure time and nutrient medium used during analysis of antibacterial action [98].

The anti-bacterial action of AgNPs is quite complex and not well studied. Its mechanism is onlytentatively explained. The antimicrobial action of AgNPs can be categorized in two types: the inhibitory action and bactericidal action. In the former strategy bacterial cells are not killed but their division is prevented whereas in the later bacterial cells will die due to the action of AgNP [58]. The antibacterial action mechanism of AgNP is summarized in **Figure 4**. The graphical presentation shown in **Figure 4** is the result of bacterial growth loaded with AgNPs synthesized from different green sources. Probable mechanism leading the differential behavior in the cases "a" to "e" is shown on the right hand part. The reason behind the bacterial cells resuming their growth after certain period of inhibitory action in cases "b", "c", "d" respectively was assumed to due to the unaffected cells, which in turn promote the growth (figure shown in inset). On the other hand a complete inhibition/bactericidal effect as in the case "e" is attributed to the complete death of cells. A shift from inhibitory action to nearly bactericidal action was observed with an increase in concentration of AgNPs loading [78] [134]. The experimental support in the form of morphological changes and perforations in cell wall has been presented as shown in **Figure 5**. The mechanism behind the bactericidal action of AgNP was illustrated by release of Ag^+ ions, which serves as reservoirs for anti-microbial action [111]. The Ag^+ cations produced interacts with the negative charge on the cell wall and affects the membrane permeability. The nano-silver cations which have greater affinity towards sulphur and phosphorus containing compounds present in the outer membrane, respiratory enzymes, proteins and DNA, penetrate through the cell wall and plasma membrane by destabilizing them and cause protein denaturation by dissipating proton motive force, respiratory inhibition, intracellular ATP depletion

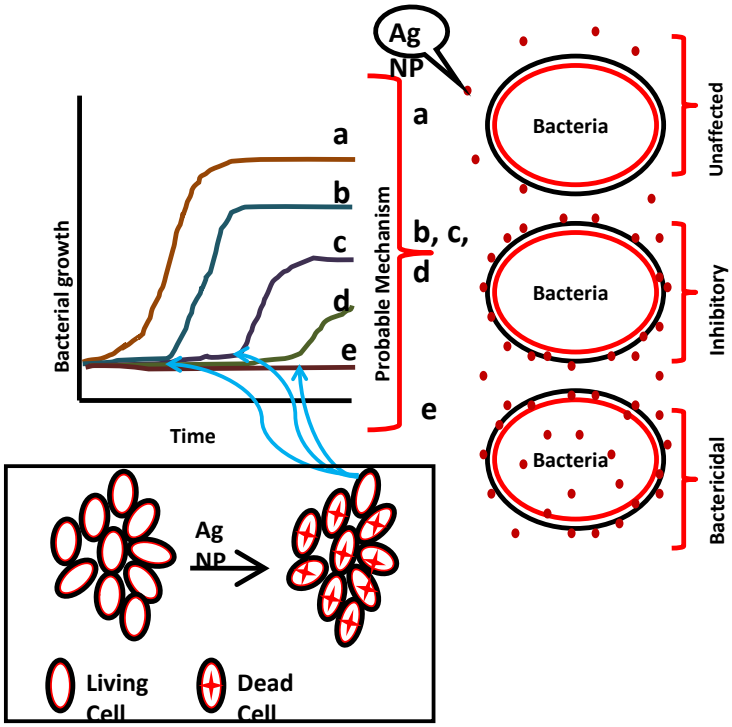

Figure 4. Mechanism of antibacterial action of AgNPs.

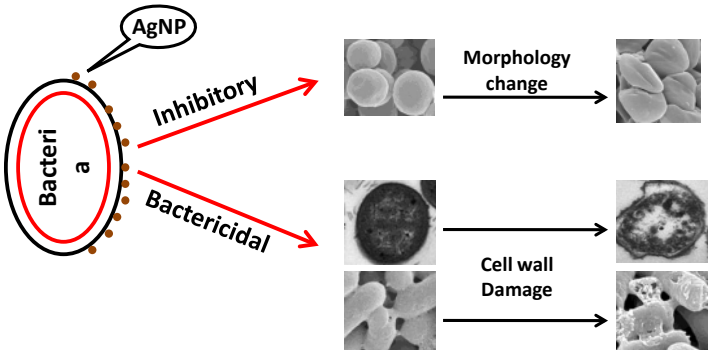

Figure 5. Morphological change and cell wall damage of bacterial cell.

and DNA damage. The above stated mechanism is in agreement with the reports of many authors [64] [72] [75] [78] [95] [98].

7.3. Anti-Fungal Action

The AgNPs exhibited antifungal action against various fungi [50] [98]. Actual mechanism behind the antifungal activity is not fully. The disrupting the structure of the cell membrane by destructing the membrane integrity, thereby the inhibition of the budding process has been attributed to be responsible for the antifungal action of AgNPs against *C. albanicans* species [150]. The shape of the AgNPs has a significant effect on the anti-microbial activity [151]

7.4. Anti-Parasitic Action

The AgNPs have been found to be effective larvicidal agents against dengue vector *Aedes aegypt* [96], and *Culex quinquefasciatus* [39], filariasis vector *C. quinquefasciatus* [120] and malarial vector *A. subpictus* [70], *Aedes aegypti* [116], *A. subpictu* [120] and other parasites [36] [152]. No attempt has been made to propose a

proper mechanism for anti-parasitic action of AgNPs. Denaturation of sulfur containing proteins and phosphorus containing DNA by AgNPs, leading to denaturation of organelles and enzymes is believed to be responsible for the larvicidal activity [117].

7.5. Anti-Fouling Action

The AgNPs synthesized from *Rhizopus oryzae* fungal species have been used for treating contaminated water and adsorption of pesticides [76] and that from *Lactobacillus fermentum* cells have been used as anti-bio fouling agent [134]. The AgNPs are being used to treat many environmental concerns like; air disinfection, water disinfection, ground water and biological water disinfection and surface disinfection [153].

7.6. Other Applications

There have been several reports on the use of AgNPs in the field of medicine. The AgNPs have been used as therapeutic agents [97], as glyconano sensors for disease diagnosis [63] and as nano carriers for drugs delivery [142]. Reports are also available on the use of AgNPs in radiation therapy [145], in H_2O_2 sensor [80], in ESR-Dosimetry [146], as heavy metal ion sensors [110] and as catalyst for reduction of dyes such as methylene blue [31].

8. Conclusion

Sufficient volume of published literature is available on the synthesis of AgNPs through green routes. Among plants, angiosperm species has been widely used in comparison with the other sources. Several characterizations methods and techniques have been used for AgNPs synthesis and confirmation. The AgNPs synthesized using biological reducing and capping agents have shown wide variation in shape and size. Among applications, the anti-microbial action of AgNPs has been widely studied. Various methods used to carry out antibacterial study and elucidate mechanism of anti-microbial have been developed. The results, however, are conflicting and there is a need for more work to resolve this issue. The potential of AgNPs for their use as drug carriers in cancer therapy, as biosensors for metabolites and pollutants, as catalyst etc. is quite high and requires intensive and integrated research activity for harnessing it.

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