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...
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 early 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 Ayurvedic medicine book medicinal literature named “Charak Samhita” [5]. Until the discovery of antibiotics by Alexander Flemming, silver was commonly used as antimicrobial agent.

In the recent past, silver nanoparticles (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 devices, 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 AgNO₃ 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 of using starch as a capping agent [60].

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 AgNO3 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.
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.
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-microbial activity. Researchers have used several novel techniques to confirm and quantify the anti-microbial 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>
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<td>1 mM, 30 min, room temp. 40 ml/200 ml, 15 min at 10000 rpm, Stirring</td>
<td>UV-Vis TEM XRD EDAX</td>
<td>Size—&lt;60 nm Shape—ph. Structure—cryst</td>
<td>Antibacterial nature</td>
</tr>
<tr>
<td>34</td>
<td>Zhang et al. (2013) [101]</td>
<td>Filtered aqueous extract of <em>Aloe vera</em></td>
<td>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</td>
<td>UV-Vis TEM XRD</td>
<td>Size—&lt;20 nm. Shape—ph. Structure—FCC</td>
<td>Antibacterial to <em>E. coli</em> and <em>S. aureus</em></td>
</tr>
<tr>
<td>No.</td>
<td>Authors (Year)</td>
<td>Method/Plant</td>
<td>Parameters</td>
<td>Characterization</td>
<td>Antibacterial/Prophylactic Properties</td>
<td></td>
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<tr>
<td>35</td>
<td>Yang et al. (2013) [74]</td>
<td>Filtered aqueous extract of Mangifera indica linn peel</td>
<td>0.5 to 4 mL, 15 to 90 min, 25 to 100°C, 0.1 to 0.2 mL/27 mL, pH: 2 - 11, Static</td>
<td>UV-Vis TEM XRD XRD XRD</td>
<td>Size—7 - 27 nm Shape—sph. Stability for 3 months, AgNPs loaded on fabrics exhibited antimicrobial property. Anti-bacterial to B. cereus, R. sballi, S. aureus and P. aeruginosa. AgNP-lectin hybrid has promising use in glycol nanoformers for disease diagnosis.</td>
<td></td>
</tr>
<tr>
<td>36</td>
<td>Jagtap and Bapat. (2013) [64]</td>
<td>Filtered aqueous extract of Aristocarpus heterophyllus lam. Seed powder</td>
<td>2 to 10 mL, 5 min, 121°C, 15 psi 2 to 10 %w/v, 1-4, Static, 15 min at 10000 rpm</td>
<td>UV-Vis FTIR SEM-EDAX TEM</td>
<td>Size—3 - 25 nm Shape—irregular Stability: 1 week, AgNPs inhibited growth of E. coli, S. aureus and P. aeruginosa</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Khalil et al. (2013) [75]</td>
<td>Filtered aqueous extract of olive leaf</td>
<td>1 mL, 2 min, 30°C to 90°C, 0.5 to 5 mL/10 mL, pH: 2 - 11, Stared</td>
<td>UV-Vis FTIR SEM TEM XRD XRD XRD</td>
<td>Size—20 - 25 nm Shape—sph. Stability—FCC</td>
<td></td>
</tr>
<tr>
<td>38</td>
<td>Karuppuiah and Rajmohan (2013) [102]</td>
<td>Filtered aqueous extract of Lxora coccinea L. leaf</td>
<td>1 mL, dark and room temp. 0.5 mL/10 mL, 1 min at 10000 rpm, Static</td>
<td>UV-Vis FTIR FE-SEM XRD XRD TGA</td>
<td>Size—13 - 57 nm Shape—sph. Structure—FCC</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>Logeswari et al. (2013) [103]</td>
<td>Filtered ethanolic extract of Solanum trivobacum, cyssicum camini, centella asiatica and citrus sinensis plant powders</td>
<td>1 mL, 24 - 48 hr, 37°C, 10 mL/5 mL, Additive: ammonium solution= 2.5 mL, agitated</td>
<td>UV-Vis FTIR XRD AFM</td>
<td>Size—41 - 53 nm. Shape—irregular Structure—FCC Antibacterial against pathogenic P. aeruginosa</td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>Geetha lakshmi and Sarada (2013) [104]</td>
<td>Sponion extracted from Trialthema decedru L.</td>
<td>1 mL, dark and incubated, 1 mL/5 mL, Static, 15 min at 10000 rpm</td>
<td>UV-Vis FTIR FE-SEM EDAX TEM</td>
<td>Size—17.9 - 59.6 mm. Shape—sph.</td>
<td></td>
</tr>
<tr>
<td>41</td>
<td>Yasin et al. (2013) [105]</td>
<td>Filtered aqueous extract of Bamboo leaf</td>
<td>3 mL, 65°C, 5 mL/5 mL, Stirring</td>
<td>UV-Vis TEM XRD EDX EDX XRD</td>
<td>Size—13 ± 3.5 nm Shape—nearly sph. Structure—cryst Antimicrobial against E. coli and S. aureus</td>
<td></td>
</tr>
<tr>
<td>42</td>
<td>Rodriguez-Leon et al. (2013) [106]</td>
<td>Ethanol/aqueous extract of Rhus homoeospergal root</td>
<td>2.5 - 15 mL, 24 - 96 hr, room temp, 5% v/v, Static</td>
<td>UV-Vis TEM EDM XRD TEM</td>
<td>Size—2 - 40 nm Shape—cub and hex Structure—FCC AgNPs synthesized in ethanol medium. Antibacterial to S. aureus, V. cholerae, M. luteus and K. pneumonia</td>
<td></td>
</tr>
<tr>
<td>43</td>
<td>Rajathi and Sridhar (2013) [107]</td>
<td>Decantated aqueous filtrate of Wrightia tinctoria leaf</td>
<td>1 mL, 2 hr, room temp, 0.5 mL/10 mL, Static, 10 min at 10000 rpm</td>
<td>UV-Vis FTIR TEM XRD</td>
<td>Size—2.5 - 20.5 nm Structure—cryst Antibacterial to P. aeruginosa, E. fasciculata, S. typhi, K. pneumonia, E. coli and C. albicans</td>
<td></td>
</tr>
<tr>
<td>44</td>
<td>Kuman et al. (2013) [108]</td>
<td>Filtered aqueous extract of codium captivea sea weed powder.</td>
<td>1 mL, 0 - 60 min, 37°C, 100°C, 5 mL/95 mL, 5 min at 5000 rpm, Static</td>
<td>UV-Vis FTIR TEM SEM-EDAX</td>
<td>Size—3 - 44 nm Nature—nano-clusters Fresh extract was more potent for AgNP synthesis.</td>
<td></td>
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<tr>
<td>45</td>
<td>Natarajan et al. (2013) [109]</td>
<td>Powdered Elannga indica leaves</td>
<td>0.5 - 2 mL, 20 - 60 min, 40°C - 100°C, 10 g/3 mL, Static</td>
<td>UV-Vis FTIR TEM DLS XRD XRD XRD</td>
<td>Size—avg 30 nm Shape—sph. Nature—MD Structures—FCC Antimicrobial against E. coli, P. putida, B. subtilis, S. aureus, A. flavus and P. oxysporum</td>
<td></td>
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<tr>
<td>46</td>
<td>Kirubaran et al. (2012) [110]</td>
<td>Filtered aqueous extract of Asdiuchilie indica(nom) leaves</td>
<td>1 mL, 90 min, room to 90°C, 1.25 mL/50 mL, pH: 6 - 8, Stired</td>
<td>UV-Vis TEM TEM XRD XRD</td>
<td>Size—15 - 20 nm Shape—sph. Structure—FCC Stability: 6 months. Heavy metal ion sensors in aqueous media</td>
<td></td>
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<tr>
<td>47</td>
<td>Satishkumar et al. (2012) [72]</td>
<td>Filtered aqueous extract of Morinda citrifolia L. leaf powder</td>
<td>1 mL, 0 - 60 min, 37°C, 100°C, 5 mL/95 mL, 5 min at 5000 rpm, Static</td>
<td>UV-Vis FTIR TEM SEM HR-TEM</td>
<td>Size—10 - 60 nm Shape—sph. Structure—FCC Stability 1 month, Inhibitory to human pathogens like E. coli, P. aeruginosa, K. pneumoniae, B. cereus, Enterococci spp. and Enterobacter aerogenes</td>
<td></td>
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<td>48</td>
<td>Edison and Sethuraman. (2012) [31]</td>
<td>Filtered aqueous extract of Terminalia chebula fruit powder.</td>
<td>10 mL, room temp, 1 mL/25 mL, pH: 4 - 9, Static</td>
<td>UV-Vis TEM HR-TEM XRD DLS ZP</td>
<td>Size—25 nm Shape—flake Nature—phyto capped Stability for 10 days. AgNPs showed catalytic activity on the reduction of methylene blue.</td>
<td></td>
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<tr>
<td>49</td>
<td>Kaviya et al. (2012) [68]</td>
<td>Aqueous filtrate of Crossandra infundibuliformis leaf</td>
<td>1 mL, 1 hr, room temp, 3 mL/40 mL, Stiring, 20 min at 4000 rpm</td>
<td>UV-Vis. FTIR FESEM-EDAX XRD XRD</td>
<td>Size—38 nm Shape—sph. Structure—FCC Stability-6 months. Antibacterial to S. pyogenes, P. aeruginosa, E. coli, S. aureus and B. subtilis</td>
<td></td>
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<tr>
<td>50</td>
<td>Gopinath et al. (2012) [111]</td>
<td>Filtered aqueous extract of Tribulus terrestris L dried fruit</td>
<td>1 mL, room temp, dark, 100 mL/150 mL, Static</td>
<td>UV-Vis FTIR TEM XRD XRD AFM</td>
<td>Size—16-28 nm Shape—sph. Stability—FCC Structures—FCC, Essential oil in T. ammi was found to be good reducing agent when compared to alkaloids in P. somniferum.</td>
<td></td>
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<td>51</td>
<td>Vijayaraghavan et al. (2012) [65]</td>
<td>Filtered aqueous extract of Trachyspermum ammi and Pippavera somniferum plant powders</td>
<td>1 mL, Trachyspermum ammi: 15 min, Pippavera somniferum: 35 min, 28°C, 1 mL/50 mL, Shaking</td>
<td>UV-Vis SEM-EDAX TEM</td>
<td>Size—.3 - 7.6 μm Shape—sph. Essential oil in T. ammi was found to be good reducing agent when compared to alkaloids in P. somniferum.</td>
<td></td>
</tr>
<tr>
<td>No.</td>
<td>Study References</td>
<td>Plant Extract/Component</td>
<td>Methods</td>
<td>Characterization</td>
<td>Applications</td>
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<tr>
<td>52</td>
<td>Sreekarth et al. (2012) [69]</td>
<td>Discoceras batatas rhizome powder</td>
<td>1 mM, 25 and 80°C</td>
<td>UV-Vis, FTIR, SEM</td>
<td>Shape—circular and flower Structure—FCC</td>
<td>Anti-bacterial to B. subtilis, E. coli, P. aeruginosa and S. pneumoniae</td>
</tr>
<tr>
<td>53</td>
<td>Chaudhary et al. (2012) [112]</td>
<td>Aqueous filtrate of Vitis vinifera fruit</td>
<td>1 mM, 10 hr, room temp.</td>
<td>UV-Vis, SEM, XRD</td>
<td>Shape—sp</td>
<td>Antibacterial against E. coli, C. bacterium, B. subtilis</td>
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<td>54</td>
<td>Aslakumar et al. (2012) [113]</td>
<td>Aqueous filtrate of Praxthimium hysterophyllum</td>
<td>1 mM, 24 hr, room temp.</td>
<td>UV-Vis, FTIR, SEM, XRD</td>
<td>Size—avg 10 nm</td>
<td>Antibacterial against E. coli, P. aeruginosa and S. aureus</td>
</tr>
<tr>
<td>55</td>
<td>Patil et al. (2012) [33]</td>
<td>Filtered aqueous extract of Ocimum tenuiflorum leaf</td>
<td>1 mM, 10 min, room temp.</td>
<td>UV-Vis, TEM, PS, ZP</td>
<td>Size—15-25 nm</td>
<td>Water-soluble organics leaf extract responsible to reduction. Wound healing applications</td>
</tr>
<tr>
<td>56</td>
<td>Arunachalam et al. (2013) [114]</td>
<td>Indigofera aspalathoides, aqueous leaf’s extracts</td>
<td>1 mM, 24 hr, 28°C</td>
<td>UV-Vis, FTIR, EDS</td>
<td>Size—70 nm</td>
<td>Anti-bacterial against S. aureus, E. coli, K. pneumoniae, B. aerues and P. aeruginosa</td>
</tr>
<tr>
<td>57</td>
<td>Mubarakali et al. (2011) [115]</td>
<td>Filtered aqueous extract of Mentha piperita plant powder</td>
<td>1 to 9 mM, 24 hr, 40°C - 80°C</td>
<td>UV-Vis, FTIR, SEM, EDS</td>
<td>Size—48-67 nm</td>
<td>Antibacterial activity against S. aureus, E. coli, and P. aeruginosa</td>
</tr>
<tr>
<td>58</td>
<td>Mukundhan et al. (2011) [32]</td>
<td>Aqueous extract of Catharanthus roseus leaf</td>
<td>1 mM, 15 min, 80°C</td>
<td>UV-Vis, TEM, XRD, EDAX</td>
<td>Size—35-60 nm</td>
<td>Stable for 6 hr</td>
</tr>
<tr>
<td>59</td>
<td>Rajakumar and Abdul Rahuman (2011) [70]</td>
<td>Filtered aqueous extract of Eclipta prostrata leaf</td>
<td>1 mM, 1 hr, room temp.</td>
<td>UV-Vis, FTIR, SEM, TEM, XRD</td>
<td>Shape—TEM, sp</td>
<td>Larvicidal to A. aegypti and C. quinquefasciatus</td>
</tr>
<tr>
<td>60</td>
<td>Kumar and Yadav. (2011) [116]</td>
<td>Filtered aqueous extract of Lonicerajaponica L. leaf</td>
<td>1 M, 10 min, room temp.</td>
<td>UV-Vis, FTIR, TEM, XRD, AFM</td>
<td>Size—40-95 nm</td>
<td>Larvicidal to A. aegypti and C. quinquefasciatus</td>
</tr>
<tr>
<td>61</td>
<td>Gnanadesign et al. (2011) [117]</td>
<td>Filtered aqueous extract of Rizophora mucronate leaf</td>
<td>1 mM, 1 min to 2 hr, room temp.</td>
<td>UV-Vis, FTIR, TEM, XRD</td>
<td>Shape—irregular Structure—FCC</td>
<td>Cytotoxic to aquatic plant D. magna</td>
</tr>
<tr>
<td>62</td>
<td>Rani and Reddy (2011) [118]</td>
<td>Decanted aqueous extract of Piper betle L. leaf</td>
<td>1 mM, 1 min to 2 hr, room temp.</td>
<td>UV-Vis, FTIR, TEM, XRD, EDAX</td>
<td>Size—25-80 nm</td>
<td>Labricidal against A. subcutis and C. quinquefasciatus</td>
</tr>
<tr>
<td>63</td>
<td>Veerasamy et al. (2011) [119]</td>
<td>Aqueous filtrate of Garcinia mangostana leaf</td>
<td>0.25 - 5 mM, 0 - 70 min, 37°C - 90°C, 5 ml/95 ml, Static, pH—4, 7, 8, 30 min (5K rpm)</td>
<td>UV-Vis, FTIR, TEM</td>
<td>Size—avg 35 nm</td>
<td>Stable for 30 days, Antibacterial against E. coli and S. aureus</td>
</tr>
<tr>
<td>64</td>
<td>Santoshkumar et al. (2011) [120]</td>
<td>Decanted aqueous filtrate of Nelsombo nucifera leaf</td>
<td>1 mM, 10 min, room temp.</td>
<td>UV-Vis, FTIR, TEM, XRD</td>
<td>Size—5-20 nm</td>
<td>Antibacterial against S. spp.</td>
</tr>
<tr>
<td>65</td>
<td>Ahmad et al. (2011) [121]</td>
<td>Aqueous extract of Deosodium triflorum</td>
<td>0.025 M, 1 hr</td>
<td>UV-Vis, FTIR, TEM, XRD, EDAX</td>
<td>Size—&lt; 50 nm and pentagon shaped</td>
<td>AgNPs were stable for 14 days. Size—XRD 18.306 nm AFM—&lt; 100 nm TEM—2550 nm DLS—153.68 nm</td>
</tr>
<tr>
<td>66</td>
<td>Prathna et al. (2011) [122]</td>
<td>Filtered and centrifuged juice of Curcuminus fruit</td>
<td>0.1 - 10 mM, 4 hr, 30°C, 14 to 41, 10 hr, 1 min to 1000 rpm</td>
<td>UV-Vis, FTIR, TEM, XRD, EDAX, ZP</td>
<td>Size—&lt; 50 nm</td>
<td>Antibacterial against E. coli, B. subtilis</td>
</tr>
<tr>
<td>67</td>
<td>Bankar et al. (2010) [30]</td>
<td>Acetone treated, aqueous extracted, filtered and precipitated powder of Banana peel</td>
<td>0.125 to 1mM, 4 min,</td>
<td>UV-Vis, FTIR, TEM, SEM-EDS, XRD</td>
<td>Size—&lt; 100 nm</td>
<td>Antifungal and antibacterial action</td>
</tr>
<tr>
<td>68</td>
<td>Njiga et al. (2010) [123]</td>
<td>Filtered aqueous extract of Sorghum bran</td>
<td>0.1 M, 1 min, room temp.</td>
<td>UV-Vis, FTIR, SEM, TEM-EDS, XRD</td>
<td>Size—10-20 nm</td>
<td>AgNPs of smaller size at 50°C of extraction temperature compared to 25°C and 80°C</td>
</tr>
</tbody>
</table>
68 Kumar et al. (2010) [124]
Filtered aqueous extract of Scyphium comnini leaf (LE) and seed (SE) powder
1 mM, 24 hr, room temp.
10% (v/v), Static,
20 min, 12k rpm
1 - 3 mM, 10 min - 3hr,
25˚C - 150˚C,
0.5 - 4.8 ml/sph, mH2O,
pH: 2 - 10,
Static
UV-Vis
FTIR
SEM
AFM
Size — LE: 30nm,
SE have higher synthesis rates
and larger size AgNP
compared to LE.

69 Dubey et al. (2010) [79]
Filtered aqueous extract of Tumataum vagum fruit.
10 m-m, room temp.
1 ml/100 ml,
Stirred,
10 min at 3000 rpm
UV-Vis
TEM
XRD
EDAX
Size — 10 - 40 nm
AgNP more stable in basic
compared to acidic medium

70 Shukla et al. (2010) [37]
Filtered aqueous extract of Piper nigrum (black pepper)
10 m-m, room temp.
1 ml/100 ml,
Stirred,
10 min at 3000 rpm
UV-Vis
SEM
TEM
AFM
Size — 20 - 50 nm
Shape — sph.
Structure — FCC
Nature — large grain,
WD, uniform and
polycrystalline

71 Krishnaraj et al. (2010) [125]
Aqueous filtrate of Alcalipha indica leaf
1 mM, 30 min, 37˚C,
dark 12 ml/100 ml,
Static,
30 min at 75000 g
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
SEM
TEM
EDS
XRD
Size — 20 - 30 nm
Structure — cub
Antimicrobial against water
borne pathogen E. coli and Vitis cholera

72 Satish Kumar et al. (2009) [82]
Aqueous bark and powder extracts of Cinnamomum zeylanicum plant
10 m-m, 24 hr,
28˚C,
1:4: 15 min at 100,000 rpm
Shaken
UV-Vis
TEM
XRD
EDAX
Size — 50 - 100 nm
Shape — irregular
Nature — PD
AgNPs loaded on cotton disks
shown antibacterial activity.

73 Tripathi et al. (2009) [34]
Aqueous filtrate of Azadarcha indica leaves
Aqueous filtrate of Azadarcha indica leaves
10 m-m, 24 hr,
28˚C,
1:4: 15 min at 100,000 rpm
Shaken
UV-Vis
TEM
SEM
EDS
XRD
Size — 20 - 30 nm
Structure — cub

74 Lee and Vivekanandan. (2008) [126]
Aqueous extract of Helianthus annus plant
Aqueous extract of Helianthus annus plant
1 mM, 24 hr,
5 ml/5 ml,
Static
UV-Vis
XRD
TEM
Shape — sph.
Structure — FCC

75 Chandran et al. (2006) [120]
Aqueous extract of Aloe vera leaf
Aqueous extract of Aloe vera leaf
1 mM, 24 hr,
5 ml/5 ml,
Static
UV-Vis
XRD
TEM
Shape — sph.
Structure — FCC

76 Ankanwar et al. (2005) [35]
Embelica Officinalis fruit extract
Aqueous extract of Embelica Officinalis fruit extract
1 mM, 24 hr,
5 ml/45 ml,
15 min at 10000 rpm.
Static
UV-Vis
XRD
TEM
Shape — spherical
Structure — FCC

77 Shankar et al. (2004) [127]
Decanted aqueous extract of Azadarcha indica leaf
Decanted aqueous extract of Azadarcha indica leaf
1 mM, 24 hr,
5 ml/45 ml,
15 min at 10000 rpm.
Static
UV-Vis
XRD
TEM
Shape — spherical
Structure — FCC

78 Shankar et al. (2003) [42]
Decanted aqueous broth of Pelargonium graveolens leaf
Decanted aqueous broth of Pelargonium graveolens leaf
1 mM, 24 hr,
5 ml/100 ml,
15 min at 10000 rpm.
Static
UV-Vis
XRD
TEM
EDAX
Size — 50 - 100 nm
Shape — nearly sph
Nature — PD
Chlorophyll of leaf extract
formed 5 nm capping around
the AgNP.

79 Das et al. (2012) [76]
Mycelial of Rhizopus oryzae
Mycelial of Rhizopus oryzae
1 to 5 ml,
72 hr, 30˚C,
0.2 to 2.5 ml,
pH — 2 to 8,
Shaken
UV-Vis
FTIR
SEM
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
Aqueous cell filtrate of Penicillium Sp. fungi
1 mM, 24 hr,
room temp,
dark 50 ml/50 ml,
Agitated,
Lysophilized
UV-Vis
FTIR
AFM
Size — 52 - 104 nm
Shape — nearly spherical

81 Balaji et al. (2009) [129]
Chlorosporum clado sporoides fungal aqueous filtrate
Chlorosporum clado sporoides fungal aqueous filtrate
72 hr, 27˚C,
10 ml,
Shaken
UV-Vis
SEM
TEM
XRD
FTIR
Size — Ave: 35 nm
Shape — sph.
Structure — FCC
Nature — PD

82 Shaligram et al. (2009) [130]
Penicillium breviciputum WA 2315 fungal aqueous filtrate
Penicillium breviciputum WA 2315 fungal aqueous filtrate
1 mM, 72 hr, 25˚C,
Shaken
UV-Vis
FTIR
SEM
TEM
XRD
Size — 58.35 ± 17.8 nm
Structure — FCC
10˚C - 2: 4 nm, sph.
27˚C
Increase in temperature led
to blue shift in UV-Vis peak,
decreased size and increased
dispersity

83 Fayaz et al. (2009) [82]
Harvested cell aqueous filtrate of Trichoderma viride fungus
Harvested cell aqueous filtrate of Trichoderma viride fungus
1 mM, dark, 16˚C - 40˚C,
Shaken.
UV-Vis,
XRD
SEM
TEM
FTIR
Size — 10 - 40 nm,
40˚C
80 - 100 nm,
Plate like
like. Structure:
Cryst Nature: MD

84 Kathiresan et al. (2009) [131]
Aqueous Cell filtrate of Penicillium fellutatum fungus
Aqueous Cell filtrate of Penicillium fellutatum fungus
0.5 - 2.5 mM, 0 - 48 hr,
0˚C - 40˚C,
dark,
pH: 5 - 7.5,
Salinity-16%, 5% NaCl,
Shaken
UV-Vis
SEM
TEM
Size — 5 - 2.5 nm
Shape — sph.
(NH4)2SO4 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
Aqueous cell filtrate of Fusarium solani fungus
1mM, room temp.
Static,
10 min, 10000 g
UV-Vis
FTIR
SEM
TEM
Size — 5 - 35 nm
Shape — sph.

86 Basavaraja et al. (2008) [132]
Aqueous filtrate of Fusarium semitectum fungus
Aqueous filtrate of Fusarium semitectum 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

S. K. Srikar et al.
<table>
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<tr>
<th>Author(s)</th>
<th>Year</th>
<th>Stressing Factor</th>
<th>AgNP Properties</th>
<th>Note(s)</th>
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<tr>
<td>Vigneswaran et al.</td>
<td>2009</td>
<td>Harvested cells</td>
<td>Size — 25 ± 12 nm</td>
<td>AgeNP were synthesized on intracellular bases.</td>
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**Bio-polymers**

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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 spectrophotometry 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], *Streptomycetes* sp. [83], *Bacillus cereus* [135] *Brevibacterium casei* [136], *S. aureus* [138] *B. licheniformis* [139], and gram negative bacteria, *E. coli* [58] *Entrobactera* [59] and *Ureibacillus thermo spharuis* [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 only tentatively 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.
The AgNPs have 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 aegypti* [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 H₂O₂ 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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Biointerfaces


