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Review Article

A REVIEW: A GREEN APPROACH FOR THE SYNTHESIS OF SILVER NANOPARTICLES AND ITS ANTIBACTERIAL APPLICATIONS

SHYLA MARJORIE HAQQ, AMIT CHATTREE

Department of Chemistry, SamHigginbottom University of Agriculture, Technology and Sciences, Allahabad - 211 007, Uttar Pradesh, India. Email: shylahaqq3@gmail.com

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ABSTRACT

This review is based on the synthesis of silver nanoparticles (AgNPs) using a green approach which is biofabricated from various medicinal plants. AgNPs were prepared from the various parts of the plants such as the flowers, stems, leaves, and fruits. Various physiochemical characterizations were performed using the ultraviolet (UV)-visible spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction spectroscopy, transmission electron microscopy, and energy dispersive spectroscopy. AgNPs were also used to inhibit the growth of bacterial pathogens and were found to be effective against both the Gram-positive and Gram-negative bacteria. For the silver to have antimicrobial properties, it must be present in the ionized form. All the forms of silver-containing compounds with the observed antimicrobial properties are in one way or another source of silver ions. Although the antimicrobial properties of silver have been known, it is thought that the silver atoms bind to the thiol groups in enzymes and subsequently leads to the deactivation of enzymes. For the silver to have antimicrobial properties, it must be present in the ionized form. The study suggested that the action of the AgNPs on the microbial cells resulted into cell lysis and DNA damage. AgNPs have proved their candidature as a potential antibacterial against the multidrug-resistant microbes. The biological agents for synthesizing AgNPs cover compounds produced naturally in microbes and plants. Reaction parameters under which the AgNPs were being synthesized hold prominent impact on their size, shape, and application. Silver nanoparticle synthesis and their application are summarized and critically discussed in this review.

Keywords: Antimicrobial activity, Ultraviolet-visible spectroscopy, Fourier transform infrared spectroscopy, X-ray diffraction spectroscopy, Scanning electron microscopy, Energy dispersive spectroscopy, High-resolution transmission electron microscopy.

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INTRODUCTION

Nanotechnology may be defined as a modern research which deals with the synthesis, design, and manipulation of the particle size ranging from 1 to 100 nm. Nanotechnology is the study of small objects which can be used across all the branches such as chemistry, biology, physics, material science, and engineering. They are used for coating medical tools and materials used in the area of surgery, cardiology, and urology [1]. Nanotechnology is on the threshold of providing a host of new materials and approaches in revolutionizing the medical and pharmaceutical field. It is rapidly growing by the production of the nanoproducts and the nanoparticles that can have novel and size related physicochemical properties differing significantly from the large matter. Among all the noble metal nanoparticles it is the AgNPs which are arch product in the field of nanotechnology as it has gained boundless interest because of their unique properties such as the chemical stability, good conductivity, antiviral, antifungal, and anti-inflammatory activities [2]. They have attracted a great deal of attention due to their unique physical, chemical, optical, and biological properties and have also found tremendous application in the field of biomedicine, drug delivery, topical ointments, creams, food industry, textile treatment, and water industry [3]. In general, there are two approaches which are involved in the synthesis of nanoparticles either from top to bottom approach or a bottom to top approach. In the case of bottom to top approach, nanoparticles can be synthesized using the chemical and biological method by self-assemble of atoms to new nuclei which grow into a particle of nanoscale while in top to bottom approach suitable bulk material breakdown into the fine particles by the size reduction with the various lithographic techniques. According to the literature survey it has been found that the biological synthesis of AgNPs using the microorganisms includes bacteria, fungi, and plants. The use of the plant extracts for this purpose is advantageous over the other microorganisms due to the ease of improvement, the less biohazard and the elaborate process of maintaining the cell cultures [4]. Greener synthesis of nanoparticles also provides advancements over other methods as they are simple, one step, cost-effective, environment-friendly, and relatively reproducible and often results in more stable materials [5]. Nano AgNPs were synthesized using *Azadirachta indica* aqueous leaf extract where the nanoparticles were found to be predominantly spherical in shape [6]. Colloidal silver is of particular interest because of distinctive properties such as good conductivity, chemical stability, catalytic, and antibacterial activities. The use of medicinal plant parts such as the stem, seed, and bark for the synthesis of AgNPs is a quite novel method leading to green chemistry in comparison to other methods such as the chemical and physical methods [7]. Plant extracts have been used as a reducing, capping and stabilizing agents for the synthesis of AgNPs due to their reducing properties [8]. Nanomaterials can provide solutions to many technological and environmental challenges in the field of solar energy conversion, medicine, and wastewater treatment [9]. Over the past few decades, there has been an increased emphasis on the synthesis of metal nanoparticles and quantum dots [10].

Physiochemical characterization of AgNPs

UV visible spectroscopy

The synthesis of AgNPs using *Albizia lebbeck* (L) *Benth* extract has been confirmed by the UV-visible spectrum. Longitudinal vibrations corresponding to the AgNPs were found to have UV-spectral peak at 407 nm. This clearly indicated the interaction between the Ag+ ions and phytochemical present in the methanolic extract. Intensity of the band increased on varying time without any shift in the peak position [11]. The synthesis of AgNPs had been confirmed by measuring the UV-visible spectrum of colloidal solutions of AgNPs which are been synthesized from *Boswellia ovaliofoliolata, Shorea tumuggaia,* and *Svensonia hyderobadensis* were found to have an absorbance peak at 350 nm, 430 nm, and 300–400 nm, respectively, and the broadening

of the peak indicated that the particles are polydispersed [12]. AgNPs were synthesized at different concentrations of *A. indica* aqueous leaf extract such as 1–5 ml using 1mM of silver nitrate which were analyzed by the UV spectra of plasmon resonance band observed at 436–446nm which is similar to those reported in the literature [13]. On synthesizing the AgNPs using the banana peel extract, an absorbance of 433 nm was observed [14]. During the synthesis of AgNPs using the seed extract of *Alpinia katsumadai*, the absorbance band was observed at 417 nm which was slightly shifted to 436nm. This shift may be attributed due to the aggregation of the smaller particles according to the Mie theory [15]. The synthesized AgNPs exhibit lowest absorption band at 400 nm [16]. AgNPs were also synthesized using the extract of saffron (*Crocus sativus* L). The synthesized AgNPs were determined by UV-visible spectrum. The UV-visible spectrum showed a peak at 450nm due to the excitation of surface plasmon vibrations. The peak indicated the reduction of silver nitrate into AgNPs [17]. The present research work describes the synthesis of AgNPs using the ethanolic leaf extract of *Curvibacter lanceolatus, Dracocephalum parviflorum*, *Eucalyptus citriodora, Melaleuca cajuputi*, *Rhodomyrtus tomentosa*, *Syzygium campanulatum,* and *Xanthostemon chrysanthus* where the SPR band appeared at 420, 417, 418, 458, 434, 462, and 424 nm, respectively. It is known that the SPR band is sensitive to the discrete dipole approximation considering different shapes and sizes of the particles formed [18]. Similar results were observed on the synthesis of AgNPs using the leaf extract of *Datura stramonium* where it was found that a narrow SPR peak appeared at 444nm which may be ascribed to the formation of isotropic spherical AgNPs. This adequate reducing biomolecules within the leaf extract greatly reduced the silver nitrate solution as silver crystal and wrapped around the AgNPs [19].

FTIR spectroscopy

The FTIR spectroscopy of the synthesized AgNPs using the flower extract of *Hibiscus rosa sinensis* showed the peak at 3403/cm, 2928/cm, 2355/cm, and 670/cm which indicates the functional groups of the plant component that is involved in the reduction and stabilization of the AgNPs. This attributes to the OH stretch, C=C bond and C-H that reveals the water-soluble heterocyclic components, polyols, and certain proteins present in the extract [20]. FTIR analysis was done to identify the possible biomolecules in case of synthesis of marigold flower where the band at 3740.10/cmcorresponds to the N-H amide stretching. The peak at 727.19/cmcorresponds to the C-H stretching. The peak at 793.73/cm corresponds to the C-Cl stretching alkyl halides and 693.43/cm corresponds to the C-H stretching strong vinyl disubstituted alkenes [21]. The FTIR analysis of *Plumeria* leaves indicated the possible biomolecules that were responsible for the reduction of Ag+ ions and the capping of the reduced AgNPs synthesized using *Plumeria* leave extract. Strong IR bands were observed at 3703/cm and 2922/cm which corresponded to the N-H, OH stretching, and aliphatic CH stretching. The bands at 2333 and 1618/cm are due to the CO_2 and C=C, respectively [22]. The FTIR spectra of synthesized silver nanoparticle using *Cassia fistula* leaf extract showed the peaks at 3403/cm, 2928/cm, 2355/cm, and 670/cm which indicated the functional group of the component that is involved in the reduction and stabilization of AgNPs [23]. To study the formation of the AgNPs using *B. globose* extract, the FTIR measurements were performed. This measurement confirms the presence of some functional groups capping the AgNPs. Three main peaks were observed at 3464/cm, 2083/cm, and 1636/cm. The broadband around 3464/cm was due to the OH stretching vibrations of phenols and carboxylic groups present in the extract. The absorbance band at 2083/cm corresponds to the alkyne groups of phytoconstituents of the extract and the band at 1636/cm can be associated to C=0 stretching vibrations. The results were found to be similar to the other AgNPs synthesized by the different plant extract [24]. The FTIR spectra of the AgNPs which were synthesized using cannonball leaves exhibited prominent peaks at 2927/cm, 1631/cm, and 1383/cm. The spectra showed sharp and strong absorption band at 1631/cm which is assigned to the stretching vibration of (NH) C=O group. The band at 1383/cm was developed for C-C and C-N stretching. The presence of a sharp peak at 2927/cm was assigned to C-H (methoxy compounds) stretching vibration [25].

TEM image

The shape and size of the resultant particles which were synthesized using *Pedalium murex* leaf extract were found to be around 50 nm. The particles were found to be spherical in shape [26]. Tem analysis revealed that the synthesized nanoparticles using the *Morinda Pubescens* showed that the synthesized nanoparticles were stable in the solution and the size of the nanoparticles ranged from 20 to 40 nm [27]. The Tem image of the synthesized AgNPs using *Calotropis procera* latex showed that the particles were spherical in shape, well dispersed with a diameter range from 4nm up to 25nm and an average particle size of 12.33 nm [28]. The Tem image of the synthesized AgNPs from the *Chrysanthemum indicum* extract showed the particle size to be 37.71–71.99nm where they had a smooth surface and polydispersed particles [29]. On the synthesis of AgNPs using *Parkia speciosa Hassk pods* by the Tem images, it was reported that the size of the nanoparticle was coming to be around 20–50 nm. This Tem image exhibited the mixture of shapes with mainly spherical shapes as predominant [30]. The result indicated that the average particle size of the synthesized AgNPs using olive leaf extract showed the size to be around 30±6 nm at the lower extract concentration and, on the other hand, at the higher concentration the majority of the AgNPs were found to be in the range of 8–15 nm. Similar studies showed that a comparatively higher extract ratio is responsible for the synthesis of symmetrical nanoparticles [31].

X-ray diffraction (XRD) spectroscopy

The XRD analysis revealed the patterns at 2Θ=32.4°, 46.4°, and 28.0° during the synthesis of the AgNPs using leaf extract of *Catharanthus roseus* Linn G. These Braggs reflections clearly indicated the presence of (111), (2,0,0) and (3,1,1) sets of the lattice planes and further on the basis they can be indexed as face-centered cubic structure of silver. Hence, the XRD pattern clearly illustrates that the AgNPs formed in the present synthesis are crystalline in nature [32]. The crystalline nature of the biosynthesized AgNPs using the *Eriobotrya Japonica* leaf extract was confirmed by the XRD pattern. The diffraction peaks at 2Θ values of 38.11°, 44, 64,° and 77° corresponded to the 111, 200, 220, and 311 crystallographic planes. The unsigned peaks may be associated with the organic compounds which originated from the *E. japonica* leaf extract and function as reductants and stabilizers for AgNPs in the mixture [33]. The exact nature of synthesized that silver nanoparticle using *Ananas comosus* formed can be deduced from the XRD spectrum. The XRD pattern of the plant-derived AgNPs showed four intense peaks in the whole spectrum of 2Θ values which range from 20° to 80°. The XRD spectrum of the AgNPs formed in our experiments at 2 Θ values of 38.45°, 44.48°, 64.69°, and 77.62° corresponding to (1,1,1), (2,0,0), (2,2,0), and (3,1,1) planes for silver [34]. The green synthesized AgNPs from the fruit extract of *Cleome viscosa L* showed the 2Θ angles at the range of 38.68°, 44.1°, 64.11° ,and 77.4° which corresponded to the 111, 200, 220, and 222 planes that confirmed the formation of face-centered cubic silver crystal [35]. The XRD pattern of the *Ricinus communis var carmencita* leaf extract showed the presence of Braggs peak at 2Θ values, i.e., 27.81°, 32.19°, 38.16°, 44.43°, 46.23°, 54.93°, 57.39°, 64.65°, and 77.61° which corresponded to (2,1,0), (1,2,2), (1,1,1), (2,0,0), (2,3,1), (1,4,2), (2,4,1), (2,2,0), and (3,1,1) plane of the silver metals which are on the faced-centered cubic structure. Thus, the XRD studies confirmed the crystallinity of *Ricinus communis var carmencita* AgNPs [36]. The pattern clearly showed the main peaks at 2Θ = 38.19, 44.37°, 64.56°, and 77.47° which corresponded to the (1,1,1), (2,0,0), (2,2,0), and (3,1,1) planes. The green synthesized AgNPs using *pedalium murex* leaf extract is found to possess an fcc structure. By determining the width of 1,1,1 Braggs reflection the estimated average size of the particle was found to be 14 nm [26]. The XRD analysis was carried out to confirm the crystalline nature of the AgNPs synthesized using Brassicaceae members. The XRD spectra scanned over a 2Θ range of 20°–80° showed the Braggs angle at 37.9°, 46.2°, 64.3°, and 76.6° which corresponded to (111), (200), (220), and (311) crystal reflection planes of the four

Table 1: AgNPs synthesized from various plant extracts

Plant	Plant part used	Particle size (nm)
Azadirachta indica	Leaf	34 nm
Plumeria rubra	Flower	20–80 nm
Marigold	Flower	46.11 nm
Orange peel extract	Peel	7.36±8.06 nm
Calotropis procera	Flower	37 nm
Olive	Leaf	20–25 nm
Carob	Leaf	5–40 nm
Andean blackberry	Fruit	12–50 nm
Morus nigra	Leaf	4–8 nm
Spinacia oleracea	Leaf	10 nm
Musa balbisiana	Leaf	200 nm
Azadirachta indica	Leaf	200 nm
Ocimum	Leaf	3–20 nm
Cassia fistula	Leaf	50 nm
Buddleja	Leaf	20 nm
Ocimum sanctum	Leaf	14.6 nm and 11.35 nm
Apple extract	Fruit	30.25±5.26 nm

AgNPs: Silver nanoparticles

faces of the face-centered cubic crystalline structure of the AgNPs. The observed results were found to be in agreement with the JCPDS No-65–2871. Reflection planes 111 and 200 were the predominant orientations while the reflections of 220 and 311 were weak and broad. The results confirmed the formation of fcc crystalline AgNPs by the reduction of Ag ions by the aqueous *B. oleracea var botrytis* and radish extracts [37].

EDS spectroscopy

The elemental analysis was performed to confirm the presence of silver nanoparticles in the solution. The EDS analysis of *S. officinarium* mediated synthesis of AgNPs showed an intense signal at 3keV which indicates the presence of elemental silver [38]. The EDS analysis of the synthesized AgNPs using *Exiguobacterium mexicanum* confirmed that the sample contained predominantly silver. The sample has other elements such as silicon, oxygen, phosphorus, chlorine, and calcium. The other elements phosphorus, calcium, chlorine, and silicon identified in EDS indicated the presence of a biological matrix present in the sample [39]. The existence of the silver element in the AgNPs which were synthesized by *Bacillus amyloliquefaciens* and *Bacillus subtilis* to control filarial vector *Culex pipiens* pallens was confirmed by the EDX instrument. This indicated the formation of the AgNPs in cell-free supernatant from bacteria D29 and A15 where the strong peak of silver ions was observed at 3keV which confirmed the reduction of silver ions Ag+ in to Ag° [40]. The AgNPs were synthesized using three medicinal plants *Musa balbisiana*, *A. indica,* and *Ocimum tenuiflorum.* The EDS profile showed a strong silver signal along with weak oxygen and carbon peaks, which may have originated from the biomolecules that were bound to the surface of the AgNPs. Carbon and copper peaks may be due to the same being present in the grids. It has been reported that the nanoparticles synthesized using plant extracts are surrounded by a thin layer of some capping organic material from the plant leaf broth. This is another advantage of nanoparticles synthesized using plant extracts over those synthesized using chemical methods [41]. In a simple reported that the AgNPs were synthesized using the leaf extract of *Aegle marmelos* and found that the EDX spectrum showed a peak of silver which confirmed its presence in the suspension. Other peaks may be due to the attached groups from the leaf extracts on the surface of the nanoparticles [42]. The AgNPs were synthesized using *Brassica oleracea* var *Botrytis* and found that the EDAX spectrum of the solution containing AgNPs was observed at 3KeV, which is typical for the absorption of the metallic nanoparticles [43].

Antibacterial activity of AgNPs

The AgNPs have potent antibacterial action against both the Gram-positive and Gram-negative bacteria. 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 the Gram-positive bacteria whereas reverse results were observed by the other researchers. The antibacterial property of the AgNPs may be due to their interactions with the cell wall of the bacteria that result in the pore formation in the cell walls where the AgNPs get deposited that causes change in the permeability of the cell membrane. The reported differential sensitivity of both the bacterial species could be attributed to the difference in the structural characteristics of the bacterial species as well as the shape and size of the silver nanoparticle, bacterial inoculum, size, exposure time, and nutrient medium used during analysis of antibacterial action [44]. In the ionized form silver is inert, but on coming in contact with the moisture, it releases silver ions. The nanoparticles have been shown to accumulate inside the membrane and can subsequently penetrate into the cells thereby leading to damage of the cell wall and cell membrane. It is thought that silver atoms bind to the thiol group of enzymes forming the stable S-Ag bonds with the thiol-containing compounds which cause the deactivation of enzymes in the cell membrane [45]. The advantage of AgNPs is that they are known to have an antibacterial effect. AgNPs were studied for their bactericidal effect against both the Gram-positive bacteria, i.e., *S. epidermidis* and Gram-negative bacteria *P. aeroginosa* [46]. The synthesized AgNPs were tested against the vector mosquitoes of *A. Stephensi*, *A aegypti,* and *C. quinquefasciatus* where the result suggested that the use of *S. acuta* synthesized AgNPs can be a rapid, environmentally safer biopesticide that can form a novel approach to develop the effective biocides for controlling the target vector mosquitoes. This is the first report on the mosquito larvicidal activity of the plant aqueous extract and the synthesized nanoparticles [47]. The antimicrobial activity of *Glycyrrhiza glabra* AgNPs was analyzed for the first time against *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli, Bacillus* spp., *Salmonella* spp., *Trichoderma* spp., *Candida albicans*, and *Rhizopus* spp. using the disc diffusion method. The culture plates were treated with *G. glabra* AgNPs thereby exhibiting the significant antimicrobial activity of 1.2 cm–1.1 cm zone of inhibition to *S. aureus* and *P. aeruginosa,* respectively, which was nearby comparable to the standard antibiotic and also was found to inhibit other bacteria [48]. The existence of the silver element in the AgNPs which were synthesized by *Bacillus amyloliquefaciens* and *Bacillus subtilis* to control filarial vector *C. pipiens pallens* was confirmed by the EDX instrument. This indicated the formation of the AgNPs in cell-free supernatant from bacteria D29 and A15 where the strong peak of silver ions was observed at 3 keV which confirmed the reduction of silver ions Ag+ into Ag° [49].

CONCLUSION

Many plants are becoming probable sources for reducing and stabilizing agents for the green synthesis of AgNPs. The constituents present in the plant cover the biomolecules that are naturally present in the plants. AgNPs have shown great attention because of their unusual physical, chemical, electronic, magnetic, antibacterial, and biological activities. The physical and chemical methods which are used for the synthesis of AgNPs have been followed over decades. The use of expensive procedures and various toxic chemicals in their synthesis makes the biological synthesis the more preferred alternative. Results of several studies indicated that the synthesized AgNPs by the plants have got more stability in comparison with those produced by the microorganisms and other methods as they are environment-friendly, cost-effective and is devoid of any complicated process of maintaining the cell cultures.

AUTHOR'S CONTRIBUTION

Dr Amit Chattree has provided the idea of designing the protocol and the individual content for writing the review along with the mentorship.

Shyla Haqq has majorly performed the experiment, analyzed the obtained data and sincerely authored the article.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of the article.

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