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REVIEW PAPER

Green synthesis of silver nanoparticles using plant extracts

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Abstract−The strategy for design of new nanometals was developed due to their wide applications in many fields. One of the most important nanometals is silver nanoparticles (AgNPs) because of their extensive applications in biotechnology and biomedical fields. AgNPs were usually synthesized by using chemical and physical methods. In the chemical methods, various toxic chemicals are used, which are harmful to the health of living organisms. Therefore, the AgNPs were synthesized by using biological methods based on green chemistry for reducing the toxic chemicals. There are various resources for green synthesis of AgNPs, such as bacteria, fungi, enzyme and plant extracts. The green synthesis of AgNPs involves three main steps: the selection of the solvent medium, the selection of environmentally reducing agents, and the selection of non-toxic substances for the stability of AgNPs. The biosynthesis of AgNPs using plant extracts is more favorable than other biological methods because of removing the elaborate process of maintaining cell cultures. It can be also suitably scaled up for large scale production of AgNPs. This review focuses on green synthesis of AgNPs using various plant extracts.

Keywords: Nanometals, Silver Nanoparticles, Plant Extracts, Biosynthesis, Green Synthesis

INTRODUCTION

The synthesis of nanoparticles has received special attention because of greater surface area to volume ratio, modified structure and more activity of nanoparticles rather than macro molecules [1-3]. Nanoparticles have many applications in optical, electronic and textile industries, medicine, cosmetic, and drug delivery [2,4]. The most important nanoproduct in the field of nanotechnology is AgNPs which in low concentrations have no toxicity against human health. AgNPs are significantly used in textiles and clothing, food packaging, medical and cosmetic ingredients, water, wastewater and air treatment, pesticides and household usage [5,6].

Green chemistry of synthetic strategies using biological methods such as enzymes [7], microorganisms [8], and plant extracts [9,10] plays a major role in the formation of AgNPs. Among biological methods, the synthesis of AgNPs using plant extracts is the best eco-friendly alternative to available traditional chemical and physical methods [11-14]. This method is mainly used for reducing the toxicity and development of green chemistry. The aim of this work is to review the green synthesis of AgNPs using various plants.

USE OF PLANT EXTRACTS FOR SYNTHESIS OF AgNPs

In the synthesis of nanoparticles using plant extracts, the extract is simply mixed with a solution of the metal salt at room temperature. The reaction is usually completed in short time. AgNPs and many other metals could be produced by this method [15].

Recently, AgNPs were synthesized using the *Crataegus douglasii* fruit extract by Ghaffari-Moghaddam and Hadi-Dabanlou [16]. To optimize the biosynthesis of AgNPs, the effect of process variables such as extract concentration, mixing ratio of the reactants, time and pH were also investigated. The scanning electron microscope (SEM) images showed AgNPs with 29.28 nm size and nearly spherical shape at 24 h interaction time. Fig. 1 represents the SEM images of the AgNPs formed after 4 h, 24 h, 5 days and 35 days interaction, respectively. The antibacterial activity of the synthesized AgNPs was also confirmed against *Staphylococcus aureus* and *Escherichia coli.*

Synthesis of AgNPs using the extract of *Artemisia nilagirica* leaves was reported by Vijayakumar et al. [17]. SEM and energy-dispersive spectroscopy (EDX) were used to characterize the AgNPs. The morphology of the AgNPs was determined by SEM, and the average diameter of the particles was found to be in the range of 70 to 90 nm (Fig. 2). The EDX results confirmed the presence of elemental silver.

Roy et al. [18] synthesized AgNPs using the fruit extract of *Vitis vinifera* as reducing agent. The reduction was attributed to the phenolic, terpenoid, polysaccharide and flavone compounds present in the extract. The particle size and lattice image of the AgNPs was investigated using transmission electron microscope (TEM) (Fig. 3). The AgNPs were nearly spherical with a weak crystalline structure and an average size of 18-20 nm. The obtained AgNPs showed an effective antibacterial activity against both gram-positive *Bacillus subtilis* and gram-negative *Escherichia coli.*

An aqueous extract of *Cynodon dactylon* leaf was used to produce AgNPs with size of 30-50 nm. The UV-Vis spectrum of AgNPs in aqueous solution shows an absorbance peak around 450 nm due to surface plasmon resonance. The AgNPs solution was centrifuged

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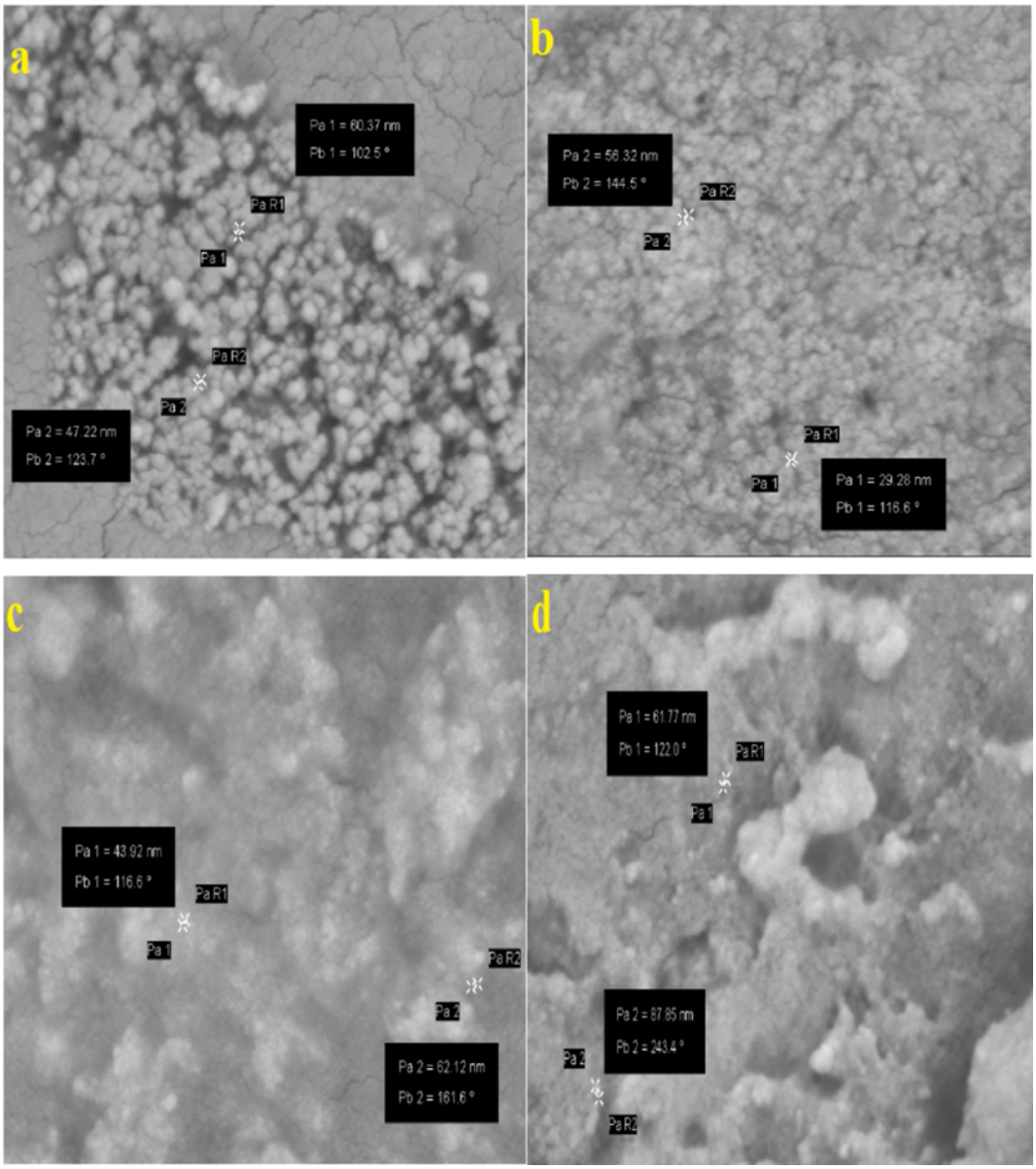

Fig. 1. The SEM images of synthesized AgNPs after 4 h (a), 24 h (b), 5 days (c), and 35 days (d) interaction time using *Crataegus douglasii* extract as reducing agent [16].

at 20,000 rpm for 20 minutes to obtain the AgNPs powder for XRD (X-ray diffraction) analysis. The XRD analysis of the AgNPs showed four distinct diffraction peaks, which can be indexed at the angle values of 111, 200, 220 and 240 [19].

In synthesis of AgNPs using a domestic microwave with the leaves extract of *Stigmaphyllon littorale*, the nanoparticles were rapidly formed with a stable size of 5-25 nm. Characterization was carried out using UV-Vis, Fourier Transform Infrared (FTIR), SEM and TEM analyses. The antimicrobial properties of obtained AgNPs were evaluated against gram positive and negative bacterial pathogens (*Pseudomonas putida*, *Escherichia coli*, *Bacillus subtilus* and *Micrococcus luteus*). The formed AgNPs showed significant antimicrobial properties [20].

Das et al. [21] reported the synthesis of AgNPs using the leaf extract of *Sesbania grandiflora*. The reduction of Ag^{+} to AgNPs was done by water soluble proteins in the leaf extract, which was confirmed by FTIR analysis. The UV-Vis spectrum of AgNPs solutions showed a peak at 422 nm. The XRD spectrum showed four distinct diffraction peaks at (2θ) 38.28°, 44.33°, 64.33°, and 77.53° corresponds to (1 1 1), (2 0 0), (2 2 0) and (3 1 1) planes of face-centered cubic silver with a lattice parameter of α=4.08 Å. The antibacterial activity of AgNPs was investigated against *Salmonella enterica* (gram-negative) and *Staphylococcus aureus* (gram-positive) by disc diffusion method. The results showed potent antibacterial activity against two bacteria.

The AgNPs were synthesized using the leaf extract of Lakshmi tulasi (*Ocimum sanctum*) as a reducing and stabilizing agent. The absorption spectrum of the AgNPs showed a peak at 406 nm. The average diameter of the AgNPs was found to be 42 nm. The structure of the synthesized AgNPs was determined by XRD analysis.

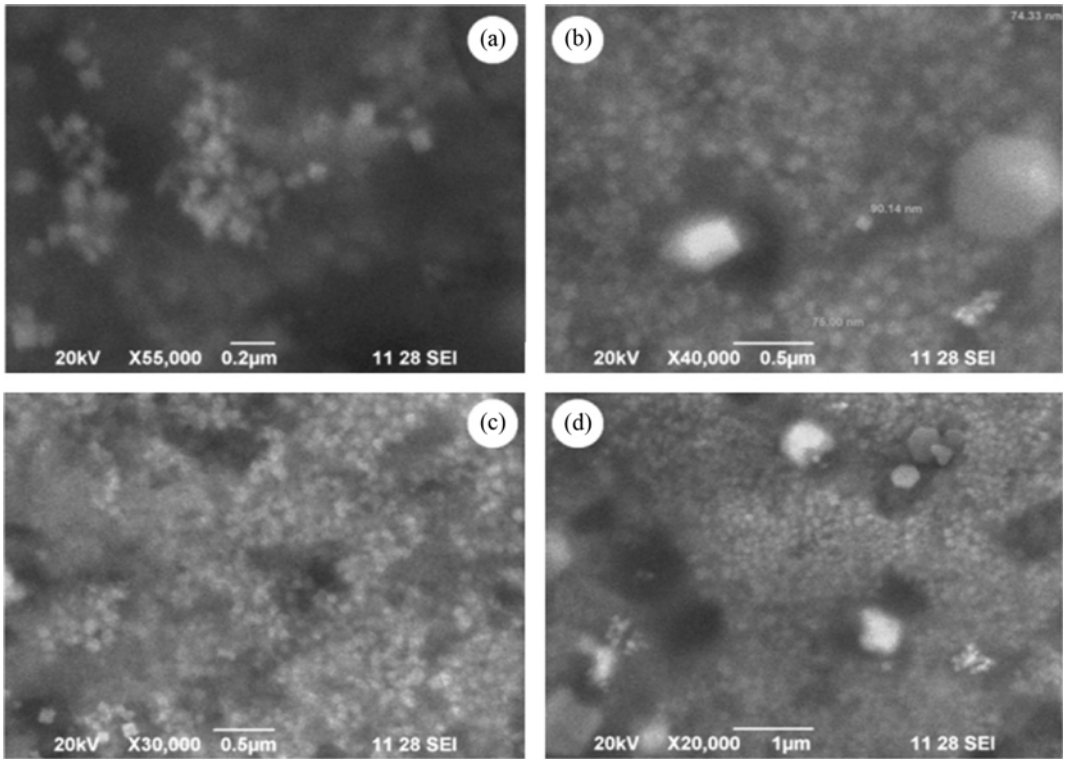

Fig. 2. The SEM images of the synthesized AgNPs using *Artemisia nilagirica* leaf extract [17].

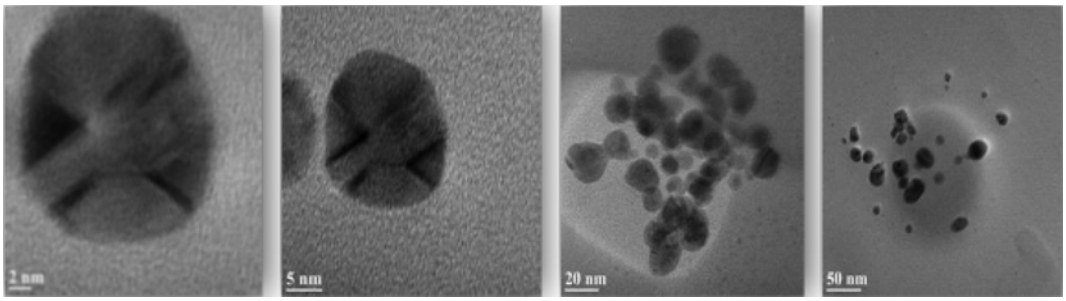

Fig. 3. TEM images of silver nanoparticles synthesized by using *Vitis vinifera* extract [18].

The XRD results showed that the nanoparticles were composed of highly crystalline Ag [22].

Bindhu and Umadevi [23] reported the synthesis of AgNPs using *Hibiscus cannabinus* leaf extracts. The UV-Vis spectrum showed surface Plasmon peak at 446 nm. The monodispersed AgNPs had spherical shape with the average size of 9 nm. The FTIR results revealed that the ascorbic acid present in *Hibiscus cannabinus* leaf extract had acted as a reducing agent. The obtained AgNPs showed a good antimicrobial activity against *Escherichia coli*, *Proteus mirabilis* and *Shigella flexneri.*

The coir extract of *Cocos nucifera* was used to synthesize the AgNPs. The UV-Vis analysis showed a peak at 433 nm due to surface plasmon resonance. The XRD spectrum confirmed the crystalline structure of AgNPs in nature. The average size of AgNPs was found to be 23±2 nm from the TEM images, which was in good agreement with the particle size calculated from XRD analysis. Gas chromatography-mass spectrometry (GCMS) of the extract showed the presence of hydrocarbons such as nonacosane and heptacosane which may possibly influence the reduction process and stabilization of AgNPs [24].

The AgNPs were prepared using the leaf extract of fresh *Ixora coccinea* L. The surface plasmon resonance band at 430 nm confirmed the biosynthesis of AgNPs by *Ixora coccinea* L. leaves extract. The SEM images showed well-dispersed AgNPs with particle sizes in the range of 13-57 nm. The AgNPs were spherical. The crystalline nature of the AgNPs was confirmed by XRD analysis. The FTIR results showed that the compounds of leaves extract such as phytosterol, flavonoids, alkaloids, triterpenoids, amino acids and proteins might be participating in the synthesis of AgNPs [25].

Synthesis of AgNPs has been also reported by using the aqueous extract of leaves and bark of *Ficus carica* as reducing and capping agent. The UV-Vis analysis showed the surface plasmon absorption in the range of 380-410 nm and 420-440 nm for *Ficus carica* bark and *Ficus carica* leaves, respectively. The scanning tunneling microscopy (STM) images showed surface morphology at the distance of 47.2 nm [26].

One-pot synthesis of AgNPs using *Aegle marmelos* leaf extract was carried out by Rao and Paria [27]. The SEM images showed the presence of large number of spherical nanoparticles with an average size of ≈60 nm. The XRD pattern showed the main peaks at (2θ) 38.08°, 44.32°, 64.45°, 77.43°, and 81.50° corresponding lattice plane value was indexed at (1 1 1), (2 0 0), (2 2 0), (2 2 2), and (3 1 1) planes, respectively, with the majority of particles showing (1 1 1) plane having face centered cubic structure.

In another study, *Ocimum sanctum* (Tulsi) leaf extract was used to produce the AgNPs with particle sizes in the range of 4-30 nm. It was observed that *Ocimum sanctum* leaf extract can reduce Ag^+ to AgNPs within 8 min of reaction time. The obtained nanoparticles were found to have a good antimicrobial activity against both gram-negative and gram-positive microorganisms [28].

Phuphansri et al. [29] reported the synthesis of AgNPs using extracts of vitamin C-rich fruits such as guava, grape and tomato. The UV-Vis analysis showed a peak located of AgNPs at 420 nm. The FTIR results of AgNPs confirmed the presence of peaks in both the spectrum of guava extract and the synthesized nanoparticles. Therefore, it was concluded the guava extract has the ability to perform both reducing agent and capping functions on the synthesis of AgNPs. The XRD pattern showed clear, sharp diffraction peaks of AgNPs. The obtained AgNPs had antibacterial activity against gram-positive (*Streptococcus aureus*) and gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa* and *Salmonella typhimurium*), which were evaluated by disc diffusion method.

Baishya et al. [30] used the leaves extract of *Bryophyllum pinnatum* (Lam.) to produce AgNPs with diameter range of 70-90 nm. The UV-Vis absorption spectrum showed a surface plasmon resonance band in nanoparticle solution at 418 nm, suggesting that the nanoparticles were dispersed in the aqueous solution with no evidence for aggregation. The XRD pattern showed that the obtained AgNPs were crystalline. The TEM analysis revealed that the AgNPs were predominantly spherical. The AgNPs showed promising antibacterial propertied against *Escherichia coli* and *Staphylococcus aureus*.

Dhanalakshmia and Rajendran [31] synthesized highly stabilized AgNPs using the leaves of extract *Tridax procumbens*. The UV-Vis adsorption spectra of obtained AgNPs solution showed the surface plasmon absorption band at 460 nm. Fig. 4 shows that the size and structure of AgNPs was further characterized by using SEM analysis. The surface deposited AgNPs are clearly seen at high magnification in the micrograph. The SEM image (Fig. 4) showed that the average size of the AgNPs was 55 nm. The antimicrobial activity of the obtained AgNPs was evaluated against human pathogenic *Escherichia coli*, *Salmonella*, *Shigella* and *Vibrio* by the standard disc diffusion method. The results showed high level of inhibition.

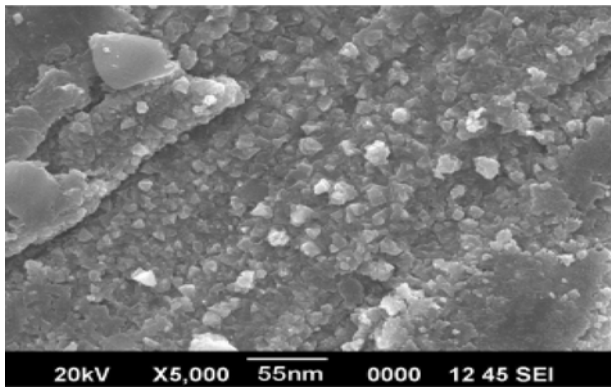

Fig. 4. The SEM image from the synthesized AgNPs using the leaf extract *Tridax procumbens* [31].

AgNPs were synthesized by using the leaves extract of Chinese tea from *Camellia sinensis* by Loo et al. [32]. The average crystallite size of AgNPs was calculated to be 3.42 nm by XRD analysis. The TEM image of AgNPs is shown in Fig. 5(A). According to the TEM image, the morphology of silver nanoparticles was found to be spherical, which is in agreement with the shape of the surface plasmon resonance band in the UV-Vis spectrum. Fig. 5(B) shows the histogram of size distribution of AgNPs. The average particle size measured from the TEM image was 4.06 nm, which is in good agreement with the particle size calculated from XRD analysis. The

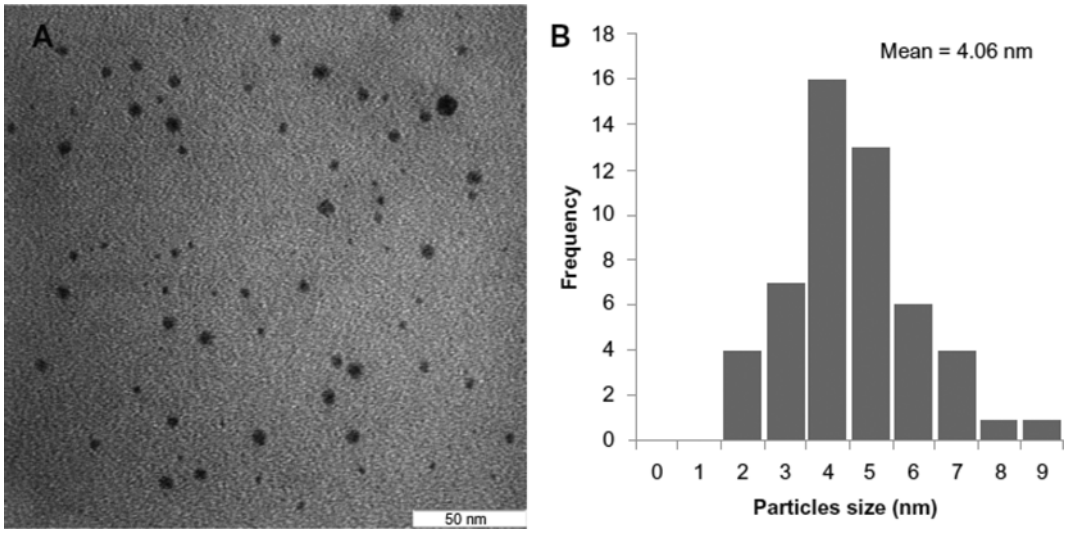

Fig. 5. (A) The TEM image of AgNPs; (B) the histogram of size distribution of AgNPs using the leaves extract of Chinese tea from *Camellia sinensis* [32].

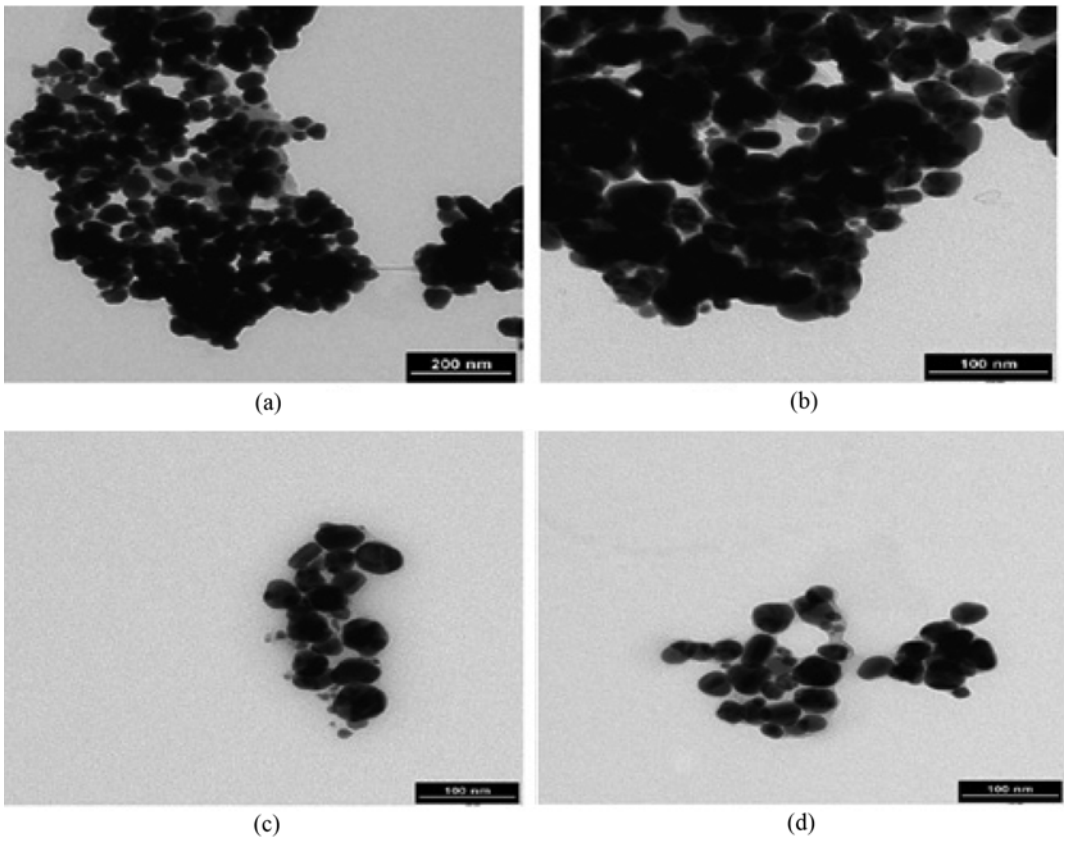

Fig. 6. The TEM images of AgNPs using the leaves extract of *Panicum virgatum* [33].

FTIR results showed the intense and broad peak at 386 cm^{-1} corresponding to the Ag metal.

Mason et al. [33] synthesized the AgNPs using the leaves extract of *Panicum virgatum*. The particle size of AgNPs was found to be in the range of 20-40 nm by TEM analysis (Fig. 6). The TEM images also show that the nanoparticles are spherical, rod-like, triangular, pentagonal, and hexagonal. The XRD pattern indicated that the synthesized AgNPs are crystallized in face centered cubic symmetry.

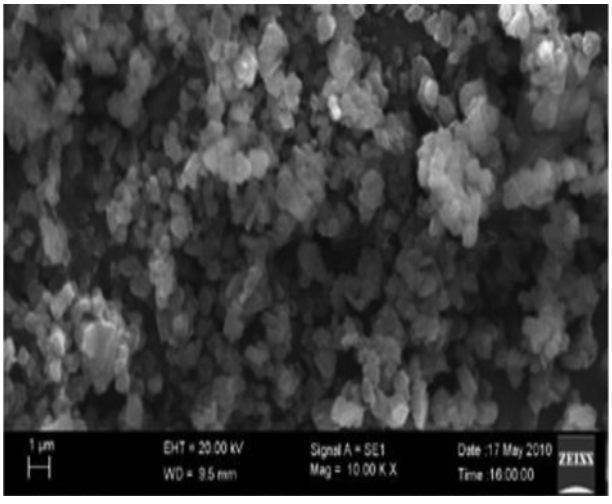

Fig. 7. The SEM image of AgNPs formed by *Ocimum sanctum* leaf extract [34].

Rout et al. [34] synthesized the AgNPs from the leaf extract of *Ocimum sanctum* as reducing agent. The AgNPs exhibited dark yellowish-brown color in aqueous solution due to the surface plasmon resonance phenomenon. The SEM image showed relatively spherical shape for AgNPs with diameter range 0 to 50 nm (Fig. 7). The XRD results confirmed that crystallization of the bio-organic phase occurs on the surface of the AgNPs or vice versa. The antibacterial and antifungal activities of the nanoparticles have also been evaluated against *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterobacter cloacae*, and *Proteus vulgaris.*

The AgNPs were synthesized by using the methanolic extract of *Adhatoda vasica* as reducing and capping agent. The UV-Vis spectra showed an absorbance peak at 395 nm due to surface plasmon absorption. The TEM analysis showed the average particle size of 15-20 nm with spherical shape (Fig. 8). The obtained AgNPs showed high DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity and reducing power activity. The AgNPs were found to be an antidiabetic agent and highly toxic agent against different human pathogens [35].

Gopinath et al. [36] synthesized the AgNPs using the fruit extracts of *Tribulus terrestris.* The UV-Vis spectrum exhibits a strong peak at 435 nm due to the excitation of surface plasmon resonance for the synthesized AgNPs. The TEM images were recorded at different magnification to find the individual particles (Fig. 9). The obtained AgNPs were observed to be spherical with average size of 22 nm. XRD analyses were performed to confirm the crystalline structure of the obtained AgNPs. The XRD spectrum showed four distinct

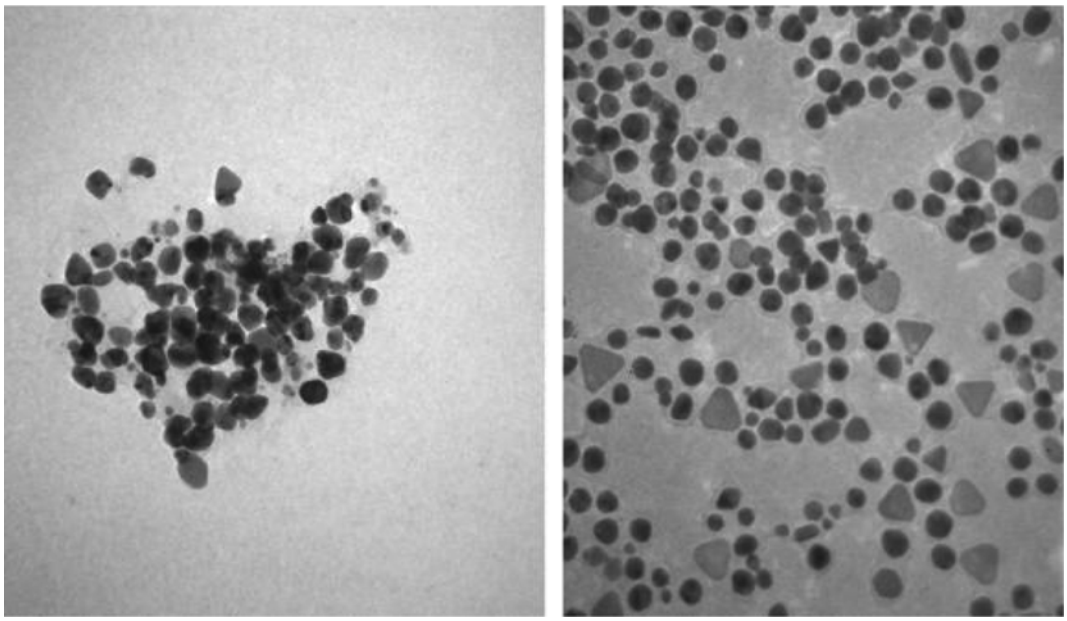

Fig. 8. The TEM images of the AgNPs using *Adhatoda vasica* [35].

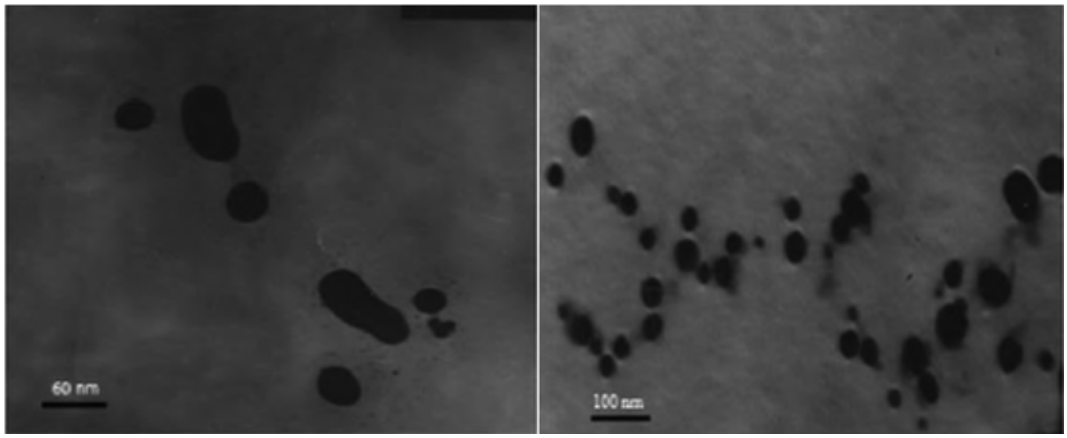

Fig. 9. The TEM images of synthesized AgNPs using the fruit extracts of *Tribulus terrestris* [36].

diffraction peaks at (2θ) 38.1°, 44.3°, 64.4° and the 77 corresponding lattice plane value was indexed at (1 1 1), (2 0 0), (2 1 1) and (2 2 0) of the cubic silver). The antibacterial property of synthesized AgNPs was determined by Kirby-Bauer method with clinically isolated multi-drug resistant bacteria such as *Streptococcus pyogens*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Bacillus subtilis* and *Staphylococcus aureus*.

OTHER PLANTS

The AgNPs were also synthesized y busing the leaf extract of *Allium sativum* (garlic) [37], the leaf extract of *Ananas comosus* [38], the methanol extract of *Solanum xanthocarpum* [39], the leaf extracts of *Indigofera aspalathoids* [40], *Chromolaena odorata* leaf extract [41], the extracts of *Curcuma longa* [42], an aqueous extract of *Terminalia chebula* [43], the *Iresine herbstii* leaf aqueous extracts [44], the aqueous extract *Trachyspermum ammi* and *Papaver somniferum* [45], an aqueous extract of *Syzygium aromaticum* [46], extracts of *Ficus benghalensis* leaf [47], the *Arbutus unedo* leaf extract [48], the *Callicarpa maingayi* extract [49], the fresh leaf extract of *Aristolochia bracteata* [50], a Mulberry leave extract [51], an aqueous extract of leaf broth of *Ocimum sanctum* [52], and the leaf extract of *Calotropis gigantea* [53]. Some other plants which were also used for synthesis of AgNPs are summarized and presented in Table 1.

GREEN SYNTHESIS OF AgNPs *VERSUS* CHEMICAL METHODS

AgNPs can be synthesized by different methods. A schematic of chemical synthesis *versus* green synthesis of AgNPs is shown in Fig. 10. In physical methods, metal nanoparticles such as AgNPs are generally prepared by evaporation-condensation process in a tube furnace at atmospheric pressure. In these methods, the source material is vaporized into a carrier gas at the furnace [90-93]. The AgNPs could also be synthesized using laser ablation of metallic bulk materials in solution [94-102]. Chemical reduction is another

Table 1. Biosynthesis of nanoparticles using some plant extracts

Plant	Size of AgNPs	Reference
Cassia angustifolia	9-31 nm	[54]
Leaf of *Curcuma long*	-	[55]
Ocimum tenuiflorum	25-40 nm	[56]
Peels of *Punica granatum*	10 nm	[57]
Alstonia scholaris	30-50 nm	[58]
Flower of *Calotropis procera*	35 nm	[59]
Leaf of *Catharanthus*	5-10 nm	[60]
Chenopodium album	10-30 nm	[61]
Rhizome of *Dioscorea batatas*	-	[62]
Leaf of *Eclipta prostrate*	10-20 nm	[63]
Gelidilla acerosa	16-40 nm	[64]
Mentha piperita	57 nm	[65]
Leaf of *Piper longum*	18-41 nm	[66]
Leaf of *Polyalthia longifolia*	15-50 nm	[67]
Leaf of *Polyalthia longifolia*	58 nm	[68]
Flower of *Rosa damascene*	10-30 nm	[69]
Leaves of *Vitex negundo*	18.2 nm	[70]
Acalyphaindica	20-30 nm	[71]
Leaf of *Argemone maxicana*	30 nm	[72]
Leaves of *Azadirachta indica*	20-nm	[73]
Banana peel	20 nm	[74]
Crude *Piper nigrum*	20-50 nm	[75]
Boswellia ovalifoliolata	30-40 nm	[76]
Desmodium trifolium	5-20 nm	[77]
Leaf of *Euphorbia hirta*	40-50 nm	[78,79]
Trianthema decandra	15 nm	[80]
Onion of *Allium Cepa*	33.67 nm	[81]
Leaf of *Eucalyptus citriodora* and *Ficus bengalensis*	20 nm	[82]
Fruit of *Carica papaya*	25-50 nm	[83]
Leaf of *Datura metel*	16-40 nm	[84]
Leaf of *Eclipta prostrate*	2-6 nm	[85]
Leaf of *Eucalyptus hybrid*	50-150 nm	[86]
Seeds of *Jatropha curcas*	15-50 nm	[87]
Aloe vera	15.2 nm	[88]
Leaf of *Pelaryonium graveolens*	16-40 nm	[89]

method for the preparation of AgNPs as stable and colloidal dispersions in water or organic solvents. The reduction of silver ions (Ag^+) was carried out using common reductants such as borohydride [103-105], citrate [106,107] ascorbate [108] and elemental hydrogen [109, 110]. Rodriguez et al. [111] synthesized the AgNPs using an electrochemical procedure based on the dissolution of a metallic anode in an aprotic solvent. The AgNPs had the particle size ranging from 2 to 7 nm. The AgNPs were prepared by microwave irradiation of silver nitrate solution in ethanol using polyvinylpyrrolidone (PVP) as a stabilizing agent. In this method, ethanol acts as a reducing agent in the presence of microwaves. The TEM images showed AgNPs with 10±5 nm diameters and spherical shape [112]. Yan et al. [113] synthesized the AgNPs using vapor deposition onto an ice matrix. The size of AgNPs was between 5 and 20 nm. The synthesis of AgNPs at the liquid-liquid using ultrasonic wave was reported by Hong et al.

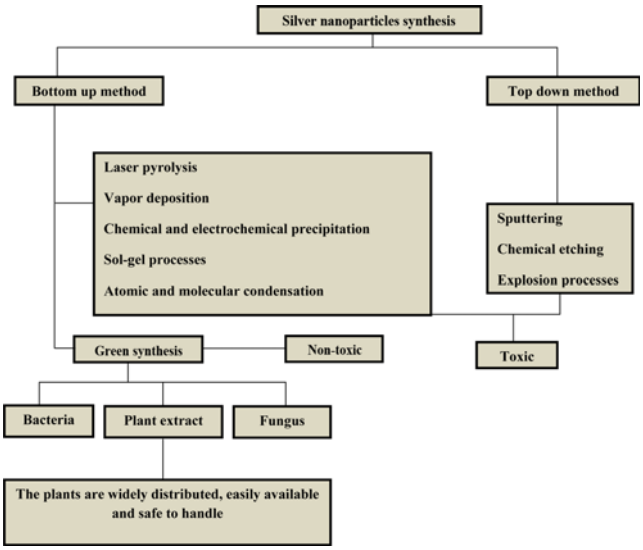

Fig. 10. Schematic of chemical synthesis *versus* green synthesis of AgNPs.

[114]. In this method, the AgNPs were prepared by adding methanol to water instead of surfactants in order to control the size of AgNPs.

Chemical methods have mostly been used for synthesis of AgNPs. However, these methods could be used to synthesize the AgNPs at large scales. In some chemical methods, a stabilizer must be added to the first solution to avoid agglomeration of AgNPs, whereas in biological methods there is no need to add a stabilizing agent. Toxicity is a disadvantage of the chemical methods. Moreover, many of these methods are energy-intensive, although synthesis of AgNPs is rapid. In contrast, biological methods are performed in eco-friendly conditions and consume no energy. Although, the time required for synthesis of AgNPs is longer compared to chemical methods, the time has recently decreased with finding suitable microorganisms or organism [115]. Therefore, the advantages of biological methods over chemical synthesis of AgNPs are summarized as follows: cost effective, environmental friendly, single step process for the large scale synthesis of nanoparticles, and no need to use high pressure, energy, temperature and toxic chemicals which are harmful to the health of living organisms [116].

There are many studies for biosynthesis of AgNPs. For example, the AgNPs were synthesized using *Psychrophilic* [117], *Bacillus* [118], *Bacillus stearothermophilus* [119], *Salmonella typhimurium* [120] and *Bacillus* sp. [121] bacteria. Silver nanoparticles were also prepared using the fungus *Aspergillus flavus* [122], *Alternaria alternate* [123], *Trichoderma Harzianum* [124], *Aspergillus terreus* [125], *Fusarium semitectum* [126], *Humicola* sp. [127] and *Penicillium diversum* [128] and *Fusarium oxysporum* [129]. Thus, the biosynthesis of AgNPs is a suitable method since it is based on green chemistry for reducing the toxic chemicals.

CONCLUSIONS

The AgNPs are synthesized using various methods from metallic silver and are generally used in food, consumer products and medical products because of their antibacterial activity. Green synthesis of AgNPs provides several advantages over chemical and

physical methods: cost effectiveness, environment friendly, easily scaled up for large scale synthesis and no need to use high pressure, energy, temperature and toxic chemicals. The synthesis of nanoparticles by using plant extracts is better than other biological methods because the elaborate process of maintaining cell cultures can be eliminated. It can be also suitably scaled up for large scale production of AgNPs. The AgNPs produced by plant extracts are usually more stable and more varied in shape and size in comparison with those produced by other methods. In summary, biosynthesis of AgNPs using plant material is a conventional and eco-friendly method compared to the chemical and physical synthesis. This method is significantly used because the plants are widely distributed, easily available and safe to handle.

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