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*Review Article*

# A Review on the Green Synthesis of Silver Nanoparticles and Their Morphologies Studied via TEM

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Silver has been recognized as a nontoxic, safe inorganic antibacterial/antifungal agent used for centuries. Silver demonstrates a very high potential in a wide range of biological applications, more particularly in the form of nanoparticles. Environmentally friendly synthesis methods are becoming more and more popular in chemistry and chemical technologies and the need for ecological methods of synthesis is increasing; the aim is to reduce polluting reaction by-products. Another important advantage of green synthesis methods lies in its cost-effectiveness and in the abundance of raw materials. During the last five years, many efforts were put into developing new greener and cheaper methods for the synthesis of nanoparticles. The cost decrease and less harmful synthesis methods have been the motivation in comparison to other synthesis techniques where harmful reductive organic species produce hazardous by-products. This environment-friendly aspect has now become a major social issue and is instrumental in combatting environmental pollution through reduction or elimination of hazardous materials. This review describes a brief overview of the research on green synthesis of silver metal nanoparticles and the influence of the method on their size and morphology.

## 1. Introduction

For over centuries, silver based compounds were used as nontoxic inorganic antibacterial agents owing to their biocidal properties in many applications such as wood preservatives, water purification in hospitals, in wound or burn dressing, and so forth. In fact, silver ions and their related compounds have low toxicity toward animal cells but present a high toxicity to microorganisms like bacteria and fungi. The recent advances in the field of nanoparticle synthesis have a strong impact in many scientific areas and the synthesis of silver nanoparticles has also followed this tendency. These unique properties of nanomaterials have spurred numerous investigations and applications in electronics, nanomedicine, biomaterials, energy, and food. In fact, silver based compounds are much cheaper than gold based one; moreover, silver nanoparticles are now considered as an important class of nanomaterials. They are presently mainly used as catalyst [1] or antibacterial/antifungal agents [2].

Environmentally friendly synthesis methods are becoming more and more popular in chemistry and chemical technologies. This trend has several origins, including the need for greener methods counteracting the higher costs and higher energy requirements of physical and chemical processes. For this reason, scientists search for cheaper methods of synthesis. The other reason is that conventional methods for nanoparticle synthesis usually require harmful reductants such as sodium borohydride or hydrazine and many steps in the synthesis procedure including heat treatments, often producing hazardous by-products. In order to reduce the environmental impact of nanoparticle synthesis, greener routes have been investigated for over a decade. The principles of green chemistry were presented by Anastas and Warner who developed 12 principles that eloquently describe green chemistry [3]. Green chemistry should aim at thwarting waste, minimizing energy use, employing renewable materials, and applying methods that minimize risk. The three main concepts for the preparation of nanoparticles in a green synthesis approach are the choice of the solvent medium (preferably water), an environmentally friendly reducing agent, and a nontoxic material for the stabilization of the nanoparticles [4].

To be energy efficient, the synthesis processes should be carried out close to ambient temperature and pressure and under neutral pH. The biological systems then appear as the most suitable factory for reaching such natural chemistry conditions. It is well known that many microorganisms can provide inorganic materials either intra- or extracellularly [5] and it was found that some of these microorganisms can be used as ecofriendly nanofactories for the production of nanomaterials, more particularly for the production of silver metal nanoparticles (Ag NPs).

Many approaches were investigated, and microorganisms such as bacteria, yeasts, fungi, and algae were used in the biosynthesis of metal nanoparticles. More recently, the utilization of plants for the production of metal nanoparticles has spurred numerous investigations in the field of green synthesis. The aim of this review is to provide a brief overview of the current research on the green synthesis of silver nanoparticles and describe how the different methods of synthesis can affect the size and the morphologies of the silver metal nanoparticles.

## 2. Green Synthesis Methods

*2.1. Green Synthesis Using Bacteria as a Medium.* Bacteria are known to produce inorganic materials either intra- or extracellularly. This makes them potential biofactories for the synthesis of nanoparticles like gold and silver. Silver is well known for its biocidal properties; however, some bacteria are known to be silver resistant [6] and can accumulate silver on the cell wall to as much as 25% of their dry weight biomass, thus suggesting their use in industrial recovery of silver from ore materials [7]. Therefore, the use of prokaryotic bacteria as nanofactories was first studied. First noble metal nanoparticle synthesis, using bacteria, was done using silver resistant bacterial strains *Pseudomonas stutzeri* AG259, which were cultured in high concentrations of silver nitrates. It was demonstrated that the cells accumulate silver in large quantities and the majority of the silver was deposited in the form of particles of 200 nanometers of diameter [8]. Significant results were observed when bacteria *Proteus mirabilis* PTCC 1710 were used for producing silver nanoparticles. It was found that depending on the type of "broth" used during the incubation of bacteria, extracellular or intracellular synthesis can be promoted. This kind of selection makes bacteria-based green synthesis flexible, inexpensive, and a suitable method for large-scale production [9]. It is important to point out that bacteria continued to grow after the synthesis of silver nanoparticles. However, the main drawback of using bacteria as nanofactories is the slow synthesis rate and the limited number of sizes and shapes available compared to the conventional chemical methods of synthesis. For this reason, fungi-based nanofactories and chemical reaction involving plant based materials were investigated (see Table 1) [10].

*2.2. Green Synthesis Using Fungi as Medium.* Similar to bacteria, due to their tolerance and metal bioaccumulation ability, high binding capacity, and intracellular uptake, fungi have been of interest in biological production of the metallic nanoparticles [11]. Compared to bacteria, fungi are simpler to handle in a laboratory process. The mechanism of nanoparticle production using fungi is different; fungi secrete large amounts of enzymes which are used to reduce silver ions that induce the formation of the metal nanoparticles [12].

The first synthesis involving fungus-mediated approaches for the metal nanoparticle synthesis was performed in the beginning of the 20th century, and Ag NPs with diameter of $25 \pm 12$ nm were synthesized using fungus *Verticillium* [13, 14]. In previous studies involving bacteria, bacteria *Pseudomonas stutzeri* AG259 isolated from silver mines were able to produce Ag NPs of well-defined size and distinct morphology within the periplasmic space of the bacteria [8]. Synthesis using *Verticillium* takes the green approach even further. During the exposure of the fungus to $AgNO_3$ solution, the reduction of ions and the formation of Ag NPs take place. Nanoparticles were approximately 25 nm in diameter presenting a rather good monodispersity and spherical morphology. Contrary to bacteria, Ag NPs were formed below the surface of the fungal cells [13]. This result differs from the work of Klaus et al. 1999, where particle morphologies synthesized using bacteria ranged from spherical, triangular to hexagonal. The mechanism of nanoparticle formation was then studied and the main hypothesis is that, in the case of fungi-based synthesis, the NPs are formed on the surface of the mycelia and not in the solution. It was then suggested that in the first step $Ag^+$ ions are adsorbed on the surface of the fungal cells due to electrostatic interaction between negatively charged carboxylate groups in enzymes present in the cell wall of mycelia and positively charged Ag ions. Finally, the silver ions are then reduced by the enzymes present in cell wall, leading to the formation of silver nuclei [13]. The shift from bacteria to fungi as a means of developing natural nanofactories offers the advantages of simpler downstream processing and handling of the biomass. Compared to bacteria, fungi are known to secrete much higher amounts of proteins, which tends to significantly increase the productivity of this biosynthetic approach; moreover, fungi could be used for the production of large amounts of metal nanoparticles.

The first report involving extracellular synthesis of silver nanoparticles using eukaryotic systems such as fungi was reported by Ahmad et al. 2003. They showed that secreted enzymes are responsible in the reduction process [15]. Before this report, all the fungi based biosyntheses were intracellular. Extracellular synthesis is advantageous as the synthesized nanoparticles will not bind to the biomass [16, 17] and it is therefore possible to extend this approach for the biosynthesis of nanomaterials over a range of chemical compositions, such as oxides, nitrides, and so forth.

When compared to other classes of microorganisms, their ecofriendliness and simplicity during handling lead to increasing the use of fungi in green synthesis. For example, fungus like white rot fungus is nonpathogenic and this contributes to the mass production of Ag NPs [18]. Another

Table 1: Size dependent synthesis of silver nanoparticles via eco-friendly synthesis routes.

| Type | Diameter | References |
|---|---|---|
| Bacteria | | |
| *Pseudomonas stutzeri AG259* | 200 nm | [8] |
| *Proteus mirabilis PTCC 1710* | 10–20 nm | [9] |
| Fungi | | |
| *Verticillium* | 25 nm ± 12 nm | [13, 14] |
| *Fusarium oxysporum* | 5–15 nm | [15] |
| *Cladosporium cladosporioides* | 10–100 nm | [16] |
| *Fusarium oxysporum* | 20–50 nm | [17] |
| *Aspergillus fumigatus* | 5–25 nm | [19] |
| *A. flavus* | 5–30 nm | [20] |
| Yeast | | |
| *MKY3* | 2–5 nm | [23] |
| Baker's yeast, *Saccharomyces cerevisiae* | 60–80 nm | [24] |
| Algae | | |
| *Sargassum wightii* | 8–12 nm | [25] |
| Plant extracts | | |
| Alfalfa sprouts | 2–20 nm | [26] |
| *Geranium* leafs | 14–46 nm | [27] |
| *Chrysanthemum indicum* L. | 38–72 nm | [38] |
| *Acacia leucophloea* | 17–29 nm | [39] |
| *Ganoderma neojaponicum Imazeki* | 5 nm | [40] |
| *Colletotrichum* sp. | 20–40 nm | [41] |
| *Caffeine and tea extract* | 60 nm | [42] |
| *Gelatin and glucose* | 3.68 nm and 5.28 nm | [43] |
| *Thevetia peruviana* (latex) | 10–60 nm | [45] |
| *Elaeagnus latifolia* | 30–50 nm | [46] |
| *Leptadenia reticulata* | 50–70 nm | [47] |
| Olive extract (1 mL) | 30 nm | [48] |
| Olive extract (5 mL) | 15 nm | [48] |
| *Terminalia chebula* fruit extract | 100 nm | [49] |
| *Tinospora cordifolia* | 17–29 nm | [50] |

important factor for choosing the method of synthesis is the reaction rate. First report of rapid synthesis using fungi was using *Aspergillus fumigatus* that allowed obtaining monodispersed Ag NPs within 10 minutes [19]. In addition, one of the most common molds *Aspergillus fumigatus* was used to make Ag NPs in a matter of minutes, when silver ions entered into contact with the cell filtrate. These investigations were clear examples describing suitability and the potential of using fungi for mass production of nanoparticles. More recently, Ag NPs were synthesized using *A. Flavus* fungi to be combined with antibiotics to enhance the biocidal efficiency against multidrug-resistant bacteria. This study demonstrated the efficiency of antibiotics combined with Ag NPs [20].

Similar to fungi, yeasts were also widely investigated for silver nanoparticle synthesis [12, 21, 22]. Silver-tolerant yeast strain MKY3 was first used for extracellular synthesis. The outcome of the synthesis was satisfying due to simplicity of the separation of the nanoparticles when using differential thawing [23]. After that, several studies followed but until recently synthesis has never been carried out by commercial baker's yeast available in grocery stores. All the aggravating steps of cultivation of the yeast were avoided, thus making the process much simpler [24].

Similar to moving from prokaryotes to eukaryotes green synthesis, the utilization of eukaryotic autotrophs widened the possibilities of green synthesis. For example, using marine algae *Sargassum wightii* allowed obtaining very stable nanoparticles compared to other biological methods [25].

*2.3. Green Synthesis Using Plants and Plant Extract as a Medium.* One of the first approaches of using plants as a source for the synthesis of metallic nanoparticles was with alfalfa sprouts [26], which was the first report on the formation of Ag NPs using a living plant system. Alfalfa roots have the capability of absorbing Ag from agar medium and transferring them into the shoots of the plant in the same oxidation state. In the shoots, these Ag atoms arranged themselves to form nanoparticles by joining themselves and forming larger arrangements. In comparison to bacteria and fungi, green synthesis using plants appears to be faster and the first investigations demonstrate that synthesis procedures are able to produce quite rapidly Ag NPs. Shankar et al. showed that using *Geranium* leaf takes around nine hours reaching 90% reaction compared to the 24 to 124 hours necessary for other reactions reported earlier [27]. Therefore, the use of plant extracts in green synthesis has spurred numerous investigations and studies up till now. It was demonstrated that the production of metal nanoparticles using plant extracts could be completed in the metal salt solution within minutes at room temperature, depending on the nature of the plant extract. After the choice of the plant extract, the main affecting parameters are the concentration of the extract, the metal salt, the temperature, the pH, and the contact time [28].

In addition to the synthesis parameters, the main issue is the choice of the plant from which the extract could be used. The advantages of using plants for the synthesis of nanoparticles are that the plants are easily available and safe to handle and possess a large variety of active agents that can promote the reduction of silver ions. Most of the plant parts like leaves, roots, latex, bark, stem, and seeds are being used for nanoparticle synthesis [10]. The most important point is the active agent contained in these parts which makes the reduction and stabilization possible. Ecofriendly plant extracts contain biomolecules, which act as both reducing and capping agents that form stable and shape-controlled nanoparticles. Main compounds which affect the reduction and the capping of the nanoparticles are biomolecules such as phenolics, terpenoids, polysaccharides, flavones, alkaloids, proteins, enzymes, amino acids, and alcoholic compounds. However, quinol and chlorophyll pigments, linalool, methyl chavicol, eugenol, caffeine, theophylline, ascorbic acid, and

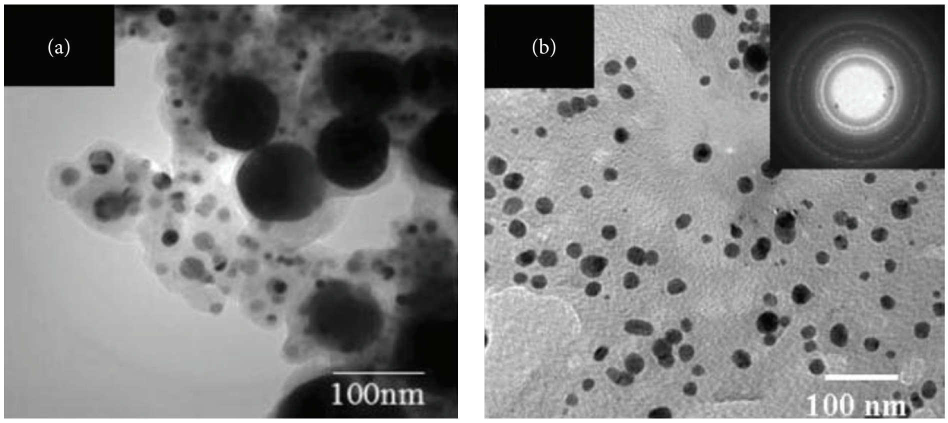


Figure 1: Ag NPs from coffee (a) and tea (b) extract [42]. Copyright license number: 3482980509111.

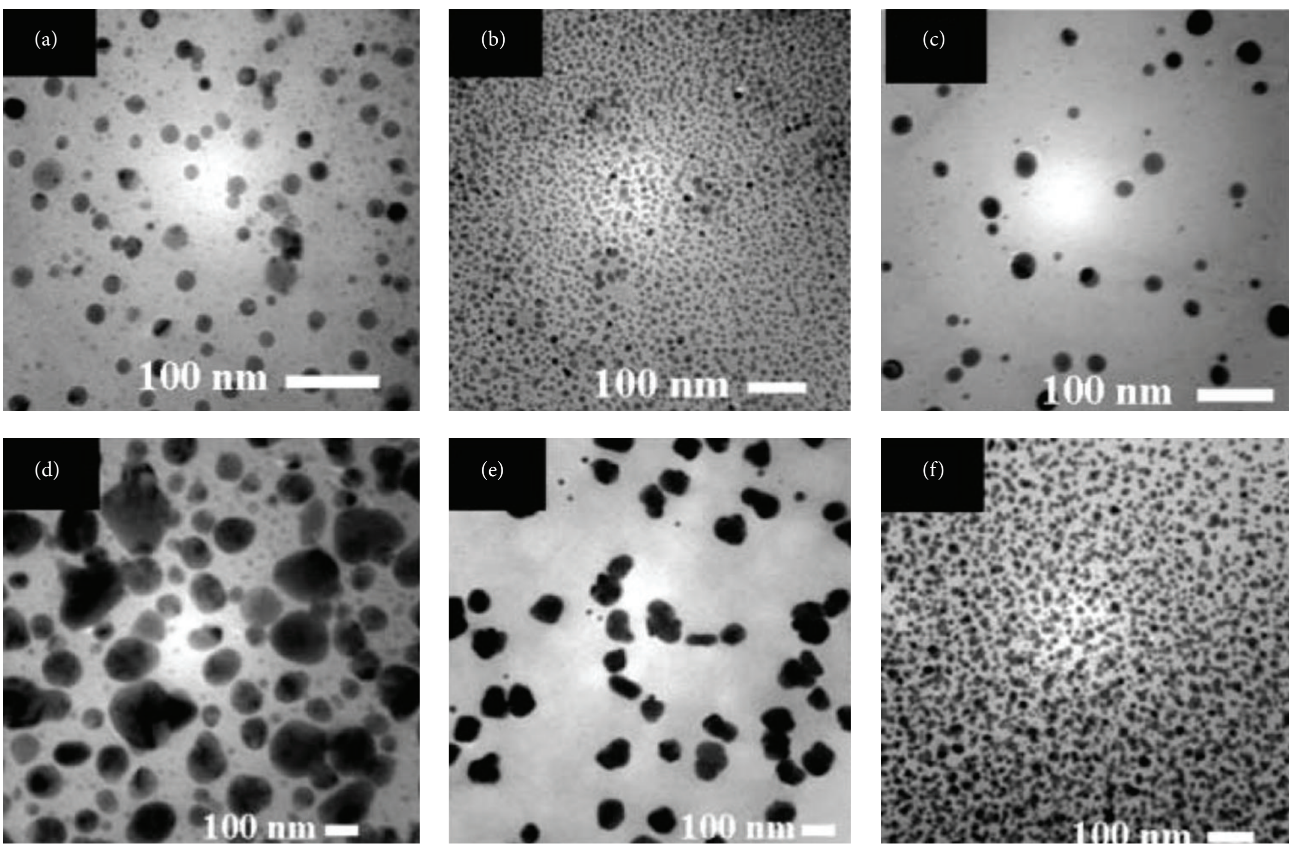


Figure 2: TEM image of silver nanoparticles synthesized using (a) Bigelow tea, (b) Folgers coffee, (c) Lipton tea, (d) Luzianne tea, (e) Sanka coffee, and (f) Starbucks coffee extract at room temperature in one step without using any hazardous reducing chemicals or nondegradable capping agents [42]. Copyright license number: 3482980509111.

other vitamins have also been reported [29–36]. The non-toxic phytochemicals including aforementioned flavonoids and phenols have unique chemical power to reduce and also effectively wrap nanoparticles, thus preventing their agglomeration. Phenolic compounds possess hydroxyl and carboxyl groups, which are able to bind to metals [37].

Most of the Ag NPs synthesized via green synthesis are investigated for biomedicine and more particularly as antibacterial agent or for cancer treatment. Recent reports showed that it was possible using *Chrysanthemum indicum* L. [38] or *Acacia leucophloea* extract [39] to synthesize Ag NPs of diameter ranging from 38–72 nm to 17–29 nm, respectively. Both samples demonstrated very good antibacterial properties. In the same manner, *Ganoderma neojaponicum Imazeki* was used for the synthesis of Ag NPs as potential cytotoxic agents against breast cancer cells [40]. As these methods

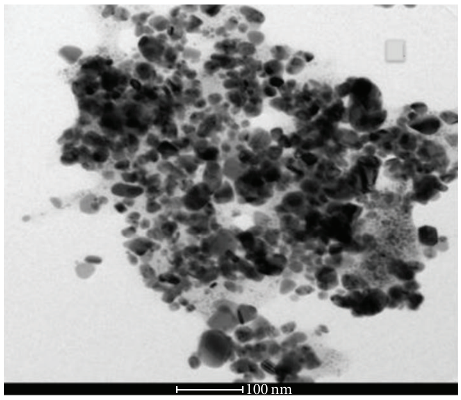


Figure 3: Representative TEM image of Ag NPs synthesized at room temperature using latex extract from the stems of fresh fruits of *Thevetia peruviana* [45] (reproduced with permission from IOP).

involving green chemistry are being more and more explored and scientists are starting to combine different options together, it was recently reported that the symbiotic biological systems such as *Geranium* leaf combined with endophytic fungus *Colletotrichum* sp. can synergize the outcome of the reaction. In fact, plants contain biomolecules which are able to stabilize unstable particles whereas fungi secrete enzymes for reduction [41].

## 3. Morphological Characterization via Transmission Electron Microscopy

The size distribution of nanoparticles in general is an important issue as nanoparticles exhibit different physical and chemical properties depending on their shape and size. Synthesis methods that generate uniformly sized and shaped nanoparticles are therefore being pursued. Transmission electron microscopy (TEM) is therefore one of the most adapted techniques to study the size and shape of the nanoparticles and provide their distribution. It is important to note that the majority of the TEM studies were performed on plant extracted green synthesis of silver nanoparticles.

Many different plant extracts have been used to prepare silver nanoparticles in the aim of producing Ag NPs presenting different capping layer molecules and presenting different morphologies. These studies in TEM have shown that the presence of a capping layer in plant mediated synthesis of Ag NPs, where the plant extract acts as capping layers, shapes the nanoparticle during its growth. It also has an effect on the size distribution of these nanoparticles. The use of medicinal plants in the synthesis of Ag NPs is not only used for size and shape control but also used to provide properties to the Ag NPs along with the antimicrobial properties of the plant. In this section, special emphasis is given to the Ag NPs morphology and size distribution as a result of synthesis parameters using TEM.

Everyday products such as tea and coffee can be used to produce Ag NPs, namely, tea leaves and coffee beans. Nadagouda et al. have explained that the caffeine and polyphenol in tea and coffee help in forming complexes with metal ions and reducing them to the corresponding metal [42]. They have used commercially available coffee and tea extract mixed with $AgNO_3$. TEM samples were prepared by diluting the nanoparticles in water. The TEM images provide monodispersed nanoparticles in each case, indicating that the polyphenols act not only as a reducing agent but also as a capping agent and therefore restrict their growth to 60 nm (Figure 1). In Figure 1, the TEM images provide size distribution of Ag NPs and indicate that tea extract reduced Ag NPs present a small particle size distribution compared to the caffeine reduced ones (Figure 1(b)). By using different types of tea and coffee extract the authors have shown that the size of the nanoparticles can be further varied (Figure 2).

Other edible products such as gelatin and glucose have also been employed by Darroudi et al. to modify the Ag NPs size and shape [43]. The synthesis was carried out by dissolving gelatin in water and adding silver solution. A small amount of glucose was also added and the solution was heat treated at different temperatures, that is, 20, 40, and 60°C. Synthesis with and without the presence of glucose was performed. The particle size showed a decrease when the temperature was increased and when glucose was absent. At 60°C, the nanoparticles had an average size of 3.68 nm and 5.28 nm. This is attributed to the rate of reduction reaction of $AgNO_3$.

In another study, ecofriendly honey was used as a reducing agent and replaced synthetic reducing agents such as hydrazine and dimethyl formamide which are not totally environmentally safe. In this study, the particle size depended on the concentration of honey and the pH of the aqueous solution [44]. Another encounter where Rupiahsi et al. used latex in the green synthesis of Ag NPs has also been reported [45]. Milky white latex was extracted from the stems of fresh fruits of *Thevetia peruviana* and filtered out. This was then mixed with $AgNO_3$. The TEM images of the particles thus obtained show spherical particles with a wide size distribution with 75% of the nanoparticles presenting particle size between 10 and 30 nm (Figure 3). Less than 10% of the nanoparticles were under 10 nm in size and between 50 and 60 nm. Most of the particles in the TEM image are polydispersed despite latex being used as a capping and reducing agent; the nanoparticles were nevertheless spherically shaped. However, other nanoparticle shapes of Ag NPs are also possible with other green synthesis methods as explained below.

Another plant mediated synthesis of Ag nanoparticles includes *Elaeagnus latifolia* a native evergreen shrub to Asia. In the work of Phanjom et al. the nanoparticles were precipitated after a room temperature reaction of the leaf extract with $AgNO_3$ and provided a size distribution of Ag NPs between 30 and 50 nm (Figure 4) [46]. However, the TEM contrasts exhibit the presence of defects such as stacking faults or twinning in them (Figure 4). The authors also suggest the formation of an organic capping layer on the surface of these nanoparticles thereby explaining the high level of monodispersion of the nanoparticles.

Swamy et al. have employed *Leptadenia reticulata* which is a medicinal plant native to the Indian subcontinent [47]. It has been actively used to alleviate symptoms of

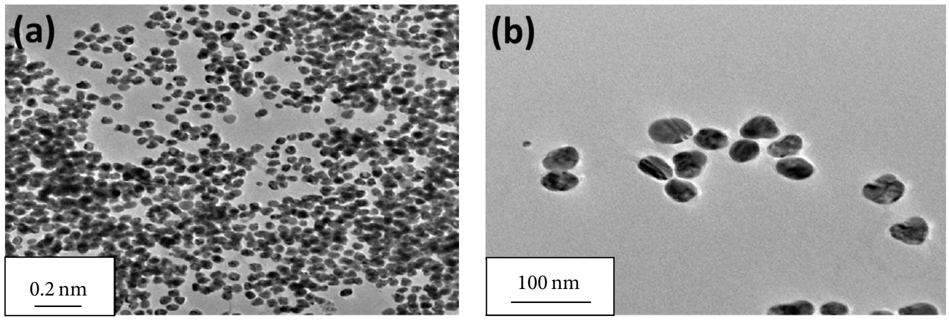


Figure 4: TEM images of Ag NPs synthesized with *Elaeagnus Latifolia* as a reducing agent: (a) overview of Ag NPs and (b) higher magnification image of the Ag NPs showing defects [46] (reproduced with permission from the journal).

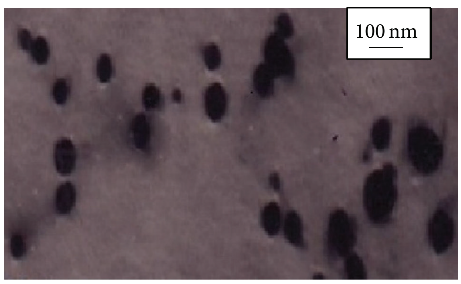


Figure 5: Ag NPs synthesized using *Leptadenia reticulata* extract [47] (reproduced with permission from Springer).

hematopoiesis, emaciation, cough, dyspnoea, fever, burning sensation, and night blindness. Extracts of this plant are also used to cure skin disorders. For the synthesis, once again $AgNO_3$ was mixed with the leaf extract in 1 : 9 proportions. The nanoparticles obtained were stored in ionized water and frozen for further study. For TEM study these nanoparticles were suspended on a carbon coated copper grid. The particles appear oblong with sizes between 50 and 70 nm (Figure 5).

Olive leaf extract with $AgNO_3$ has been used by Khalil et al. for the synthesis of Ag NPs and their antibacterial properties have been evaluated [48]. It is well known that olive leaf is effective against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli*. Their studies focused first of all on the effect of concentration of the leaf extract on the particle size. In this context they observed a decrease in the particles size with increase in the olive leaf extract concentration during reaction as indicated in Figure 6. The authors have calculated that for the 1 mL concentration average particle size was 30 nm (Figure 6) while for 5 mL of olive leaf extract concentration the particle size was less than 15 nm (Figure 6).

Moreover, the effect of pH of the reaction solution on the particles size was also evaluated and they noticed a clear decrease in particle size with increase in pH. Here Ag NPs with 2 different pH values (Figure 7) were studied with TEM and smaller particle sizes were obtained for higher pH (Figure 7). It is therefore possible to vary the pH and the extraction concentration and tailor the size of the NPs.

*Terminalia chebula* fruit extract has also been used by Kiran Kumar et al. for the production of silver nanoparticles [49]. In their reaction $Ag_2SO_4$ was mixed with the extraction liquid compared to other methods presented here where $AgNO_3$ was used.

The nanoparticles produced by this method showed sharp facets. All the nanoparticles were under 100 nm in size. The shape anisotropy is believed to be due to lack of protective biomolecules which assists in the homogeneity of the shape during growth, thus forcing them to attain thermodynamic stability by acquiring shapes such as triangles and hexagons. The monodispersed aspect of these nanoparticles was again attributed to the capping layer of polyphenols which are known to reduce $Ag^{+2}$ to $Ag^{+0}$ and the oxidized polyphenol binds to the Ag NPs via –C=O bonds and also simultaneously stabilizes them.

Other plant mediated Ag NPs synthesis involves *Tinospora Cordifolia* by Anuj and Ishnava [50]. Here they used the stem extract to obtain a suspension that was then mixed with $AgNO_3$. The nanoparticles produced in their study were spherical except for larger particles that presented nonuniform shapes. They also noticed a fine film on the TEM grid which corresponded to the capping layer produced from the *Tinospora Cordifolia* extract (Figure 8).

More recently, it was shown that Ag NPs synthesized using *Acacia Leucophloea* extract present a spherical morphology with a diameter ranging from 17 to 29 nm. The authors observed an enhancement of the antibacterial activity of Ag NPs synthesized using this method [39].

## 4. Conclusion

During the last decades, many efforts were put into the development of new green synthesis methods. Living organisms have huge potential for the production of nanomaterials that can be applied to many fields and more specifically to biomedicine. Organisms ranging from simple bacteria to highly complex eukaryotes can all be used for the production of nanoobjects with the desired size and shape. Prokaryotes are the simplest forms of biomass and therefore are easy to manipulate genetically to make them produce more desired substances for synthesis. However, the cultivation of the bacteria and large scale production remains problematic

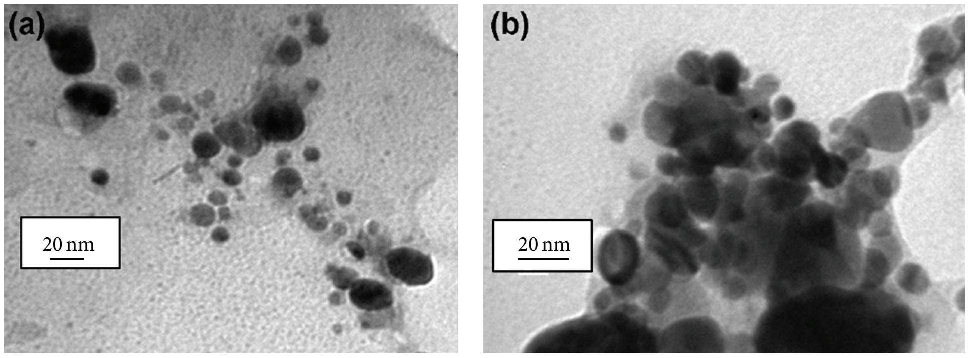


Figure 6: TEM micrographs with (a) 1 mL and (b) 5 mL olive leaf extract [48] (reproduced with permission from the journal).

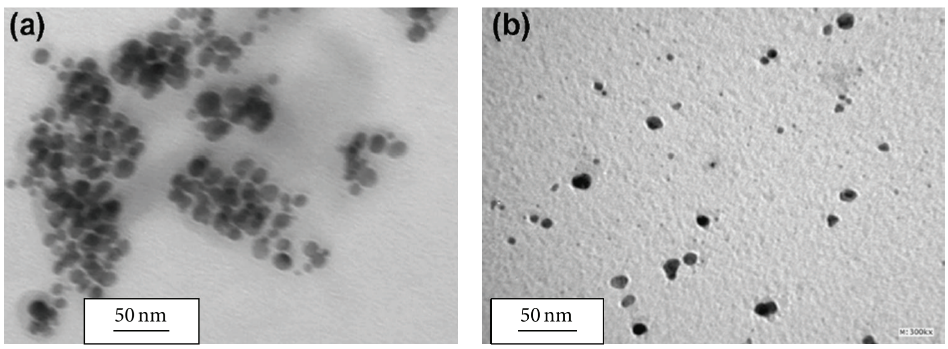


Figure 7: TEM micrograph of the silver nanoparticles [48]: (a) at pH 3, nm and (b) at pH 8 (reproduced with permission from the journal).

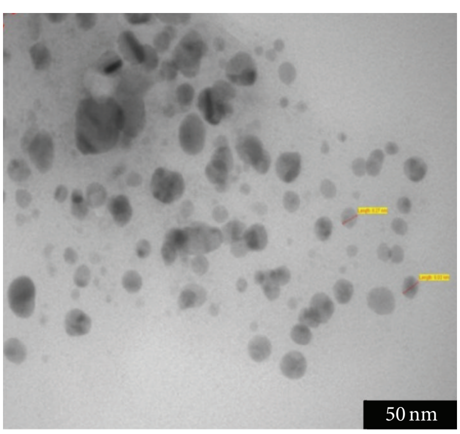


Figure 8: Ag NPs synthesized using *Tinospora Cordifolia* extract as a reducing agent [50] (reproduced with permission from the journal).

compared to others. Therefore, as a first approach, bacteria were studied as first nanofactories for the production of noble metal nanoparticles. However, the low synthesis rate and the limited number of size and shape distributions available oriented the investigations to the use of fungi and algae.

Fungi present a suitable option for large-scale green nanoproduction. They are easy to handle during downstream processing and they secrete large amounts of enzymes needed in the reduction. They also present filamentous tolerance towards metals, high binding capacity, and intracellular uptake. Nevertheless, the genetic manipulation to overexpress specific enzymes in order to intensify synthesis is much more difficult among eukaryotes.

More recently, many investigations were carried out on the possible use of plant extracts and the number of research papers published in this field has increased exponentially during the last 2 years due to their easy availability, environmental friendliness, and cost-effectiveness. Moreover, plants contain the most effective compounds for synthesis and therefore enhance the synthesis rate. Size and shape distribution of the nanoparticles as obtained from TEM studies shows that many factors affect their morphologies including the nature of the plant extract, the pH of the solution, and the temperature of the reaction. Nevertheless, obtaining uniform size and shape distribution of Ag NPs remains a subject of investigation.

## Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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