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Survey

SURVEY

Biosynthesis, characterization and therapeutic applications of plant-mediated silver nanoparticles

ANDREIA CORCIOVA[1]* and BIANCA IVANESCU[2]

[1]*Department of Drugs Analysis, "Grigore T. Popa" University of Medicine and Pharmacy, 16 Universitatii Street, 700115, Iasi, Romania and* [2]*Department of Pharmaceutical Botany, "Grigore T. Popa" University of Medicine and Pharmacy, 16 Universitatii Street, 700115, Iasi, Romania*

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Abstract: Nanotechnology is one of the most studied domains, and nanoparticle synthesis, especially of silver nanoparticles, has gained special importance due to their properties, biocompatibility and applications. Today, the processes of nanoparticles synthesis tend toward the development of inexpensive, simple, non-toxic and environmentally friendly methods. Thus, the use of plants in the synthesis of silver nanoparticles has attracted considerable interest because biomolecules can act as both reducing and stabilizing agents. This survey aims at discussing the conditions for obtaining silver nanoparticles using plants and their characterization by several methods, such as FTIR and UV–Vis spectroscopy, X-ray diffraction, and scanning and transmission electron microscopy. In addition, it examines some of the most common biological uses of silver nanoparticles: antibacterial, antioxidant and cytotoxic.

Keywords: green chemistry; synthesis; antibacterial; antioxidant; cytotoxic.

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* Corresponding author. E-mail: acorciova@yahoo.com
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1. INTRODUCTION

Nanotechnology, which refers to the manipulation of particles with sizes smaller than 100 nm, named nanoparticles, is a developing domain. Special attention is attributed to zero-valent metal nanoparticles that can serve as the basis for various physical and biological systems. Metallic nanoparticles have numerous biological applications and can be used as therapeutic and diagnostic agents, as carriers for targeted drug delivery, for biomolecules detection, and they can be employed in other fields too, such as food, agricultural or treatment of waste.[1]

In this survey, the focus is on the silver nanoparticles (AgNPs), considered among the most potent antimicrobial agents, as evidenced by the numerous studies that have explored their antibacterial[1,2] and antifungal activity[3] in the past decade.

AgNPs can be prepared and stabilized by physical and chemical methods. Besides the fact that these methods raise problems of toxicity and have a relatively inadequate cost–efficiency ratio, many experimental conditions affect their size, morphology, stability and properties.[1,4] Preparation of nanoparticles involves the reduction of metal ions by a reducing agent and their stabilization at the zero valence state through capture by a stabilizing agent. This process requires the use of chemicals that pose a potential risk to the environment and influence the size and shape of the particles.[3] Therefore, the current trend is the biological synthesis of AgNPs through the use of various biological agents, such as yeasts, enzymes, bacteria, polysaccharides, algae, oligosaccharides, fungi, DNA and human cell lines.[1,4–8] Perhaps one of the most accessible and environmentally safe methods compared to others methods for nanoparticles production consists in the use of plant extracts and whole plants. The plants are easily accessible, the process is less laborious and shorter, the synthesis can be realized on a large scale and the method is environmentally friendly.[3,9]

Thus, the paper presents an analysis of the conditions for obtaining AgNPs, with plants, followed by a presentation of the methods by which they could be characterized and their biological applications.

2. SYNTHESIS OF SILVER NANOPARTICLES

An extract of unicellular green algae *Chlorella vulgaris* was used for the first time to synthesize single-crystalline AgNPs at room temperature. The components that participated in the synthesis were proteins that had two functions: reduction of Ag^+ (*via* the carboxyl groups in aspartate or glutamate residues and hydroxyl groups in tyrosine residues) and control of the shape of nanoparticles (anisotropic growth of silver nanocrystals into nanoplates). This synthesis process gave a good yield (> 55 %) of low polydispersity AgNPs.[10]

Obtaining AgNPs using plants was first reported by Gardea-Torresdey *et al*.[11] They demonstrated that silver ions Ag^+ are reduced to Ag in a solid agar medium and then adsorbed from alfalfa roots and transferred into shoots, where the silver atoms are rearranged to form nanoparticles that could form further various other arrangements.

The majority of plants used for the synthesis of AgNPs belong to the following families: *Acanthaceae*, *Amaranthaceae*, *Apocynaceae*, *Asteraceae*, *Burseraceae*, *Dioscoreaceae*, *Euphorbiaceae*, *Fabaceae*, *Lamiaceae*, *Moraceae*, *Myrtaceae*, *Poaceae*, *Ranunculaceae*, *Rutaceae*, *Solanaceae* and *Asphodelaceae*.[1]

Among the plants used for the synthesis of AgNPs, the most frequent were: *Punica granatum*, *Cydonia oblonga*, *Castanea sativa*, *Ficus carica*, *Juglans cinerea*, *Morus nigra*, *Morus alba*,[12] *Piper nigrum*,[13,14] *Morinda citrifolia*,[15] *Calendula officinalis*,[16] *Glycyrrhiza glabra*,[17] *Artemisia annua*,[18] *Sida acuta*,[18] *Emblica officinalis*, *Terminalia catappa*, *Eucalyptus hybrida*,[19] *Choerospondias axillaris*, *Nyctanthes arbortristis*, *Moringa oleifera*, *Zanthoxylum armatum*, *Areca catechu*,[20] *Argemone Mexicana* and *Ocimum tenuiflorum*.[21]

Biomolecules that participate in reduction and capping of the nanoparticles are distributed in the leaves, stems, fruit, seeds and roots, and belong to the following classes of compounds: alcoholic compounds, alkaloids, amino acids, phenols, flavones, enzymes, polysaccharides, proteins, terpenoids, some vitamins, chlorophyll and other metabolites.[22]

In the phytosynthesis of AgNPs, primary and secondary metabolites from plants act as both reducing and capping agents. Primary metabolites, such as carbohydrates, proteins, peptides, amino acids and vitamins, are always present in plants and are involved in the reduction and stabilization of metallic silver in nanoparticles.[23] Li *et al*.[24] showed that amine groups in proteins from the aqueous extract of *Capsicum anuum* anuum played an important role in the formation of AgNPs. Furthermore, the carbonyl group of amino acids and proteins has the

ability to bind metal ions, to cap nanoparticles and to prevent agglomeration, thereby stabilizing the medium.[25]

Some secondary metabolites with biological activities, such as terpenoids, alkaloids, flavonoids, phenolic acids and other polyphenols, have been reported to act as either reducing or stabilizing agents in the formation of AgNPs. Sathishkumaret *et al*.[26] reported that the terpenoids (linalool, eugenol, methyl chavicol) present in *Cinnamomum zeylanicum* bark are responsible for the reduction of AgNPs. The alcohols, ketones, aldehydes, and lactones of terpenoids are involved in both the reduction and capping of silver nanoparticles. In adequate concentration, terpenoids could be adsorbed on the surface of AgNPs, possibly by interaction through π-electrons or carbonyl groups.[27]

Secondary metabolites are active principles from plants and their inclusion in silver nanoparticles could potentially enhance their biological activities or help target the compounds. For example, numerous studies focused on the synthesis of AgNPs with extracts from *Artemisia* species[28,29] and evaluation of their antibacterial, antifungal, antioxidant and anticancer effects.[18,30,31] *Artemisia* is a widely spread genus including numerous medicinal and aromatic plants known to be a rich source of bioactive compounds (sesquiterpene lactones, flavonoids, phenolic acids, coumarins) with antimicrobial, anticancer and anti-inflammatory activities.[32] In addition to having intrinsic therapeutic value, these compounds are also involved in the synthesis of nanoparticles.

Various functional groups of flavonoids can actively chelate and reduce metal ions into nanoparticles.[33] Zargar *et al*.[34] showed that the hydroxyl groups in the structure of flavonoids present in an aqueous extract of *Vitex negundo* are responsible for the bioreduction of metal ions during the synthesis of AgNPs. Moreover, polyphenols and caffeine present in tea or coffee extracts play a crucial role in the synthesis of AgNPs.[35] In the synthesis of AgNPs in a *Lycopersicon esculentum* leaf extract, the hydroxyl and carbonyl groups of phenols reduced the silver ions to silver, while the amines present in the extract capped the AgNPs.[36] Polyols in a papaya fruit extract are the compounds implicated in the bioreduction of silver ions and formation of AgNPs.[37] Different antioxidants with varied structures present in *Ananas comosus* juice act synergistically in reducing silver ions.[38]

For the extraction of biomolecules, solvents such as water,[12,16] ethanol,[39,40] 70 % ethanol,[41] *etc*. are used. Among these, water is preferred, because it is the most polar solvent, easy to use for active principles extraction and further is a non-toxic solvent.[42]

The synthesis of nanoparticles has several stages:

- preparation of the plant extract and silver salt solution;

- preparation of AgNPs by mixing those two solutions in different proportions, at certain pH and temperature values and for different times. In the presence

of plant compounds, Ag^+ is reduced to Ag, and then oligomeric clusters are formed, which lead to silver nanoparticles;

- AgNPs purification is realized by centrifugation to remove the unreacted plant extract, followed by resuspension in distilled water and again centrifugation, repeatedly, to remove unwanted substances;
- confirmation of AgNPs formation through different analytical methods.

2.1. Conditions for AgNPs synthesis

For the AgNPs synthesis, various parameters need to be taken into account in order to achieve the maximum yield, nanoparticles with a certain shape and size and to achieve stability.

Parameters that require investigation:
- concentration and amount of plant extract;
- concentration of silver salt;
- pH;
- temperature;
- reaction time.

2.1.1. Concentration and amount of plant extract

To obtain AgNPs from *Aegle marmelos* leaves, Rao and Paria[43] used an initial 5 % concentration extract that was diluted 4–50 times. As or the 20- and 50-fold dilutions, unreacted $AgNO_3$ was observed in the medium, a dilution of 17 times (0.3 %) was considered appropriate for the production of AgNPs. Concerning the concentration of $AgNO_3$, concentrations of 1–4 mM were tested. A concentration of 1 mM was considered appropriate, since an increase in the particle size was observed when the concentration of $AgNO_3$ was further increased. This situation could be explained on the one hand by the small concentration of active principles in the extract as capping agent, insufficient to prevent agglomeration. On the other hand, a high concentration of $AgNO_3$ decreases the rate of reaction and nuclei formation.

Another example is the generation of AgNPs with tealeaf extract of different concentrations: 1, 5, 10, 25, 50 and 100 % and 10 mM solution $AgNO_3$, by stirring at 700 rpm, at 25 °C for 120 min. It was observed that the yield of nanoparticles production was above 94 % in all cases. In the following studies, a 5 % plant extract concentration was used, as the zeta potential was found to increase with increasing extract concentration. Example: from –20.7 mV (1 % extract), –21.3 mV (5 % extract) to –12 mV (50 % extract) and –11.3 mV (100 % extract). With the increase of the zeta potential, AgNPs dispersion becomes unstable due to particles precipitation from the dispersion.[44]

Halawani[42] showed that at a higher concentration of the plant extract, the biocompounds act both as reducing agents and as surface coatings of nanoparticles to protect them from aggregation.

Moreover, utilization of different amounts of extract can control certain properties of the resulting nanoparticles. In the case of AgNPs synthesis from *Malus domestica* extract, by using a different ratio extract, $AgNO_3$ (1–5 mL extract: 50 mL of 0.1 M $AgNO_3$), it was observed that the formation of uniformly dispersed AgNPs was significantly influenced by this ratio. Peak absorption increased with increasing amount of extract, which showed that as the amount of extract added was increased, the possibility of reduction of silver nitrate increased, which will lead to the formation of well defined and stable silver nanoparticles.[45]

The same was observed for AgNPs from *Artemisia absinthium*, *i.e.*, the maximum absorbance increased with increasing extract concentration (from 10 to 50 %) and decreased with decreasing of extract concentration (from 60 to 10 %). Augmentation of the extract concentration raises the average hydrodynamic sizes of AgNPs.[28]

In the case of *Pinus eldarica* bark, on raising the amount of extract, the absorbance increased, and production of nanoparticles grew, but no directly proportionally, and the AgNPs size decreased.[46]

The use of equal volumes of plant extract and silver salt led to comparatively smaller size and more stable nanoparticles.[28]

In some cases, stabilizing agents such as sodium dodecyl sulfate (SDS) and sodium citrate were used. These were used to prepare AgNPs from *Artemisia nilagirica* leaf extract. The authors/researchers also used silver nitrate as the precursor metal and hydrazine hydrate as a reducing agent, thereby obtaining particles with a diameter of 70–90 nm.[29]

2.1.2. Concentration of silver salt

For the preparation of AgNPs, silver acetate[41] or silver nitrate may be used, the most common being silver nitrate ($AgNO_3$), of various concentrations (0.1––10 mM). For *Garcinia mangostana* leaf extract, the optimal concentration to obtain AgNPs was found to be 1 mM.[47] In the case of AgNPs obtained from *Angelica pubescens*, the most effective conditions were: equal volumes of the two solutions, 5 % extract (obtained at 100 °C, for 30 min) and 5 mM $AgNO_3$, temperature 80 °C and a reaction time of 50 min. The average crystallite size was 12.48 nm.[48]

Generally, by augmenting the concentration of $AgNO_3$, the production of AgNPs increases[46] and the color of the solution intensifies, because aggregation of silver ions occurs and larger nanoparticles are obtained.[14]

In the case of AgNPs from *Solanum trilobatum* using 1 mM $AgNO_3$, monodisperse nanoparticles were formed without aggregation. In the 2–4 mM $AgNO_3$

concentration range a band at 460 nm was observed with aggregation and at 5 mM $AgNO_3$ concentration a broad band at 500 nm was observed, indicating that the particles were polydisperse. The plasmon resonance band broadened with increasing $AgNO_3$ concentration.[49]

2.1.3. pH

The pH plays an important role in the synthesis of AgNPs, particularly influencing the size and morphology of the nanoparticles. To demonstrate the effect of pH on the formation of AgNPs, Iravani and Zolfaghari[46] used *Pinus eldarica* bark extract (20 %, boiled for 15 min), $AgNO_3$ of different concentrations and phosphate buffer with a pH in the range of 3–11. On increasing the pH, the reduction intensified, the absorbance was augmented and the production and stability of AgNPs enhanced. More4over, the pH influences the size of AgNPs, *i.e*., at low pH values, large AgNPs were obtained while small-sized AgNPs were obtained at high pH values. The same conclusion was reached in the case of AgNPs obtained from *Garcinia mangostana* leaves extract. Acid pH values suppress the formation of AgNPs, with aggregation of nanoparticles and formation of large nanoparticles (pH 4), while alkaline pH (pH 8) favors the formation of AgNPs, with nucleation and formation of small highly dispersed AgNPs. The optimal pH was found to be neutral.[47]

Another example of the influence of pH is given by *Acalypha indica* fresh leaves extract, at 2–13 pH range. In this case, at acid pH values, AgNPs were not obtained while at alkaline pH values, a rapid color formation was observed, which proves the formation of AgNPs with the absorption peak shifted to 500 nm, but at pH 13, agglomeration was immediate. At neutral pH the reaction started after the addition of $AgNO_3$ and AgNPs formation was complete within 30 min of incubation.[50]

In the case of AgNPs production using *Crataegus douglasii* fruit, for a period of 24 h, the absorption wavelength of the sol decreased from pH 2 to pH 6. At pH 2, AgNPs were not generated and flocculation observed, while in alkaline media an important decrease in the flocculation parameter occurred, which may have been the result of aggregation.

The stability of the nanoparticles in the presence of additives depends on the pH of the solution because hydroxide ions may change the surface charge of the nanoparticles. Cluster distribution stability was improved in the alkaline pH range due to the complete charging of the cluster, thus maximizing the electrostatic/electrosteric repulsive interactions. At pH values above 8, Ag^+ partially hydrolyzes in solution to form bioorganic–$Ag(OH)_x$ or bioorganic–$Ag(NH_3)_2$ complexes on the surface of the particles and $AgOH/Ag_2O$ colloid in the medium. The degree of hydrolysis and the formation of colloids are enhanced by an increase in the solution pH.[51,52]

For AgNPs from *Solanum trilobatum*, pH 5.8 suppressed the formation of the nanoparticles due to the limited availability of functional groups of the compounds in the extract. At pH 8.8, a broad absorption band peaking at 480 nm was obtained, while at pH 7.8, a narrow peak positioned at 440 nm was observed, with the maximum yield of AgNPs.[49]

2.1.4. Temperature

The reaction temperature influences the production and size of AgNPs. In order to obtain AgNPs, various temperatures could be used. Room temperature was found to be optimal because small and spherical AgNPs are formed, having a single surface plasmon band, at lower wavelengths.[1] Thus, Kumar *et al*.[53] demonstrated the production of AgNPs with 35 ± 5 nm mean particle size and irregular spherical shape using a 10 % *Annona squamosa* extract and 1 mM $AgNO_3$ at room temperature for 4 h.

Ndikau *et al*.[54] synthesized AgNPs from 0.001 M *Citrullus lanatus* fruit peel extract, and 250 g L^{-1} $AgNO_3$ in 4:5 ratios at 80 °C and pH 10. The AgNPs were stable, spherical, with diameters of 17.96±0.16 nm.

Das *et al*.[55] prepared AgNPs from *Sesbania grandiflora* leaf extract and 1.0 mM $AgNO_3$ solution by incubation at 37 °C in the dark. The AgNPs were stable for 6 months at room temperature, had a spherical shape and a size in the 10–25 nm range.

Preparation of AgNPs from *Solanum trilobatum* by varying the temperature led to the following conclusions: at 20 °C no plasmon resonance band was observed, at 35, 45 and 70 °C characteristic peaks of the formation of AgNPs were observed, and the maximum yield was obtained at the highest temperature. The plasmon resonance peaks of the formed AgNPs shifted significantly toward the blue region from 460 nm to 440 nm, suggesting that the same form of nanoparticles strongly influences the plasmon resonance bands at higher temperatures.[49]

At high temperatures (25–150 °C), a rise in AgNPs production, an increase of absorbance and a reduction of nanoparticles size, which resulted in an increase in the sharpness of their plasmon resonance band, were observed.[46] In the case of AgNPs from *Garcinia mangostana* leaf extract, by varying the temperature (37––90 °C), the production of nanoparticles was enhanced with increasing temperature. The AgNPs initial size was reduced because of the decrease in the aggregation of the growing nanoparticles, while at over 75 °C, the crystal grew around the nucleus that led to decrease in the absorbance.[47]

2.1.5. Reaction time

Reaction time influences the production and stability of AgNPs. Thus, by testing some different reaction times (0–70 min), it was found that the production of AgNPs increased with increasing reaction time. However, AgNPs instability

should be taken into account, because after an optimal period of 60 min, the AgNPs agglomerate and the particle size increases.[47]

Logeswari *et al*.[56] obtained AgNPs using ethanol extracts of *Solanum trilobatum*, *Syzygium cumini*, *Centella asiatica* and *Citrus sinensis*, 1 mM $AgNO_3$, ammonium solution, at 37 °C using reaction times of 24–48 h. The silver ions were reduced to AgNPs, which could be seen from the change in the color of the solution from yellow to dark brown. The AgNPs had irregular shapes and the nanoparticle size was between 41–53 nm.

In addition, Vanaja *et al*.[49] studied the generation of AgNPs from *Solanum trilobatum* extract and observed, at 20 min, the appearance of a sharp, narrow peak at 420 nm indicating the formation of an isotropic shape and a uniform size of the nanoparticles. After 20 min, the peak had shifted to 440 nm. On increasing the reaction time, the synthesis of nanoparticles was enhanced and the maximum yield was confirmed by the absorption maximum. The reaction was completed in 4 h and was visually observed through the occurrence of a precipitate at the bottom of the vial.

In case of AgNPs obtained from *Ocimum sanctum*, the bioreduction of silver ions into nanoparticles was observed to begin at 3 min and reached an optimum level within 30 min (optimum absorption at 430 nm). The absorption band measured at 60, 90 and 120 min intervals had not achieved better values. The widening of the absorption peak with increased time indicated increased polydispersity of the nanoparticles.[57]

Besides using the stirring method, ultrasonic cavitation technique could be used on a mixture of extract and silver salt to obtain AgNPs. Thus, Shimpi *et al*.[3] prepared AgNPs from an extract of *Alstonia scholaris* leaves (25 %) obtained by the same method and 0.01M $AgNO_3$ for 40 min at an on–off pulse rate of 5 s at an intensity of 20 KHz. The ultrasonic cavitation technique is based on cavitation, which consists in the formation, growth and collapse of bubbles due to the transmission of acoustic vibrations through a medium. According to Shimpi *et al.*[57] the silver ions diffuse into the cavitation bubbles and concentrate at the interface where they react with high-energy species, with the start of nucleation. During cavitation, the bubbles grow and when the maximum size is reached they collapse by implosion, and AgNPs are created. These nanoparticles have a large number of dangling bonds on their surface. In order to gain surface stability, it is necessary to establish connections, which leads to the development of nuclei. When growth is terminated prematurely, AgNPs stabilization occurs through the formation of bonds with the stabilizers present in the plant extract, which prevents aggregation of the nanoparticles.

Some examples of plants that have been used to obtain AgNPs are given in Table I, together with the conditions used to obtain AgNPs.

TABLE I. Plants and conditions used for AgNPs synthesis

Plant	Vegetal material	Extract concentration (in water)	$AgNO_3$ concentration	Method	Temperature °C, pH	Time of reaction	AgNPs size (nm), shape distribution	Reference
Peumus boldus	Leaves	5 %, Soxhlet extraction	2 mM	Stirring	ambient	3 h	18 nm, spherical	58
Heritiera fomes, Sonneratia apetala	Leaves barks	10 %, boiled for 10 min	1 mM	Stirring, under direct sun light	ambient	12 h	50, 400 nm 20–30, 70–100 nm	59
Urtica dioica	Leaves	20 %, boiled for 15 min	10^{-3}M	Incubation in the dark	40 °C	60 min	20–30 nm, crystallized in face-centered cubic	60
Acalypha indica	Leaves	10 %, heated at 65 °C for 5 min	1 mM	Incubation in the dark, under static conditions	37 ºC	–	–	50
Hedera helix	Leaves	20 %, boiled for 20 min	1 mM	Stirring, under sun light	ambient	15 min	more or less spherical, 10–30 ± 2 nm in diameter	61
Ziziphus spina-christi	Leaves	25 %, boiled for 10 min	1 mM	Shaking, under dark conditions	ambient	30 min	21.5–59.67 nm, hexagonal shape	42
Sambucus nigra	Fruits	3 %, stirred for 1 h	1 mM	Mixed	ambient, pH 8	10 min	8–33 nm spherical	62
Artemisia annua, Sida acuta	Leaves	10 %, boiled 15 min	0.1 M	Manual shaking, under sunlight	ambient	10 min	–	18
Ocimum sanctum	Leaves	10 %, boiled for 10 min	10^{-2} M	Kept under direct sunlight	ambient	30 min	7–11 nm, circular shape	57
Ocimum tenuiflorum	Leaves	1.5 %, boiled	1 mM	Stirring	37 °C, in presence of ammonium solution	24–48 h	26 nm (XRD)[a]	63

TABLE I. Continued

Plant	Vegetal material	Extract concentration (in water)	$AgNO_3$ concentration	Method	Temperature °C, pH	Time of reaction	AgNPs size (nm), shape distribution	Reference
Solanum trilobatum	Leaves	1.5 %, boiled	1 mM	Stirring	37 °C, in presence of ammonium solution	24–48 h	20–22.3 nm	63
Syzygium cumini	Leaves	1.5 %, boiled	1 mM	Stirring	37 °C, in presence of ammonium solution	24–48 h	26–26.5 nm	63
Centella asiatica	Leaves	1.5 %, boiled	1 mM	Stirring	37 °C, in presence of ammonium solution	24–48 h	24–28.4 nm	63
Citrus sinensis	Peel	1.5 %, boiled	1 mM	Stirring	37 °C, in presence of ammonium solution	24–48 h	59–65 nm	63
Crataegus douglasii	Fruit	10 %, boiled 15 min	0.01 M	Shaking	28 °C	24 h	29.28 nm, nearly spherical shape	51
Carica papaya	Peel	10 %, refluxed for 1 h, at 80 °C	1 mM	Stirring	ambient	1 h	3–5 nm, polydispersed spherical in shape	64
Matricaria chamomilla	Plant	25 % boiled for 5 min	20 mM	Kept in a dark place	–	–	60–65 nm, different forms	65

[a]X-Ray diffraction

2.2. Methods for the analysis of AgNPs

Among the methods used to characterize the AgNPs encountered in the literature, the following will be mentioned:

- Visual inspection;
- UV-vis spectroscopy;
- Dynamic light scattering and determination of zeta potential;
- FTIR spectroscopy;
- X-Ray diffraction (XRD)
- Scanning and transmission electron microscopy (SEM and TEM);
- Photoluminescence spectroscopy.

2.2.1. Visual inspection

First, the demonstration of AgNPs formation is made visually by the color change from a colorless,[47] pale yellow,[66,67] yellow,[49,67] yellow–brown[46] or yellowish–brown[45,67] solution to a brown,[44,47,49] or dark brown sol[45] due to excitation of the surface plasmon resonance in the AgNPs.[46,47,66,67]

The color is due to surface plasmon resonance that occurs due to the presence of collective oscillation of conducting free electrons induced by an electromagnetic field that interact,[17] and electrons gathering around the surface of the metal particles.[20] The frequency and width of the surface plasmon resonance depends on the size and shape of the metal nanoparticles, the dielectric constant of the metal and the environment.[19]

2.2.2. UV–Vis spectroscopy

UV–Vis spectroscopy is used to observe the change in AgNPs size, based on the wavelength of the surface plasmon resonance band.[49]

Allafchian *et al*.[66] synthesized 25-nm spherical AgNPs using a *Phlomis* sp. extract rich in flavonoids, phenylpropanoids and other phenolic compounds and a 0.01 M $AgNO_3$ solution at room temperature. The UV–Vis spectra showed the a peak corresponding to the surface plasmon resonance AgNPs at 440 nm, after 5 min of reaction and the absorbance increased up to 30 min of reaction.

For AgNPs obtained from *Pinus eldarica* bark, monitoring was performed at a wavelength of 430 nm, observing an increase in the intensity of the absorbance over time, which demonstrates the increase in the concentration of AgNPs. A broad peak, with maximum absorbance at 438 nm showed that AgNPs from *Garcinia mangostana* leaf extract were polydisperse.[47]

The UV–Vis spectra obtained from a 5 % extract of the fresh fruits of *Malus domestica* and 0.001 M $AgNO_3$ proved the production of nanoparticles with different sizes: a large absorption peak was registered after a reaction time of 1 h and sharp peaks after 6 and 24 h, with the wavelength of the absorption peak shifting to higher values with increasing reaction time.[45]

In the case of the AgNPs synthesized using olive leaves extract, it was observed that as the concentration of the extract increases, the absorption peak becomes more defined and sharper, and the wavelength shifts to blue, 458–441 nm. This behavior indicates a reduction of the average particle diameter and a spherical and homogeneous distribution of AgNPs.[67] On the other hand, AgNPs from *Piper nigrum* leaves extract, with increasing concentration of $AgNO_3$ solution (1–5 mM) showed a blue shift of the absorption maximum, indicating an increase in the average diameter of nanoparticles in the range of 5–50 nm.[14]

The blue shift of the absorption peak located at 420 nm could be explained by the small particle size $\leq$ 25 nm.[68,69]

2.2.3. Dynamic light scattering and determination of the zeta potential

The particle size is determined by dynamic light scattering, a technique that characterizes the size of colloidal dispersion, by studying the illumination of a particle or molecule suspension in Brownian motion with a laser beam. Fluctuations in the intensity of scattered light in dependence on time are analyzed with the help of an autocorrelator that determines the autocorrelation depending on the signal.

The zeta potential describes the degree of stability of a colloidal dispersion of nanoparticles.[66] The zeta potential value may be positive or negative; a negative value may occur due to the possible incorporation of bioactive components present in the extract.[45,70–72] In addition, a negative potential suggests that the surface of the nanoparticles is negatively charged, which causes a strong rejection force between the particles, thus preventing their aggregation.[73] A strong negative potential may be due to a shielding effect determined by the bioactive compounds in plants.[74]

Gengan *et al*.[74] are of the opinion that a zeta potential higher than 30 mV or lower than –30 mV demonstrates a stable system.

In the case of *Malus domestica*, the AgNPs obtained were polydisperse mixtures with sizes in the range of 50–300 nm, and a mean size of about 150 nm in diameter. The zeta potential of the synthesized AgNPs was determined in water as dispersant and was –65.07 mV; the high value confirms a particle repulsion phenomenon and an increase in the stability of the formulation.[45]

2.2.4. FTIR spectroscopy

FTIR is a useful method in the analysis of the chemical composition of the AgNPs surface and the local molecular environment of nanoparticle incorporation agents. Thus, in order to demonstrate the production of AgNPs by FTIR analysis, the FTIR spectra of the extract and AgNPs are compared to observe the common peaks and the changes that had occurred.

The FTIR spectrum of AgNPs from *Malus domestica* had a broad band at 3450 cm^{-1}, attributed to OH stretching vibrations due to the presence of hydroxyl groups in the reducing agent, and intense peaks at 1379 and 1625 cm^{-1} corresponding to C–N stretching vibrations and to amide I band of proteins from the apple fruit extract. This confirms that *Malus domestica* extracts have the ability to reduce and stabilize AgNPs.[45]

FTIR spectra of AgNPs and *Calendula officinalis* extract showed common but displaced bands: the bands at 3350 (extract) and 3323 cm^{-1} (AgNPs) are assigned to the OH groups, the bands at 2928 (extract) and 2943 cm^{-1} (AgNPs) are assigned to the alkyl and CH groups, the bands at 1622 (extract) and 1640 cm^{-1} (AgNPs) are assigned to type I amides, 1237 (extract) and 1217 cm^{-1} (AgNPs) are assigned to type III amides. In addition, in AgNPs spectra, there are

bands not found in the extract spectrum: at 1406, 1021 and 840 cm^{-1}, bands specific for the presence of silver.[40]

Shifted peaks were also observed in the FTIR spectra of AgNPs synthesized from the tea leaf extract: from 3420 to 3371 cm^{-1} due to N–H stretching amides, from 2931 to 2925 cm^{-1} due to C–H stretching alkanes, from 1383 to 1371 cm^{-1} for hydroxyl groups and from 1051 to 1044 cm^{-1} for C-stretching from ether groups. In the AgNPs, new peaks were observed at 1695, 1452, 1241, and 926 cm^{-1} related to alkene groups (C–C stretching), tertiary ammonium ions, polyphenols, aliphatic amines (C–N stretching vibrations) and alkene groups (C–H stretching).[44]

In the case of AgNPs from *Garcinia mangostana* leaf extract, in addition to the stretching absorption bands corresponding to C–C (aromatic ring), C–O–C (ethers) and C–O (C–OH), at 1160 cm^{-1}, a band corresponding to C–O from the –OH group of hydroxyflavones and hydroxyxanthones was observed. This band was not present in the spectrum of the AgNPs because these compounds were responsible for reducing Ag^{+}, thereby becoming oxidized to an unsaturated carbonyl group, resulting in a broad peak at 1660 cm^{-1}.[47] The same explanation is given for the AgNPs from *Morinda pubescens*, *i.e.*, the band at 1226 cm^{-1} most likely corresponding to C–O group of hydroxyflavones and catechins disappeared after the bioreduction, and a peak at 1650 cm^{-1} was observed.[75]

The presence of secondary metabolites acting as reducing agents in the synthesis of AgNPs from *Solanum nigrum* was demonstrated by a peak at 2357 cm^{-1}, attributed to hydrogen-bonded OH stretching.[69]

The FTIR spectra of AgNPs and *Glycyrrhiza glabra* root extract demonstrate that the potential biomolecules responsible for the synthesis and stabilization of AgNPs are flavonoids, terpenoids, thiamine and reducing sugars.[17]

2.2.5. X-Ray diffraction (XRD)

The mean size of the crystallized particles of AgNPs can be calculated using the Debye–Scherrer formula.[66,76]

In order to apply XRD testing, the samples were prepared as follows: the dispersion resulting in the process of nanoparticles production was centrifuged at 10000 rpm for 30 min. The AgNPs residue was washed and resuspended in absolute ethanol that was evaporated to dryness at 80 °C to obtain AgNPs powder, which was used for the X-ray diffraction measurements.[45]

The XRD spectrum of AgNPs from *Malus domestica* showed four distinct peaks at degrees (2θ) 38.28, 44.330, 64.33 and 77.53°, and from *Solanum trilobatum* at 38.13, 46.2, 64.44 and 77.36°. These values correspond to the (111), (200), (220) and (311) planes of face-centered-cubic (FCC) silver, with a network parameter of 4.08 Å, which is consistent with the Joint Committee Pow-

der Diffraction Standards (JCPDS) Card No-087-0720.[44,45,49] Another peak was also observed at 2θ 31.9°, possibly due to Ag_2O.[44]

The XRD spectrum of AgNPs synthesized from *Solanum nigrum* showed 6 peaks at 2θ 10.00, 11.51, 12.00, 27.75, 32.15 and 45.94°, corresponding to six diffraction facets of silver.[69]

In the case of AgNPs obtained from the *Mussaenda erythrophylla* leaf extract, the pattern showed characteristic Bragg reflections. The XRD spectrum shows only one clear signal at 2θ 38.50°, corresponding to (111) plane of face-centered cubic silver, demonstrating that the synthesized nanoparticles were highly pure.[70]

2.2.6. Scanning and transmission electron microscopy (SEM and TEM)

SEM was used to determine the surface morphology and topography of the synthesized silver nanoparticles. Samples are prepared by placing a drop from the colloidal dispersion onto a support and drying at room temperature.

The SEM images for AgNPs obtained from *Malus domestica* show flower structures[45] and those from *Solanum trilobatum* have spherical and monodisperse forms, well distributed with aggregation in the range 50–70 nm.[49]

AgNPs synthesized using *Phlomis* sp. extract were spherical, well dispersed, and the size ranged from 19 to 30 nm, with an average size of 25 nm, confirmed by SEM and TEM.[66]

SEM and TEM analyzes of AgNPs obtained from *Musa balbisiana, Azadirachta indica* and *Ocimum tenuiflorum* demonstrated the production of nanoparticles of different shapes due to the different availability, quantity and nature of the capture agents present in the extracts. The nanoparticles were predominantly spherical but also in the form of triangles, pentagons and hexagons with dimensions up to 200 nm.[77]

A spherical form of nanoparticles was observed for the AgNPs obtained from *Garcinia mangostana*[47] and tea leaf extract[44]. In the case of AgNPs obtained from *Alstonia scholaris* leaf extract, TEM analysis demonstrated the production of spherical and triangular pyramidal shaped nanoparticles with minimal aggregation and diameters of 15–38 nm.[3]

For the AgNPs synthesized from olive extract leaves, the TEM images showed that the average particle size was influenced by the concentration of the plant extract: as the concentration of extract increases, the particle size decreases. In addition, small amounts of extract reduce silver ions, but do not protect most of the quasi-spherical AgNPs from aggregation, because there is a deficiency of biomolecules that act as protective agents. At high concentration, biomolecules act as reducing agents and cover the surfaces of nanoparticles, protecting them from aggregation.[67]

High resolution TEM images for the AgNPs obtained from a *Terminalia catappa* extract, showed that each nanoparticle was surrounded by a material with a lower contrast, which could be a bio-organic extract component that acts as a stabilizer agent for the nanoparticles.[19] For the AgNPs obtained from *Couroupita guianensis* leaf extract, Devaraj *et al*.[78] consider that the same phenomenon in SEM images were due to hydrogen bonding interactions and electrostatic interactions between organic biomolecules bound to the surface of the AgNPs, and hence, the nanoparticles were not in direct contact, even within aggregates, indicating stabilization of the nanoparticles by a coating agent.

2.2.7. Photoluminescence spectroscopy

The visible luminescence of AgNPs is due to the excitation of electrons from occupied bands into states above the Fermi level.[79,80]

The AgNPs obtained from *Alstonia scholaris* leaf extract were photoluminescent at a 370 nm excitation wavelength, the obtained peak having a bathochromic shift to 447 nm.[3]

3. THERAPEUTIC APPLICATIONS OF AgNPs

3.1. Antimicrobial activity

AgNPs were shown to be active against both Gram-positive and Gram-negative bacteria, as well as fungi.

For example, the antifungal activity of AgNPs synthesized from *Acalypha indica* leaf extract intensified with increasing concentrations of AgNPs. The mechanism of action includes the destruction of membrane integrity of fungal spores, the interaction of AgNPs with compounds containing phosphorus and sulfur, leading to the destruction of DNA and proteins of the microorganisms and ultimately to cell death.[50]

The AgNPs obtained from *Solanum trilobatum* inhibited the growth of pathogenic bacteria, the highest percentage of inhibition being observed against *Escherichia coli, Klebsiella planticola, Klebsiella pneumoniae* and *Streptococcus* sp.[49]

The antibacterial action could be explained by several mechanisms. Thus, the antibacterial effect of the AgNPs obtained from *Garcinia mangostana* leaf extract against *E. coli* and *Staphylococcus aureus* has a double mechanism of action, namely, the bactericidal effect of Ag^+ and the damaging effect on some polymeric subunits of the membrane.[47]

Kvitek *et al*.[81] and Feng *et al*.[82] suggested that AgNPs attach themselves to the surface of the bacterial cell membrane by interacting with sulfur-containing proteins, disrupting the membrane permeability and the respiratory functions of the cell leading to cell death. In this regard, Li *et al*.[83,84] demonstrated the

interaction of AgNPs with compounds containing thiol from the respiratory enzymes of bacterial cells, thus inhibiting the respiration process in bacteria.

It is also evident that the binding of the particles to the microorganisms depends on the surface available for interaction. Generally, small nanoparticles have a larger surface area for bacterial interaction compared to larger particles.[85–87] For example, the stronger bactericidal effect of AgNPs containing higher amounts of *Olea* extract was justified by the extremely large surface area of small nanoparticles that provide for better contact and better interaction with bacterial cells than larger nanoparticles.[67]

Morones *et al*.[88] explained the antibacterial action of AgNPs not only by interaction with the membrane surface, but also by penetration into the bacteria. Li *et al*.[84] showed that AgNPs enter the bacterial cells and condense the DNA so that DNA replication and cell replication are prevented.

Since antimicrobial activity proved to be different for Gram-positive bacteria and Gram-negative bacteria, the difference in sensitivity was explained by the difference in the thickness of bacterial cell membrane and the constituents of the membrane. This fact was demonstrated by Khalil *et al*.[67] who tested the antimicrobial activity of AgNPs obtained from *Olea* leaves extract against *S. aureus, Pseudomonas aeruginosa* and *E. coli*. Gram-negative bacteria were less sensitive to AgNPs than Gram-positive bacteria. This effect was also observed for AgNPs obtained from *Piper nigrum* leaves extract due to the difference between the cell wall of Gram-positive and Gram-negative bacteria.[14] Augustine *et al*.[14] tested AgNPs obtained from *Piper nigrum* leaves extract and $AgNO_3$ with different concentrations. It was observed that by increasing the $AgNO_3$ concentration, the antibacterial activity decreased, which was attributed to the larger size of the AgNPs, and thus, the antibacterial activity was dependent on the size of AgNPs.

Moreover, in the case of AgNPs obtained from the *Phlomis* sp. leaf extract, it was observed that the antimicrobial action decreased in the order *E. coli*, *Salmonella typhimurium, S. aureus* and *Bacillus cereus*.[67] This may be due to the Gram-positive bacterial cell wall composed of a thick layer of peptidoglycan with a rigid structure made of linear polysaccharides chains cross-linked by short peptides, which prevents nanoparticles from entering the cell wall.[89,90]

Variation in the chemical composition of the bacterial cell wall also explains the varied degree of antibacterial activity of AgNPs synthesized from *Avicennia alba* leaves *versus* Gram-positive and Gram-negative bacteria. The best activity was observed for *Arthrobacter protophormia* and *Proteus mirabilis*, even better than streptomycin, as a positive control.[91]

AgNPs obtained from *Cassia roxburghii* stem were tested against bacteria and fungi in combination with an antibiotic/antifungal agent and compared with antibiotic/antifungal agent alone. A synergistic action was observed. Antibac-

terial activity was higher in the case of Gram-negative bacteria than Gram-positive bacteria, and the antifungal activity was moderate.[92]

Some examples of antifungal and antibacterial activity of AgNPs from plant extracts are Presented in Table II.

TABLE II. Antifungal and antibacterial activity of AgNPs from plant extracts

Plant species and part of plant used for AgNPs synthesis	Sensitive fungi	Sensitive bacteria		Reference
		Gram-positive bacteria	Gram-negative bacteria	
Acalypha indica leaf	*Alternaria alternata*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Rhizoctonia solani*, *Botrytis cinerea*, *Curvularia lunata*			50
Solanum trilobatum leaf		*B. subtilis,* *Streptococcus* sp.	*Serratia* sp., *E. coli*, *K. pneumoniae*, *K. planticola*	49
Olea europaea leaf		*S. aureus*	*E. coli*, *P. aeruginosa*	67
Piper nigrum leaves		*S. aureus*	*E. coli*	14
Avicennia alba leaves		*Micrococcus luteus*, *Arthrobacter protophormia*, *Rhodococcus rhodochrous*, *Enterococcus faecalis*, *Streptococcus mutans*, *B. subtilis*	*Enterobacter aerogenes, Alcaligenes faecalis, Proteus vulgaris*, *Proteus mirabilis*, *P. aeruginosa, Salmonella enterica*	91
Cassia roxburghii stem	*Candida glabrata*, *Candida albicans*, *Cryptococcus neoformans*	*S. aureus*, *B. cereus*	*E. coli*, *P. aeruginosa*	92

3.2. Antioxidant activity

One of the methods used for testing the antioxidant activity of AgNPs is the DPPH (2,2-diphenylamine-1-picryl hydrazyl) scavenging method. Thus, AgNPs synthesized from *Heritiera fomes* and *Sonneratia apetala* and 1 mM $AgNO_3$, by stirring, under exposure to sunlight, for up to 12 h showed potent antioxidant activity, exhibiting IC_{50} (the effective concentration that shows 50 % inhibition activity) values in the range 53.64–169.71 μg mL^{-1}, due to the ability to donate electrons or hydrogen ions to neutralize DPPH free radicals.[59] In the case of

AgNPs obtained from *Angelica pubescens*, a dose-dependent activity was observed, the IC_{50} value was 1.01 mg mL^{-1}. The free radical scavenging activity was due to biomolecules such as flavonoids, sesquiterpenes, and phenols.[48]

Abdel-Aziz *et al*.[94] determined the antioxidant activity of AgNPs (30–50 nm) obtained from *Chenopodium murale* extract and 5 mM $AgNO_3$ by the DPPH scavenging and *β*-carotene bleaching assays. The results showed significant differences between the antioxidant values of the plant aqueous extract and AgNPs due to the higher total phenolic content and total flavonoids content in the case of AgNPs. The IC_{50} values for the two methods increased in a dose-dependent manner.

Goodharzi *et al*.[95] determined the antioxidant potential by the DPPH method and the reducing capacity by the Folin–Ciocalteu method for some AgNPs synthesized from extracts of the following plants: *Rosmarinus* sp., *Zataria multiflora*, *Pelargonium graveolens*, *Chamaemelum nobile*, *Francoeuria undulata*, *Achillea wilhelmsii* and *Carthamus tinctorius*. The highest antioxidant and reduction capacity were observed in the case of *Rosmarinus sp*. and *Z. multiflora* nanoparticles. The lowest antioxidant activity and reduction capacity were observed in the case of *C. tinctorius* nanoparticles. In addition, the relationship between antioxidant and reduction capacity demonstrates that plants with high antioxidant activity have high reducing capacity and high capacity for AgNPs synthesis.

3.3. Cytotoxic activity

Currently, the development of molecules that could safely transport active principles inside the human body is a top priority and the synthesis of nanoparticles derived from plants could prove to be an efficient solution. However, the major concern is still the safety of these carriers.

Evaluation of cytotoxic activity can be achieved by the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) method. The 50 % cytotoxic concentration (CC_{50}) is defined, as the concentration of a compound (μg mL^{-1}) required to reduce cell viability by 50 %, which is calculated by regression analysis.[96]

AgNPs obtained from *Salvia officinalis* and *Ricinus communis* were tested for cytotoxicity. The MTT test indicated that treatment with different plant extracts and nanoparticles reduced the cell growth of the Vero cell line in a concentration-dependent manner. CC_{50} values for the Vero cell line were 22.7, 11.7, 16.5, 10.0, 14.0 and 15.0 mg mL^{-1} for *Salvia officinalis* leaf extract, AgNPs obtained from *Salvia officinalis* leaf extract, *Ricinus communis* leaf extract, AgNPs obtained from *Ricinus communis* leaf extract, *Ricinus communis* fruit extract and AgNPs obtained from *Ricinus communis* fruit extract. The results did not involve the activity of the extracts on other cell lines (primary or transformed), or

the antiproliferative actions *in vivo*. However, the cytotoxic effect of these nanoparticles must be taken into account for future use.[96]

AgNPs synthesized from *Tanacetum vulgare* leaf extract, at concentrations ranging from 0.5 to 35 μg L^{-1}, were tested for their cytotoxic action compared to the aqueous leaf extract. It was observed that both samples show cytotoxicity against breast cancer (MCF-7) cell lines. The cytotoxicity of AgNPs was dose dependent while that of the plant extract was not because some of the bioconstituents may increase cell growth, while others may decrease it.[97] The same dose-dependent cytotoxicity on the MCF-7 breast cancer cell line was observed for AgNPs synthesized from *Couroupita guianensis* leaf extract.[78]

Piao *et al*.[98] explained the mechanisms of AgNPs cytotoxicity as follows: the destruction of cellular components by AgNPs is determined by the induction of generation of reactive oxygen species and depletion of intracellular glutathione. It also leads to c-Jun N-terminal kinase activation, mitochondrial disruption, caspase activation and apoptosis. By testing the AgNPs obtained from *Podophyllum hexandrum* leaf extract against human cervical carcinoma HeLa cells, it was shown that the synthesized nanoparticles act by decreasing cellular proliferation, induction of oxidative stress, DNA damage and apoptosis by caspase-mediated and mitochondria-dependent pathways.[99]

4. CONCLUSIONS

The emergence of AgNPs synthesis by biological methods has been a significant development in the field of nanoparticles. The interest for this type of synthesis has increased rapidly because these methods do not require the use of hazardous/toxic chemicals, as did previously employed methods. The use of plants, easily accessible factors, has led to the production of AgNPs through a simple, fast, cost-effective and eco-friendly process. The synthesis of nanoparticles was attributed to the abundance of biomolecules in plant extracts. Studies have shown that the size, morphology, stability and biological properties of metallic nanoparticles are strongly influenced by the type of molecules found in plant extracts and by the experimental conditions, and hence, designing the synthesis of AgNPs with desired characteristics is a real challenge in the production of nanoparticles from plants.

ИЗВОД

БИОСИНТЕЗА ПОТПОМОГНУТА БИЉКАМА, КАРАКТЕРИЗАЦИЈА И ТЕРАПИЈСКА ПРИМЕНА НАНОЧЕСТИЦА СРЕБРА

ANDREIA CORCIOVA[1] и BIANCA IVANESCU[2]

[1]Department of Drugs Analysis, "Grigore T. Popa" University of Medicine and Pharmacy, 16 Universitatii Street, 700115, Iasi, Romania and [2]Department of Pharmaceutical Botany, "Grigore T. Popa" University of Medicine and Pharmacy, 16 Universitatii Street, 700115, Iasi, Romania

Нанотехнологија, поготову синтеза наночестица сребра, привлачи посебну пажњу због њихових својстава, биоподударности и примене. Савремени процеси синтезе

наночестица заснивају се на јефтиним, једноставним и нетоксичним методама, које не нарушавају животну средину. Синтеза наночестица сребра коришћењем биљака привлачи велико интересовање зато што биомолекули могу да делују као редукујуће, али и стабилишуће врсте. Услови за добијање наночестица сребра употребом биљака, као и карактеризација ових честица помоћу неколико метода, као што су инфрацрвена спектроскопија са Фуријеовим трансформацијама, UV–Vis спектроскопија, дифракција X-зрака и скенирајућа и трансмисиона електронска микроскопија, дискутовани су у овом прегледном раду. Поред овога, разматрани су и неки од уобичајених примера биолошке примене наночестица сребра, као што су: антибактеријски, антиоксидативни и цитотоксични.

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