Antioxidant silver nanoparticles green synthesized using ornamental plants

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The aim of this study was to describe a *green* approach to prepare new antioxidant silver nanosystems using some ornamental plants (*Hyacinthus orientalis* L. and *Dianthus caryophyllus* L.) as promising tools for biomedical applications. The potential ability of these plants for the bioreduction of Ag^+ to Ag^0 was investigated by spectral methods (UV-VIS absorption, FTIR, DLS). The stability of the herbal silver nanoparticles was checked by ξ-potential measurements and their antioxidant properties were determined by chemiluminescence method.

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1. Introduction

Nowadays it is a growing interest in the development of *nanotechnology* – the science which deals with the creation, production, characterization and manipulation of materials at the nanoscale [1, 2].

Nano-sized materials, known as *nanoparticles*, possess unique and improved properties because of their larger surface area to volume ratio and are considered *building blocks* of nanotechnology to design materials with interesting properties.

In the last decade, noble metal nanoparticles have attracted much attention of scientific researchers due to their applications in medicine, biology, optoelectronics, material science [3-7]. Among the noble metals, silver is the most extensively used and studied because of their unique properties and its use in the biomedical field especially [8-11].

A lot of strategies are employed for the synthesis of silver nanoparticles (AgNPs), but the *green* methods have been gained considerable interest because of use of environmentally benign materials [12-15].

So, the synthesis and design of nanomaterials through biological routes (called *biosynthesis*) have attracted great interest. Among the biological systems, the living plants [16-20] are considerably preferred for biosynthesis of silver nanoparticles due to the diversity richness of plant kingdom that provides phytochemicals with strong antioxidant properties. It is well known that plants have been used by humans for a very long time to treat many diseases.

This paper aims to demonstrate that ornamental plants can be used not only for decorative purposes, but also in therapeutic applications. *Dianthus caryophyllus* L. and *Hyacinthus orientalis* L., the mostly cultivated ornamental plants around the world, were used to synthesize silver nanoparticles.

The petals of *Dianthus caryophyllus* L. contain many active constituents (flavonoids, anthocyanins, dianthramides, antiretroviral proteins and phenols) and have many medicinal uses (skin toner, antifungal, antimicrobial, antispasmodic, acute dermatitis relief, tooth pain, vomiting and gastritis, digestive function stimulant) [21]. The dried flower buds of *Dianthus caryophyllus* L. are an oriental drug with cardiotonic, diaphoretic, antibacterial and vermifuge properties, being used also in the treatment of gastro-intestinal disorder and to treat tooth aches [22].

Polyhydroxylated alkaloids isolated from *Hyacinthus orientalis* bulb inhibit some glycosidases, having therapeutic applications as anti-diabetic agents [23].

Soare *et al*. concluded that the extracts obtained from *Hyacinthus orientalis* flowers have antimicrobial activity and exhibit antioxidant properties similar to those of different medicinal plants [24].

Our study reports a simple, eco-friendly and economical method to phytosynthesize silver nanoparticles using aqueous petal extracts obtained from these ornamental flowers: *Dianthus caryophyllus* L. and *Hyacinthus orientalis* L.

The phyto-AgNPs prepared were monitored by spectral methods (UV-VIS absorption, FTIR, DLS) and their physical stability was checked by ξ-potential measurements. The antioxidant properties of these nanostructures were evaluated using chemiluminescence assay.

2. Experimental part

2.1. Reagents

Silver nitrate, luminol (5-amino-2,3-dihydro-phthalazine-1,4-dione), Tris(hydroxymethylaminomethane base), HCl, H_2O_2 were purchased from Merck (Germany).

2.2. Preparation of plant extracts

Plant materials (*Hyacinthus orientalis* and *Dianthus caryophyllus*) were purchased from local flowershops.

The samples used in our experiments (rose and white hyacinth, white and red carnation) were cleaned in distilled water and cutted into small pieces. 1 gram of petals were weighed and transferred into a 50 mL Erlenmeyer flask with 10 mL of distilled water and boiled for 10 minutes in order to release the intracellular material into solution. The aqueous petal extracts thus obtained were then filtered through a filter paper to obtain a clear extract.

2.3. Silver nanoparticle green synthesis

The ornamental plant extracts were used as reducing agents for Ag^+ as well as capping agents for silver nanoparticles.

In order to obtain silver nanoparticles, 5 mL of flower extracts were added and mixed with 5 mL $AgNO_3$ 10^{-3} M. Samples were kept in the dark at 4°C.

The colour of the ornamental flower extracts changed from yellowish to brown in aproximatelly 30 minutes after the addition of $AgNO_3$ 10^{-3} M, indicating the formation of silver nanoparticles.

2.4. Characterization methods

UV-VIS spectroscopy analysis

The absorption spectra of the plant extracts and of the silver nanoparticles were obtained with a double beam UV-VIS spectrophotometer Lambda 2S Perkin Elmer (PECSS software), in the wavelength range of 200-800 nm.

ATR-FTIR spectroscopy

Fourier transformed IR spectroscopy (FTIR) spectra were collected using a Perkin Elmer Spectrum GX instrument with Attenuated Total Reflectance (ATR) diamond crystal in the range of 700–4000 cm^{-1} (at a spectral resolution of 4 cm^{-1}).

DLS technique

The size of the silver nanoparticles was measured by dynamic light scattering technique (Zetasizer Nano ZS, Malvern Instruments Ltd., U.K.), in the range between 0.6 nm and 6.0 μm, at a scattering angle of 90^0C and 25°C temperature, using intensity distribution.

ξ-potential determination

The ξ-potential measurements of the silver nanoparticles were realized by applying an electric field across the analyzed aqueous dispersions using the appropriate accessory of Zetasizer Nano ZS (Malvern Instruments Ltd., U.K.).

The zeta potential is related to the electrophoretic mobility and to the stability of the of silver nanoparticle suspensions. All measurements were performed in triplicate.

Chemiluminescence assay

The *in vitro* antioxidant activity of plant extracts and of herbal silver nanoparticles has been determined by chemiluminescence (CL) measurements using a Chemiluminometer Turner Design TD 20/20, USA.

Luminol - a cyclic hydrazide, has been used as a light amplifying substance which emits light when oxidized in the presence of oxidizing species. As a free radicals generator system, it has been used H_2O_2 in TRIS-HCl solution buffer (pH = 8.6).

The antioxidant activity (percentage of free radical scavenging) of each sample was calculated using the expression:

$$AA = \frac{I_0 - I}{I_0} \cdot 100\%$$

where I_0 is the maximum CL intensity for *standard* (the reaction mixture without the sample) at t = 5 s and I is the maximum CL intensity for sample at t = 5 s [25, 26].

3. Results

3.1 Characterization of silver nanoparticles by UV-VIS absorption spectroscopy

The absorption spectra of aqueous petal extracts obtained from four kinds of ornamental plants: red and white *Hyacinthus orientalis* (Figure 1), pink and white *Dianthus caryophyllus* (Figure 2) were compared with the absorption spectra of silver nanoparticles prepared using these extracts in order to reveal the formation of silver phyto-nanoparticles.

The absorption spectra of silver phyto-nanoparticles were recorded after 24 hours after their preparation and exhibited absorbance peaks at 458 nm (rose and white hyacinth), 440 nm (red carnation) and 415 nm (white carnation) which are specific for silver nanoparticles [27] and do not appear in the spectra of the aqueous petal extracts.

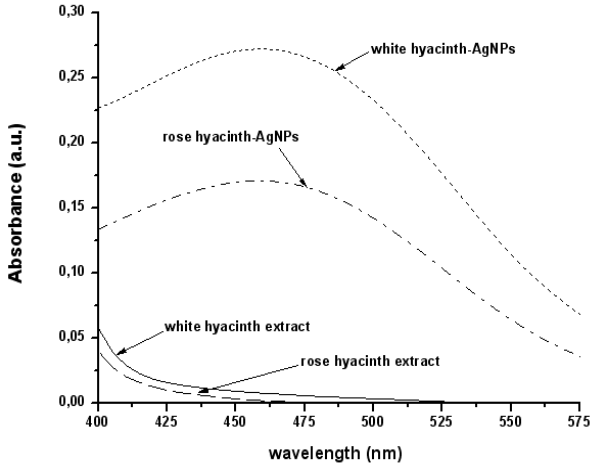

Fig. 1. Absorption spectra of the rose and white hyacinth extracts, of the rose hyacinth-AgNPs and white hyacinth-AgNPs

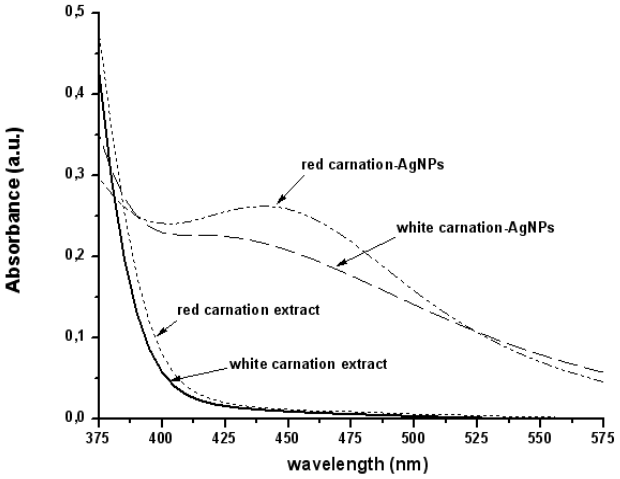

Fig. 2. Absorption spectra of the white and red carnation extracts, of the white carnation-AgNPs and red carnation-AgNPs

3.2 Characterization of ornamental plant-AgNPs by ATR-FTIR analysis

ATR-FTIR spectra of silver nanoparticles synthesized using aqueous petal extracts from: *Dianthus caryophyllus* (Figures 3 and 4) and *Hyacinthus orientalis* (Figures 5 and 6) were recorded in order to study the formation of herbal silver nanoparticles and to identify the possible biomolecules responsible for Ag^+ bioreduction and for capping the resulted silver phyto-nanoparticles.

The FTIR spectra of silver nanoparticles exhibited IR bands located in the region 1300-1350 cm^{-1} (corresponding to asymmetric and symmetric stretching vibrations of the nitrate group): 1330 cm^{-1} for red carnation-AgNPs, 1317 cm^{-1} for white carnation-AgNPs, 1348 cm^{-1} for pink hyacinth-AgNPs, 1339 cm^{-1} for white hyacinth-AgNPs.

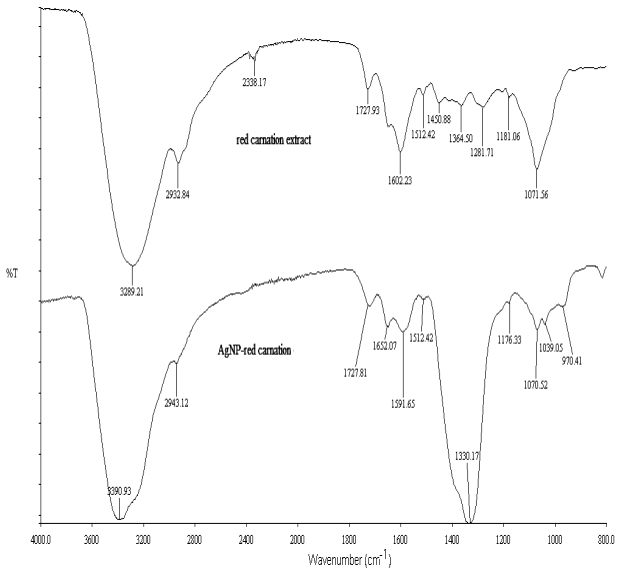

Fig. 3. ATR-FTIR spectra of red carnation extract and red carnation-AgNPs

The IR bands in the region 1652-1657 cm^{-1} may result from stretching vibration of -C=C-. The peaks around 1650 cm^{-1} are assigned to the amide I bonds of proteins [28, 29].

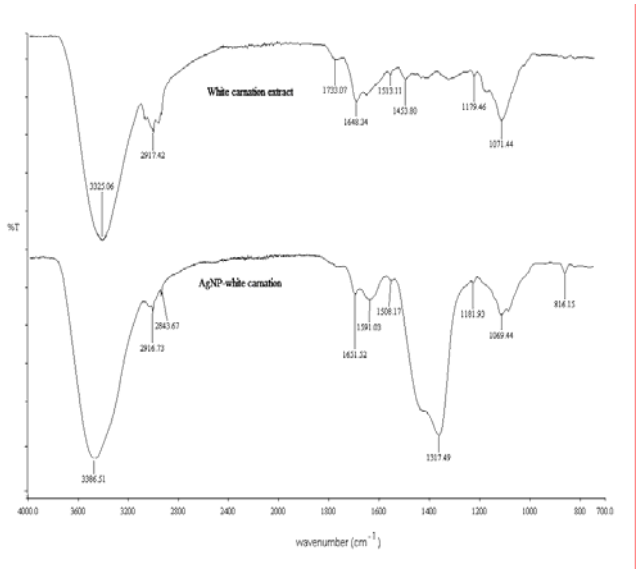

Fig. 4. ATR-FTIR spectra of white carnation extract and white carnation-AgNPs

ATR-FTIR spectra could provide useful information about the possible biomolecules responsible for Ag^+ ion reduction and for capping the phytosynthesized silver nanoparticles. These spectra revealed various functional groups present at different positions.

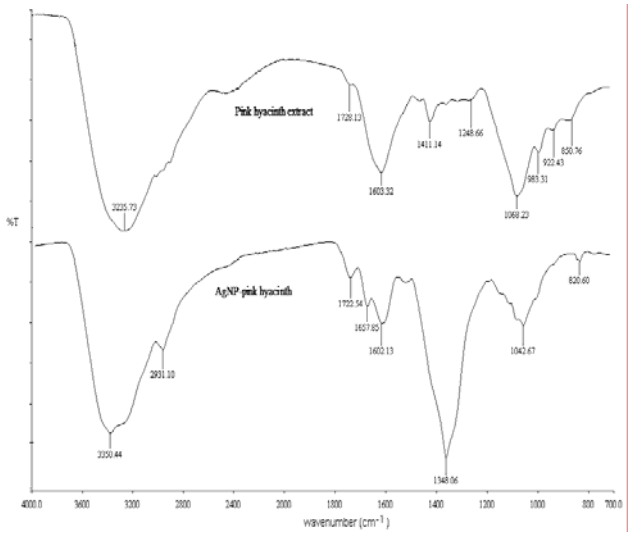

Fig. 5. ATR-FTIR spectra of pink hyacinth extract and pink hyacinth-AgNPs

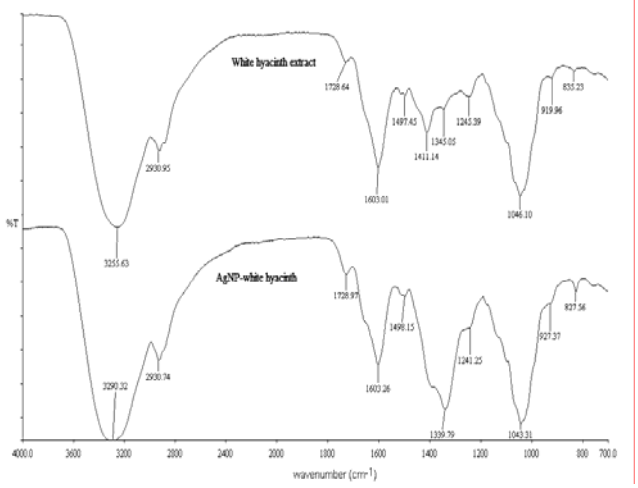

Fig. 6. ATR-FTIR spectra of white hyacinth extract and white hyacinth -AgNPs

Table 1. DLS data of AgNPs and the ξ-potential values of AgNPs

Sample	$Z_{average}$ (nm) ± SD	PDI ± SD	ξ-potential (mV) ± SD
rose hyacinth-AgNPs	61.45 ± 0.308	0.367 ± 0.041	-24.7 ± 0.385
white hyacinth-AgNPs	62.12 ± 0.212	0.349 ± 0.137	-23.6 ± 0.245
white carnation-AgNPs	65.65 ± 0.118	0.329 ± 0.045	-22.3 ± 0.208
red carnation-AgNPs	89.6 ± 0.135	0.337 ± 0.092	-21.6 ± 0.203

3.3 Characterization of herbal AgNPs by DLS technique

The size of the herbal silver nanoparticles was determined by dynamic light scattering measurements.

The hydrodynamic diameters, $z_{average}$ and the polydispersity index, PdI are presented in Table 1.

The polydispersity index is the measure of the distribution of nanoparticle population and high values for PdI indicate a large size distribution with multiple AgNP population.

3.4 Evaluation of physical stability of silver phytonanoparticles

The physical stability of the silver nanostructures was evaluated in terms of ξ-potential. The zeta potential values of the phytonanosilver suspensions are shown in Table 1.

The surface charge of all the silver nanostructures was negative, ranging from -21.6 to -24.7 mV.

3.5 The antioxidant properties of herbal AgNPs

The chemiluminescent method was used to evaluate the antioxidant properties of the silver nanoparticles phytosinthesized using ornamental plants.

Fig. 7 shows the antioxidant activity values of the plant extracts compared with AgNPs prepared from them.

All of the samples exhibited strong antioxidant properties ranging between 86.46 and 95.16% for plant extracts and 88.30 and 97.38%, for herbal silver nanoparticles.

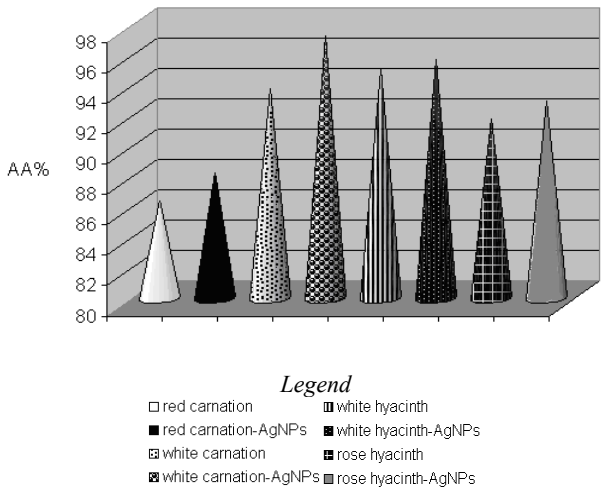

Fig. 7. Antioxidant activity(AA%) of ornamental plant extracts and of ornamental plants-AgNPs

4. Discussion

The present study reported the bioreduction of silver ions through ornamental flower extracts and testing for their antioxidant activity.

The phytosynthesis of silver nanoparticles was confirmed firstly by visual observation: the yellowish colour of petal extracts turned to brown after addition of $AgNO_3$ 10^{-3} M solution due to excitation of surface plasmon vibrations indicating the formation of silver nanostructures [30].

The synthesis of silver nanoparticles using ornamental flowers were detected also by UV-VIS absorption spectra showing a strong plasmon resonance which was centered between 415-458 nm, depending on the type of petal extract.

The UV-VIS absorption spectra of the plant extract alone showed no absorption in the spectral window between 400-600 nm, whereas the aqueous petal extracts exposed to silver ions (from $AgNO_3$ 10^{-3} M solution) presented distinct absorption at around 415-458 nm.

ATR-FTIR spectra were recorded in order to identify the possible biomolecules responsible for silver ion bioreduction and for capping the phytosynthesized silver nanoparticles.

The ATR-FTIR spectra of aqueous flower extracts showed strong IR bands characteristic of O-H stretching of hydroxyl (3289 cm^{-1} for red carnation, 3325 cm^{-1} for white carnation, 3235 cm^{-1} for pink hyacinth, 3255 cm^{-1} for white hyacinth; 3391 cm^{-1} for red carnation-AgNPs, 3386 cm^{-1} for white carnation-AgNPs, 3350 cm^{-1} for pink hyacinth–AgNPs; 3290 cm^{-1} for white hyacinth-AgNPs) [31, 32], C-H stretching (alkyls) (2917 cm^{-1} in white carnation and white carnation-AgNPs, 2933 cm^{-1} in red carnation, 2943 cm^{-1} in red carnation-AgNPs and 2931 cm^{-1} for pink and white hyacinth flower extracts and (pink and white)hyacinth-AgNPs), –C–O alcohols, C–OH alcohols, –OH aromatic, C–O alcohols (1039,05 cm^{-1} for red carnation-AgNPs; 1055 cm^{-1} for white carnation-AgNPs; 1042,08 cm^{-1} for pink hyacinth-AgNPs; 1043,31 cm^{-1} for white hyacinth-AgNPs) [15], C=C aromatic ring or amides I, amides II (between 1451 and 1513 cm^{-1} for red carnation and red carnation-AgNPs; 1508-1513 cm^{-1} for white carnation and white carnation-AgNPs; 1411-1498 cm^{-1} for white hyacinth and (pink and white)hyacinth-AgNPs), C-O bending (esters, polyols: hidroxiflavones, catechins) or amide III (1265 cm^{-1}). Specific IR bands characteristic for catechins were found at 970-1071 cm^{-1} for red carnation and red carnation-AgNPs, 816-1071 cm^{-1} for white carnation and white carnation-AgNPs, 851-1068 cm^{-1} for pink hyacinth, 820-1042 cm^{-1} for pink hyacinth-AgNPs, 835-1046 cm^{-1} for white hyacinth and 827-1043 cm^{-1} for white hyacinth-AgNPs [33]. Specific peaks for ketones were observed at 1727 cm^{-1} for red carnation and red carnation-AgNPs, at 1730-1733 cm^{-1} for white carnation and white carnation-AgNPs, between 1722-1728 cm^{-1} for pink hyacinth and pink hyacinth-AgNPs, at 1728 cm^{-1} for white hyacinth and white hyacinth-AgNPs.

All of the phytosynthesized silver nanoparticles presented FTIR bands characteristic for polyphenols in the wavenumber range between 1651 and 1658 cm^{-1} [33-36].

ATR-FTIR spectra showing the presence of IR peaks assigned to polyphenols and also the existence of IR bands characteristic of amide I and amide II groups specific for proteins/enzymes suggest that flavonoids and proteins present in aqueous petal extracts of ornamental plants could be responsible for the reduction of silver ions and for the stabilization of the phytosynthesized noble metal nanoparticles [37-39].

The DLS results indicated that all the particles are nano-sized with average diameters ranging between 61.45 and 89.6 nm and a good polydispersity index.

The zeta potential values (ranging between -21.6 and -24.7 mV) of herbal silver nanoparticles revealed a moderate physical stability of the samples due to interparticle repulsion.

All silver phyto-nanoparticles presented higher values of antioxidant activity than plant extract alone. In all the tested samples, the antioxidant activity of petal extracts was amplified after silver nanoparticles synthesis. This enhancement was more pronounced in the case of *Dianthus caryophyllus* – AgNPs samples.

The herbal silver nanoparticles exhibited high values of antioxidant activity ranging between 88.30 and 97.38%, white carnation–AgNPs having the strongest antioxidant properties (AA = 97.38%).

Nanoparticles smaller in size, then having a larger total surface area, were more efficient in the antioxidant activity tests as compared with nanoparticles with bigger size. Thus, red carnation-AgNPs, the smallest AgNPs, possess the lower antioxidant activity: AA = 88.3%).

The antioxidant behavior of these silver phyto-nanosystems makes them useful in therapy of many diseases caused by oxidative stress.

5. Conclusions

This paper described a new facile, low-cost and ecological method to prepare silver nanoparticles using ornamental plants.

Scientific literature reveals no data concerning the synthesis of noble metal nanoparticles using *Hyacinthus orientalis* and *Dianthus caryophyllus*.

The phytosynthesis of silver nanoparticles was demonstrated by visual inspection and by performing some spectral techniques (UV-VIS absorption, ATR-FTIR spectroscopy).

ATR-FTIR results proved that bioactive compounds responsible for silver bioreduction could be proteins and flavonoids (present in the aqueous petal extracts of ornamental flowers) presumed to act as reducing and capping agents for the silver nanoparticles preventing the agglomeration of the particles and thereby stabilizing the nanoparticles.

The herbal silver nanostructures presented average diameters ranging between 61.45 and 89.6 nm and a moderate stability indicated by the zeta-potential values.

The silver phyto-nanoparticles exhibited high antioxidant properties compared with the plant extract alone, so these metal nanoparticles could be used in treatment of many diseases caused by oxidative stress.

This study demonstrated that these beautiful flowers could be used not only as decorative elements, but also for biomedical purposes.

These plants contain active ingredients, so it is obvious and necessary to exploit the vast therapeutic potential of plants.

In conclusion, this study proved the ability of ornamental plant extracts to reduce Ag^+ to Ag^0, the ingredients of these flowers acting as reducing agents for Ag^+ as well as capping agents for phytosynthesized AgNPs.

The method for silver nanoparticle synthesis described in this paper is a *green* procedure (using environmentally benign natural resources) with a lot of advantages such as eco-friendliness, biocompatibility and cost-effectiveness allowing large scale commercial production of these herbal AgNPs to be used in biomedical applications.

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