Introduction

Synthesis of metal nanoparticles (NPs) is extensively researched due to their wide applications in different fields such as medicine, electronics, and energy. One of the most principal classes of metal NPs is that made of noble metals such as gold (Au), silver (Ag), and platinum (Pt). Among the noble metal NPs, Ag NPs are probably the most widely applied NPs in photonics, microelectronics, and photocatalysis. In addition to the wide use of Ag NPs in medicine due to their potent antimicrobial activity against a variety of pathogenic microorganisms.

Various physical, chemical, and biological approaches have been developed to synthesize silver nanoparticles. High temperature, pressure, and energy are usually required during preparation by physical methods. In case of chemical processes, majority of the used chemicals are toxic and probably unsafe for both the environment and the biological systems. Therefore, production of silver nanoparticles by these methods has been restricted. So, attention has been paid for biological approaches in which silver nanoparticles have been done by the use of microorganisms and plants. The microbe mediated synthesis routes have numerous limitations such as identification of powerful strain, preservation of aseptic circumstances, possibility of infection and contamination and time consuming. Therefore, scientists shift their attention toward plant extracts for the green synthesis of Ag NPs due to the major benefit of this synthesis technique in protecting the environment.

In this study, new series of stable densely dispersed Ag NPs were obtained using an entirely green synthesis approach. In this approach, the natural non-toxic ingredients extracted from the callistemon viminalis plant were used with assistance of heating to create the Ag NPs.

Materials and Methods

Materials

Fresh Callistemon viminalis leaves were collected from Orman garden in Giza. Silver nitrate, (AgNO₃) and Chitosan (medium molecular weight) were obtained from Sigma-Aldrich (Germany). All of the other chemicals and solvents were of analytical grade and used as provided.

Methods

Callistemon viminalis leaves were washed several times using tap water, distilled water and then deionized water to remove any impurities attached to the leaves. They were then air dried for 3 days at room temperature before cutting them into fine pieces. Twenty gram of the leaves were then transferred to a 500-ml flask with 100 ml of sterile distilled water. The suspension was then boiled for 10 min and the...
extract was collected using vacuum filtration through Whatman number 1 filter paper. The final extract was then stored at 4°C to be used within 1 week.  

Chitosan nanoparticles were prepared using the ionic gelation with TPP anions. Chitosan was dissolved at 0.2% level (w/v) in 3% (v/v) acetic acid before stirring. TPP was dissolved at 0.5% level (w/v) in distilled water. Then, the TPP solution was added drop wise to the chitosan solution during sonication (amplitude 60, pulse-on 5 sec, pulse-off 5 sec).

Various concentrations, durations and temperatures were tested to determine the optimum method for the preparation of AgNPs. In brief, mixtures of different v/v ratios (1:40, 2:40 and 3:40) of the plant extract and aqueous 2 mM AgNO₃ were prepared. Mixtures were heated for 1 min, 5 min, 10 min, 20 min, 30 min, 45 min and 60 min at 60°C, 70° C, 80° C and 100°C.

After determination of the optimum criteria for the synthesis of AgNPs, the process was repeated again with the addition of 1 ml of 3% (wt/v) chitosan solution. The synthesized nanoparticles were then separated by centrifugation at 20,000 rpm for 20 min. The obtained pellet is then washed 3 times using 20 ml of deionized water each.

Several characterization methods were used to evaluate the synthesis of AgNPs. Reduction of the AgNO₃ into AgNPs was monitored using UV-Vis spectrometry (Evolution 600, ThermoScientific, MA, USA). The absorption of the AgNPs suspensions resulting from various synthetic criteria was measured in the range of 200-800 nm. Samples were measured using the plant extract as the blank solution. Additionally, the plant extract and the AgNPs solution with the extract were evaluated using FTIR. Samples were dried and ground with KBr pellets and then analyzed in the range 400 – 4000 cm⁻¹ at resolution of 8 cm⁻¹. Transmission electron microscope, TEM (JEOL TEM-2100) was used to evaluate the morphology and size of the synthesized nanoparticles at an accelerating voltage of 120 kV. The samples were also analyzed using X-ray diffraction using CuKα radiation at λ =1.5406 Å with the voltage set to 40 kV and the current intensity to 40 mA sec. The 20 angles are measured in the range of 40 to 60°.

Results

A. UV-vis spectrophotometry

UV-vis spectrophotometry was also utilized to confirm formation of the Ag NPs at different parameters as shown in Fig. (1 a,b,c and d) Increasing callistemon viminalis extract concentration from 0.25 ml to 1ml increased the intensity of the peak then decreased with extract concentration of 2 ml as shown in Fig.1a. In Fig.1b, peak intensities increased as temperature of heating was arised from 60 °C to 100 °C. From Fig.1c, peak intensities of samples increased steadily with increasing time of heating from 1 min to 45 min. With addition of 1 ml chitosan 3% (wt/v) together with 1ml extract, the peak intensity increased more than 1 ml extract

B. X-ray diffraction (XRD) analysis

XRD pattern of callistemon viminalis/chitosan-Ag NPs prepared by heating at 100°C for 45 min using 2 mM AgNO₃ is illustrated in Fig. (1e). As apparent from the figure, there is a broad band that appeared at 2θ = 20° which indicates the amorphous phase of callistemon viminalis and chitosan. Additionally, the pattern showed Bragg reflections with 2θ values of 38.18°, 44.18°, 64.1°.

C. Fourier Transform Infra-Red (FT-IR) analysis

FTIR analysis was used to confirm the composition of the callistemon viminalis extract and the formation of the AgNPs with the extract. The FTIR spectra of the extract with and without the Ag NPs are shown in Figure (1f). Both spectra are very similar and showed bands around 1025, 1354, 1538, 1609, 2942, and 3449 cm⁻¹. A peak at 1025 cm⁻¹ can be attributed to C–O–C and C–OH vibrations of the protein in the leaf. The band appearing at 1354 cm⁻¹ corresponds to aliphatic.
Figure 1 Physicochemical characterization of AgNPs (a - d) UV-Vis absorption spectra showing optimization of various synthesis parameters, (e) XRD pattern, (f) FTIR absorption spectra and (g) TEM images showing the morphology of the nanoparticles CH2 and CH3 as well as the bending of O-H bonds. The amide I and II peaks can be observed at 1609 and 1541 cm⁻¹, respectively. Vibration of secondary amine of the proteins appeared at 1538 cm⁻¹. While the band that appeared around 2,942 cm⁻¹ corresponds to the C-H asymmetric stretching. OH stretching appeared as a broad strong band at 3240 cm⁻¹ which was slightly shifted to 3260 cm⁻¹ with the formation of AgNPs.

D. Transmission electron microscopy

The morphology of the nanoparticles was investigated using TEM for the nanoparticles developed after 45 min of heating at 100°C using 2 mM AgNO₃ in presence of chitosan (Figure 1g). As shown, the nanoparticles exhibited uniform spherical shape.

Discussion

In this study, Synthesis of Ag NPs has been achieved using various concentrations of plant aqueous extracts and chitosan with heating. The formation and the stability of the nanoparticles was confirmed using UV-Visible spectroscopy. The peak appearing at 420-440 nm for the Ag NPs result from the coherent oscillation of the surface electrons (surface plasmon resonance). Intensity of the peak increased by increasing the heating temperature to 100°C for time period up to 45 min which might be related to the concentration, size and shape of the AgNPs. It was also observed that the addition of chitosan resulted in increasing the intensity of the peak. This can be attributed to the ability of chitosan to chelate Ag⁺ through OH and NH₂ the β (1–4)
d-glucosamine units. Additionally, chitosan acts as a stabilizer for the Ag NPs preventing its aggregation at the macroscopic level due to the ion-dipole intermolecular forces.18 It was also obvious from the XRD pattern that the synthesized nanoparticles have crystalline nature.

FTIR was used to investigate the presence of reducing and stabilizing biomolecules in the callistemon viminalis extract. The strong broad band appearing at around 3240 cm⁻¹ suggests the presence of –OH groups which are responsible for the reduction of Ag⁺ into Ag⁰ and accordingly forming the nanoparticles.

**Conclusion**

The current study demonstrated that the callistemon viminalis extract can be used as a green reducing and capping agent for the eco-friendly synthesis of Ag NPs. The developed Ag NPs showed good stability. this preliminary study revealed that the developed Ag NPs can be tailored and used, after more investigations, as potential antibacterial agents for various biological and biomedical applications.

**References**