Novel Synthesis of Phyto-Mediated Silver Nanoparticles and Their Antioxidant Activity

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ABSTRACT

In recent science, nanotechnology is a burning field for researchers. Nanotechnology deals with nanoparticles having a size of 1-100 nm in one dimension used significantly in medical chemistry, atomic physics, and all other known fields. Nanoparticles are used immensely due to their small size, orientation, and physical properties, which are reportedly shown to change the performance of any other material which is in contact with these tiny particles. These particles can be prepared easily by different chemical, physical and biological approaches. But the biological approach is the most emerging approach to preparation because this method is easier than the other methods, eco-friendly, and less time-consuming. The green synthesis was done by using the aqueous solution of Wedelia Chinensis leaf extract and $AgNO_3$. Silver was of particular interest for this process due to its evocative physical and chemical properties. A fixed ratio of plant extract to metal ion was prepared and the color change was observed which proved the formation of nanoparticles. The nanoparticles were characterized by UV-vis Spectrophotometer, FTIR, XRD, and TEM. The nanoparticles were found to have sizes ranging from 160-180 nm.

Keywords: *Nanotechnology, Nanoparticles, Green Synthesis, Wedelia chinensis, Antioxidant activity.*

Introduction

The field of nanotechnology is one of the upcoming areas of research in the modern field of material science. Nanoparticles show completely new or improved properties, such as size, distribution, the morphology of the particles, etc. Novel applications of nanoparticles and nanomaterials are emerging rapidly in various fields [1]. Metal nanoparticles have a high specific surface area and a high fraction of surface atoms. Because of the unique physicochemical characteristics of nanoparticles, including catalytic activity, optical properties, electronic properties, antibacterial properties, and magnetic properties [2-5] they are gaining the interest of scientists for their novel methods of synthesis.

Silver is well known for possessing an inhibitory effect on many bacterial strains and microorganisms commonly present in medical and industrial processes. In medicines, silver and silver nanoparticles have ample application including skin ointments and creams containing silver to prevent infection of burns and open wounds, medical devices, and implants prepared with silver-impregnated polymers. In the textile industry, silver-embedded fabrics are now used in sporting equipment [6].

In recent days several attempts have been made to fabricate AgNPs with controlled sizes and shapes [14]. However, most of the wet chemical methods reported rely heavily on organic solvents and use hazardous reducing agents [7]. Hence, there is an increased interest to design a green chemistry route to minimize or eliminate the use and generation of toxic substances in synthetic processes [8]. Even though a number of plants have already attempted to synthesize AgNPs, synthesizing nanoparticles with controlled size and various morphologies is still a great challenge.

To date, no reports have been documented on a biogenic synthesis of AgNPs using the abundantly and commercially available *Wedelia chinensis* as a biomaterial. The plant has been claimed to have significant therapeutic effects in the management of cancer, inflammation, wound healing, CNS disorder, ulcer, etc. *Wedelia* is also known for its antioxidant and antimicrobial activities due to the presence of active constituents such as triterpenoids, flavonoids, and wedelolactones, and research is still in progress to find uses for them [9]. Hence, the present research aimed to investigate the synthesis and characterization of the AgNPs from the aqueous extract of *Wedelia chinensis* and evaluated its antioxidant activity.

Methodology

Sample Collection

Fresh green and mature leaves of Wedelia Chinensis were collected from Veliavillai, Kanniyakumari District (Tamilnadu, India), and used for the preparation of extract. Silver nitrate and $AgNO_3$ were procured from Merck Specialties Private Limited, Mumbai. Double distilled deionized water was used as a solvent for the synthesis.

Preparation of Leaf Extract

In a general manner, 10g of healthy and matured leaves of plant portion were selected and then washed thoroughly with deionized water. These leaves were ground well and collected using glass vessels. Afterward, it was boiled in 100ml of deionized water in a water bath at 600^0C for 20 minutes. The mixture was cooled and this extract is filtered using Whatman's no.1 filter paper. The filtrate was collected in a clean and dried conical flask by standard sterilized filtration method and was stored in a refrigerator for further use.

Synthesis of Silver Nanoparticles

1 mM aqueous solution of $AgNO_3$ was prepared and used for the synthesis of silver nanoparticles. 10 ml of leaf extract was added to 90 ml of 1 mM aqueous $AgNO_3$ solution in a 250 ml Erlenmeyer flask and incubated at room temperature. The sample colour changes from colourless to light grey within 10 minutes indicating the formation of AgNPs. Ninety-

five percent of the bioreduction of Ag^+ ions occurred within 1 hour. The AgNPs obtained by leaf extract was centrifuged at 15,000 rpm for 5 min and subsequently dispersed in sterile distilled water to get rid of any uncoordinated biological materials. The pellet of AgNPs collected at the bottom of the centrifuge tube was collected, dried, and stored at -40^{0}C.

Results and Discussion

The characterization of the silver nanoparticles and the detailed analysis on antioxidant activities are reported in this session.

UV – Visible Analysis of Silver Nanoparticle

Bioreduction of plant extracts to reduce silver ions into silver nanoparticles during exposure to the leaf extract could be followed by colour change **(Fig. 1)**. Silver nanoparticles exhibit light grey colour in an aqueous solution due to the surface plasmon resonance phenomenon, which results from collective oscillations of their conduction band electrons in response to electromagnetic waves. The strong absorption peak located at 400 nm confirms the reduction of silver ions to form metallic silver nanoparticles (AgNPs).

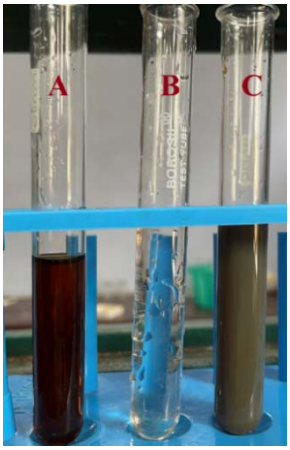

Fig. 1. Visual Observation of silver nanoparticles synthesis

(A- Plant extracts, B- Salt solution, C- Reaction mixture)

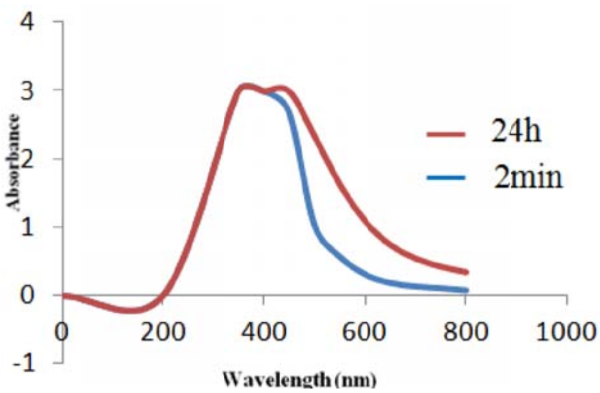

Fig. 2. UV-Visible spectra of AgNPs at different incubation times

FT-IR Analysis of Silver Nanoparticles

The FTIR measurements were carried out to identify the possible biomolecules present in aqueous Wedelia leaf extract responsible for the bioreduction and stabilization of silver nanocrystals. The spectra of leaf extract were recorded and compared before and after the addition of silver nitrate **(Fig. 3)**. The interferogram of dried leaf extract before the reaction shows a prominent peak at 1022, 1326, 1696, 2929, 3409 cm^{-1} represent the complex nature of the biomolecules **(Fig. 3a)**. The very strong absorption bands centered around 1022, 1326, 1696 cm^{-1} may arise from -C-O, C-O-C and C=O stretching modes of vibration. A moderate intense peak located at around 2929 cm^{-1} is due to the presence of C-H deformation vibration. Additionally, a broad band centered at 3409 cm^{-1} was observed confirming the O-H stretching vibrations, present in the leaf extract. The FT-IR spectrum of biosynthesized AgNPs exhibited few distinct peaks in the range of 1080, 1421, 1626, 2940, and 3463 cm^{-1} **(Fig. 3b)**. Further, a comparison study between the FTIR spectrum of leaf extract and biosynthesized AgNPs showed only minor changes in the position of absorption bands. On the basis of IR data, it may be inferred that the biomolecules present in WLE significantly have a bioreduction property to synthesize AgNPs.

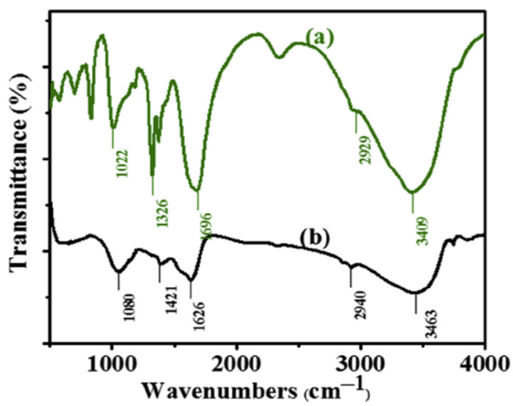

Fig. 3. FT-IR spectrum of: (a) WLE (alone) and (b) synthesized AgNPs from WLE

X-ray diffraction (XRD) Analysis

X-ray diffraction (XRD) analysis is carried out for synthesized AgNPs. The lattice constant a = 4.086 A^0 calculated from the XRD spectrum was in good agreement with standard diffraction data JCPDF Card No. 03-0921. The average diameter of particle (D) was estimated to be 31.68 nm using Scherrer's equation $D = K\lambda/\beta s\cos^{\theta}$. A wide base corresponding to the peak indirectly indicates the presence of small particles where little changes in the peak positions confirm the presence of biomolecules on the crystal. XRD pattern of AgNPs shows clear peaks at 38.080, 44.260, 64.670, and 77.540 **(Fig. 4)** correspond to miller indices of (1 1 1), (2 0 0), (2 2 0), and (3 1 1) of zero-valent silver, respectively. The crystalline size of AgNPs is 25 nm. The XRD study thus confirmed face-centered cubic geometry for the silver nanoparticles.

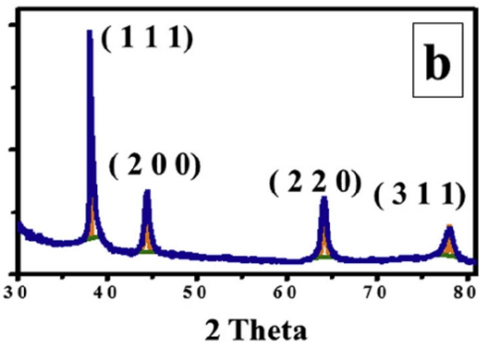

Fig. 4. XRD spectra of AgNPs synthesized using WLE

Transmission electron spectroscopy (TEM)

The TEM analysis was performed to visualize the size and shape of AgNPs formed. TEM micrograph shows the particles were predominantly spherical within 18-68.76 nm and distributed with little aggregation in solution **(Fig. 5a and b)**. The distribution of AgNPs observed in the images could be due to the surface capping hence stabilizing effect of leaf extract over the AgNPs.

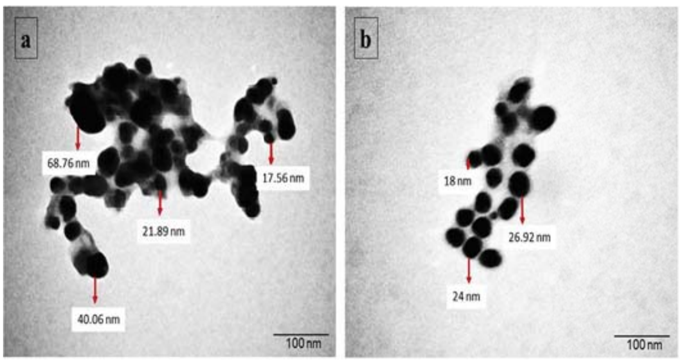

Fig. 5. (a and b) TEM micrographs of biosynthesized spherical-shaped AgNPs

Antioxidant Assay

Antioxidant activity of the biosynthesized AgNPs was evaluated using two different methods as follows, where Wedelia leaf extract and L-ascorbic acid (AA) were considered as control and reference, respectively. The mechanism of the reduction of Ag^+ions to Ag^0 is due to the presence of water-soluble antioxidative substances like ascorbic acid which is present in the gooseberry extract. Ascorbic acid is the reducing agent and can reduce and thereby neutralize, reactive oxygen species leading to the formation of ascorbate radical and an electron. This free-electron reduces the Ag^{+} ions to Ag^{0}.

DPPH free radical scavenging assay

The efficiency of antioxidants to scavenge the free radical from DPPH was carried out by DPPH assay. The reduction of DPPH was assessed spectrophotometrically by observing the decrease in absorbance due to the formation of a stable DPPH-H molecule (reduced form). The free radical scavenging activity of AgNPs increased gradually with an increasing concentration of AgNPs (12.25-200mg/mL) **(Fig.6)**. The maximum scavenging activity of AgNPs (200 mg/mL) was found to be 80.2 ± 0.15% as compared to control leaf extract (67.53 ± 0.34%), and reference L-ascorbic acid (96.32 ± 0.33%).

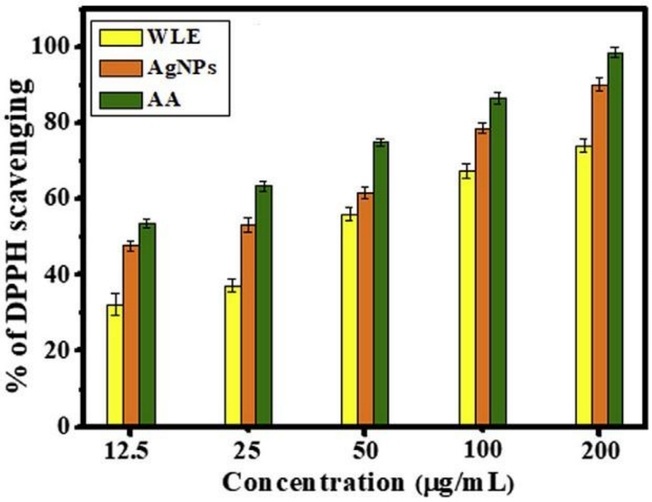

Fig. 6.DPPH free radical scavenging activity of WLE, biosynthesized AgNPs, and AA (L-ascorbic acid) at different concentrations (12.5-200 mg/mL).

Reducing power assay

The reducing power of Wedelia leaf extract, AgNPs, and AA was determined based on the reduction of Fe^{3+} ions to Fe^{2+} ions. The reducing capacity of the biogenic AgNPs (200 mg/mL) was observed higher (absorbance 0.81 ± 0.146) compared to control leaf extract (absorbance 0.18 ± 0.006) and lower than the reference L-ascorbic acid (0.86 ± 0.048) **(Fig. 7)**. Because of their free radical scavenging properties, AgNPs can be considered as a potential candidate in the management of cancer, diabetes, AIDS, neurodegenerative disease, etc.

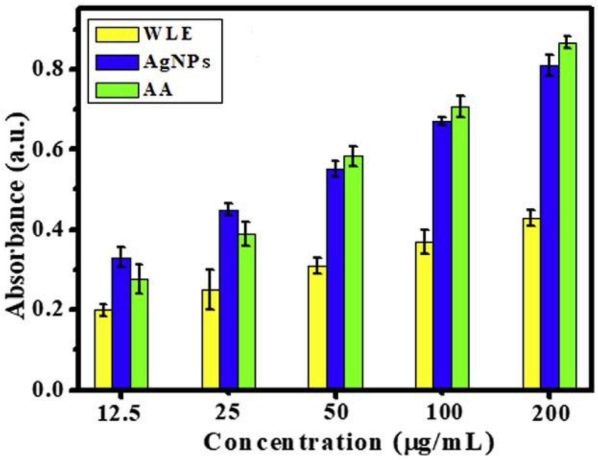

Fig. 7.Reducing power of WLE biosynthesized AgNPs and AA (L-ascorbicacid) at different concentrations (12.5-200mg/mL).

Conclusion

The present investigation deals with the leaf extract mediated green synthesis of fabricated silver nanoparticles using Wedelia Chinensis. Photosynthesis of spherical nanosilver particles was effectively established without using any templates, additives, or accelerants. Flavonoid/wedelolactone comprises functional groups as evident from the FT-IR analysis might have been associated with the reduction and stabilization.

The leaf extract of Wedelia Chinensis was used as a reducing agent for the synthesis of silver nanoparticles from aqueous silver nitrate. A greater conversion of silver ions to nanoparticles was achieved by employing leaf broth. The successful formation of silver nanoparticles has been confirmed by UV-Vis spectrophotometer, Fourier Transform Infrared Spectroscopy (FTIR), X-Ray Diffraction (XRD), and Transmission Electron Microscopy (TEM).

FTIR studies confirmed the presence of silver nanoparticles which may responsible for the reduction of silver ions to silver nanoparticles. Most of the characteristic vibrational bands originated from water-soluble compounds like polyphenols, flavonoids, triterpenoids, wedelolactones, etc. present in the Wedelia leaf extract. The XRD pattern revealed the complex crystalline nature of silver nanoparticles. While the TEM image confirms the formation of a spherical shape. Synthesized AgNPs possess significant antioxidant activity.

These nanoparticles may have a great application in the field of pharmacology and other industries. Silver nanoparticles are a promising material for future cancer therapy.