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Phytofabrication of silver nanoparticles by using aquatic plant *Hydrilla verticilata*

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Abstract. *Sable N, Gaikwad S, Bonde S, Gade A, Rai M. 2012. Phytofabrication of silver nanoparticles by using aquatic plant* Hydrilla verticilata*. Nusantara Bioscience 4: 45-49.* In the context of current drive to developed new green technology in nanomaterials, synthesis of nanoparticles is of considerable importance. There has been considerable work done in the field of nanoscience and Nanotechnology during the last decade due to the introduction of various protocols for the synthesis of nanoparticles by using plants and microorganisms. Here we firstly report the extracellular phytosynthesis of silver nanoparticles (Ag-NPs) using aquatic plants *Hydrilla verticilata.* The characterization of the phytosynthesized Ag-NPs was done with the help of UV-Vis spectroscopy, FTIR, Nanoparticle Tracking Analysis (NTA), Zeta potential and SEM. The SEM micrograph revealed the synthesis of polydispersed spherical nanoparticles, with the average size of 65.55 nm. The phytofabricated Ag-NPs can be used in the field of medicine and agriculture, due to their antimicrobial potential.

Key words: phytofabrication, *Hydrilla*, Ag-NPs, SEM, FTIR

Abstrak. *Sable N, Gaikwad S, Bonde S, Gade A, Rai M. 2012. Fitofabrikasi nanopartikel perak menggunakan tumbuhan akuatik* Hydrilla verticilata*. Nusantara Bioscience 4: 45-49.* Dalam konteks mendorong pengembangan teknologi hijau yang baru pada nanomaterial, sintesis nanopartikel sangat penting. Selama dekade terakhir terjadi perkembangan yang cukup pesat dalam bidang nanosains dan nanoteknologi karena diperkenalkannya berbagai protokol untuk mensintesis nanopartikel menggunakan tumbuhan dan mikroorganisme. Dalam penelitian ini, dilaporkan fitosintesis ekstraseluler nanopartikel perak (Ag-NP) menggunakan tumbuhan akuatik *Hydrilla verticilata* untuk pertamakalinya. Karakterisasi Ag-NP yang difitosintesis dilakukan dengan bantuan spektroskopi UV-Vis, FTIR, Analisis Pelacakan Nanopartikel (NTA), potensial Zeta dan SEM. Mikrograf SEM menunjukkan hasil sintesis nanopartikel berbentuk bulat yang tersebar, dengan ukuran rata-rata 65,55 nm. Fitofabrikasi Ag-NP dapat dimanfaatkan dalam bidang kedokteran dan pertanian, karena memiliki potensi antimikroba.

Kata kunci: fitofabrikasi, *Hydrilla*, Ag-NPs, SEM, FTIR

INTRODUCTION

Nanotechnology is a relatively recent development in scientific research, the development of its central concepts happened over last decades. The development of experimental procedures for the synthesis of nanoparticles of different chemical compositions, sizes, shapes, and controlled polydispersity is vital for its advancement. Currently, there is an ever-increasing need to develop environmentally benign processes in the field of nanoparticle synthesis, therefore focusing attention on biological systems. Nanobiotechnology is combination between nanotechnology and biology and which refers to the ability to create and manipulate biological and biochemical materials, devices, and systems at nano level (Kholoud et al. 2010).

Different microorganisms such as bacteria, fungi, and yeasts can be used as nanofactories for the biosynthesis of nanoparticles. It has been shown that fungi are good candidates for synthesis of metal and metal sulphides nanoparticles, near about 20 different fungi has been investigated for the synthesis of metal nanoparticles. *Verticillum* sp. reduces metal ions into Au and Ag nanoparticles (Mukherjee et al. 2002). *Fusarium oxysporum* produces high stable gold, silver and platinum nanoparticles (Mukherjee et al. 2002, Riddin et al. 2006). Other reports of nanoparticles synthesis by fungi includes by *Aspergillus niger* (Gade et al. 2008), *Fusarium acuminatum* (Ingle et al. 2009).

But, plants as a system for synthesis of nanoparticles are rapid and eco-friendly biosynthesis process. Plants are used to synthesize Nanoparticles either intracellularly or extracellularly (Bonde et al. 2012). Living plants (Torresdey et al. 2002, 2003) are used for synthesis of gold and silver nanoparticles, part of a plant like from geranium leaf broth (Shivshankar et al. 2003, 2004, 2005) or by fruits (Li et al. 2007) or even by sundried leaves (Huang et al. 2007).The rapid synthesis of silver nanoparticles by using different plant extracts of *Pinus, Persimmon, Ginkgo, Magnolia* and *Platanus* were used and compared for their extracellular metallic Ag-NPS synthesis (Song et al. 2008) and the other reports of utilization of plant for the synthesis

of metal nanoparticle includes; *Azadirachta indica* (neem) (Shankar et al. 2004), *Aloe vera* (Chandran et al. 2006), *Emblica officinalis* (amla). (Amkamwar et al. 2005), *Capsicum annuum* (Li et al. 2007), *Cinnamomum camphora* (Huang et al. 2007), *Gliricidia sepium* Jacq. (Raut et al. 2009), *Carrica papaya* (Mude et al. 2009), *Opuntia ficus-indica* (Gade et al. 2010), *Murraya koenigii* (Bonde et al. 2012), *Ocimum sanctum* (Mallikarjum et al. 2011), *Saururus chinenis* (Nagajyoti et al. 2011), *Foeniculum vulgare* (Bonde 2011).

The phytofabrication (fabrication by plants) of Ag-NPs from plant extracts has received some attention as a simple and viable alternative to bacterial and fungal system, also metal ions reduces much faster using plant system as compare to microbes (Rai et al. 2008). The reduction of metal ions is known to using enzyme extracted from the plant extract, owing to this property the plant is selected for bioreduction of silver ions in present study.

In the present study we have for the first time exploited aquatic plants for the synthesis of Ag-NPs. The aquatic weed *Hydrilla verticilata* was used for the synthesis of Ag-NPs using 1mM silver nitrate ($AgNO_3$). The characterization of the phytofabricated Ag-NPs were carried out with the help of UV-Vis spectroscopy, FTIR, NTA, Zeta Potential and SEM.

MATERIAL AND METHODS

Extraction

The 20 g *Hydrilla verticilata* (Figure 1) plant part was washed 2-3 times with sterilized distilled water to avoid any microbial contamination, and then surface sterilized by $HgCl_2$ (0.1%) for 1 min, cut into small pieces and ground with 100 mL of sterilized distilled water in an omnimixer. Later, crude extract was filtered through muslin cloth and centrifuged at 10,000 g for 15 min to obtain clear leaf extract which was later used for the fabrication of Ag-NPs.

Fabrication of Ag-NPs

For the fabrication of Ag-NPs extract was challenged with $AgNO_3$ (1 mM) solution and incubated at room temperature. Control (without treatment with $AgNO_3$) (1 mM) i.e only extract) was also maintained.

Detection of Ag-NPs

Visual observation

In conical flask 99 mL of plant filtrate was taken and 1 mL of $AgNO_3$ (100 mM) was added into it (final concentration becomes 1 mM). After incubation of filtrate at room temperature for 24 hrs the colour of filtrate changes from light green to dark brown. This colour change indicates the formation Ag-NPs.

UV-Visible Spectroscopy

The preliminary detection of Ag-NPs was done with the help of UV-Visible spectrophotometer (Perkin-Elmer, Lambda 25) by scanning the absorbance spectra in the range of 250-800 nm wavelengths.

Fourier Transform Spectroscopy

FTIR measurements of Ag-NPs synthesized from *Hydrilla verticilata* was carried out on a Perkin-Elmer FTIR Spectrophotometer in the range 450- 4000 cm^{-1} at resolution of $4cm^{-1}$. Scanning electron micrographs were taken using a JEOL 6380A instrument. The samples were fixed with 2.5% glutaraldehyde overnight at room temperature. The dehydration of fixed samples were carried out with gradient alcohol (10% to 95%), incubated for 20 min in each gradient and dipped in absolute alcohol for 2-5 min. The final specimen was prepared by placing a drop of dehydrated sample on a glass slide followed by coating with monolayer platinum for making the surface conducting.

NanoSight LM-20 analysis

Liquid sample of Ag-NPs at the concentration range of 107-109/mL were introduced into a scattering cell through which a laser beam (approx. 40 mW at k = 635 nm) was passed. Particles present within the path of the laser beam were observed via a dedicated non- microscope optical instrument (LM-20, NanoSight Pvt. Ltd., UK) having CCD

Figure 1. *Hydrilla verticilata* plant

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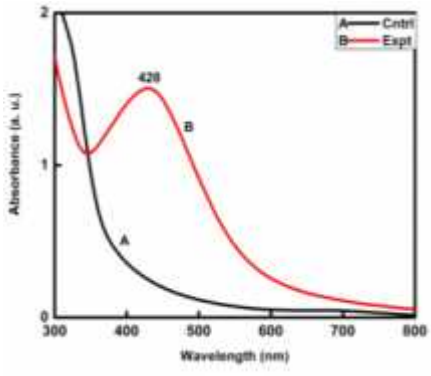
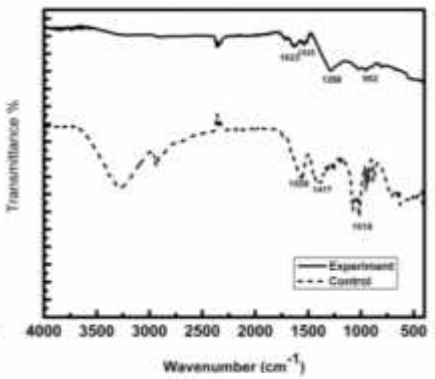
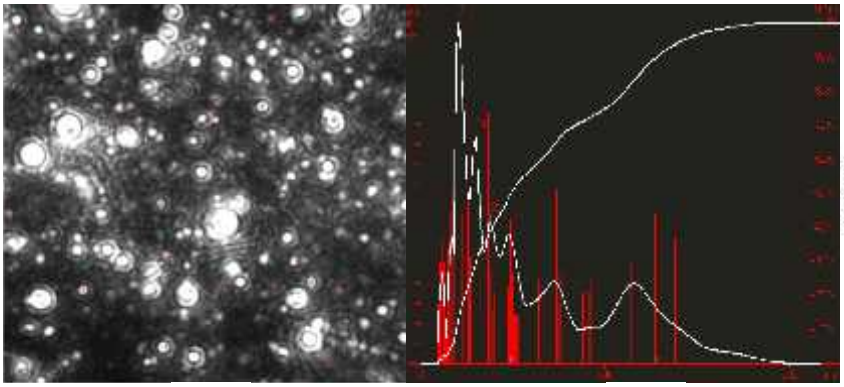




3 **4**

Figure 2. Control (left) and Ag-NPs (right) fabricated from *Hydrilla verticilata*
Figure 3. UV-Vis spectra of (A) leaf extract (control) and (B) Ag-NPs showing absorbance at about 428 nm.
Figure 4. FTIR spectrum for extract (control) and experimental after treatment with 1mM silver nitrate solution.



5A **5B**

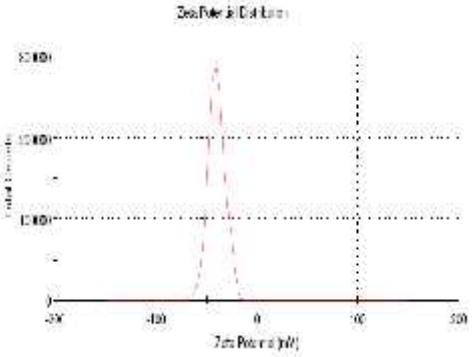


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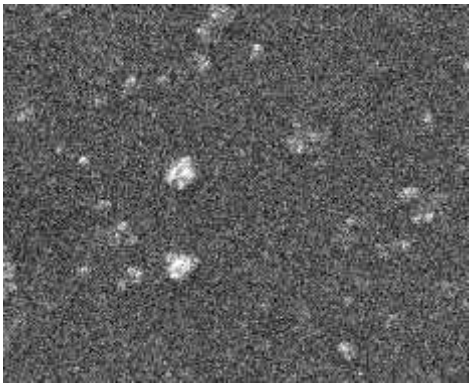
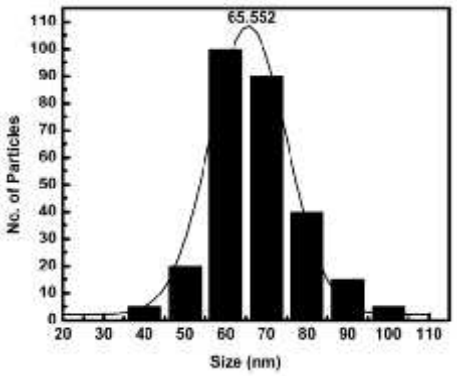
Figure 5. A. Particle size/concentration of Ag-NPs, B. Particle populations of Ag-NPs using NanoSight LM-20
Figure 6. Particle size distribution of Ag-NPs by intensity with Zeta Analyzer.



7A



7B

Figure 7. A. SEM micrograph of Ag-NPs (65.55 nm) (scale bar-100nm). B. A particle size distribution determined from the SEM images.

camera. The motion of the particles in the field of view (approx. 100 X 100 μm) was recorded (at 30 fps) and the subsequent video and images were analyzed.

Particle size measurement

Particle sizing experiments were carried out by means of laser diffractometry, using Zetasizer nano series (Malvern). Measurements were taken in the range range between 0.1-1000μm.

Scanning electron microscopy

Scanning electron microscopy of Ag-NPs was carried out by fixation of 2.5% glutaraldehyde overnight at room temperature. Then cell filtrate was dehydrated with gradient alcohol (10% to 95%) and incubated for 20 min. for each gradient. It was dipped in absolute alcohol for 2-5 min. A drop of dehydrated sample placed on glass slide (1 cm x 1 cm). The sample was coated with monolayer platinum. The slide was observed under scanning electron microscope.

RESULTS AND DISCUSSION

The change in colour of plant extract from light green to dark brown when challenged against silver ions (1 mM $AgNO_3$) at room temperature. The colour change in the extract was noted by visual observation (Figure 2). The characterization of Ag-NPs fabrication was done by using UV-visible spectrophotometer which confirms the presence of the absorbance peak at 428 nm (Figure 3).

Further characterization was done by Fourier Transform Infrared Spectroscopy (FTIR) measurements to identify the possible biomolecules responsible for the reduction of the Ag^+ ions and capping of the bioreduced Ag-NPs by protein. The amide linkages between amino acid residues in proteins give rise to the well-known signatures in the infrared region of the electromagnetic spectrum (Basavaraja et al. 2007). FTIR spectrum showed peaks in the range 1000-2000cm^{-1}. Representative spectra of obtained nanoparticles manifest absorption peaks of respective functional groups and indicated the presence of stabilized protein molecules (Figure 4).

Nanoparticle Tracking and Analysis (NTA) was used to measure the dispersion characteristics i.e. size and size distribution. In particular, it is the most recently developed system, NTA, was assessed in-depth due to its ability to see and size of particles individually on a particle-by-particle basis. NTA allows individual nanoparticles in a suspension to be microscopically visualized and their brownian motion to be separately but simultaneously analyzed and from which the particle size distribution can be obtained on a particle-by-particle basis (Figure 5A). The Figure 5b showed particle populations by size and intensity. The distribution data were mean 64 nm, mode 21 nm and standard deviation 43 nm. This result corroborates the results obtained by Montes-Burgos et al. 2010.

Particle size determination of the formulated nanoparticles was shown under different categories like size distribution by volume, by intensity (Figure 6). The average zeta potential of peak was found to be -40.1 mV, area 100% and width 8.51 mV.

The formed Ag-NPs are well distributed with respect to volume and intensity is an indication of the formation of well built Ag-NPs and their monodispersity.

Scanning Electron Microscopy (SEM) study reveals the synthesis of spherical polydispersed Ag-NPs in the reaction mixtur e, which showed the spherical nanoparticles of size of 65.55 nm (Figure 7A). The particle size histogram showed average size of Ag-NPs (Figure 7B). Ag-NPs analyzed in NTA and scanning electron microscopy corroborates in their size.

CONCLUSION

It has been demonstrated that the extract of plant *Hydrilla verticilata* is capable of fabricating Ag-NPs and these Ag-NPs are quite stable in solution due to capping likely by the proteins present in the extract. This is an efficient, eco-friendly and simple process and more efficient with appreciable control over size, composition and even the shape of the nanoparticles. Ag-NPs have more applications as antimicrobial agents.

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