Available online: www.notulaebiologicae.ro
Print ISSN 2067-3205; Electronic 2067-3264
Not Sci Biol, 2017, 9(3):443-448. DOI: 10.15835/nsb9310107
Original Article

Green Nanotechnology: Synthesis of Silver Nanoparticles Using Aqueous Leaf Extract of *Swertia chirayita*

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Abstract

In the present study the synthesis of silver nanoparticles mediated by aqueous leaf extracts of *Swertia chirayita* is reported. Nanoparticles are particles having diameters below 100 nm. The nanoparticles formed were characterized using Uv-Vis spectrophotometer, scanning electron microscope, Ft-IR and light scattering & Zeta potential analysis. A Surface Plasmon Resonance (SPR) peak was observed at 450 nm in Uv-Vis analysis. SEM analysis showed that the particles were spherical and cuboidal in shape and had diameter from 85-120 nm. The FT-IR analysis spectra peak were observed at 3,610.74 cm^{-1}, 3,089.96 cm^{-1}, 2,125.56 cm^{-1}, 166.50 cm^{-1} and 864.11 cm^{-1}, which corresponds to the presence of capping agents such as primary and secondary amines, hydroxyl compounds, flavonoids, alcoholic and phenolic compounds. The results of light scattering analysis also confirmed the formation of nanoscale particles. The zeta potential analysis showed a peak of -25 mV which demarcates the stability of the synthesized nanoparticles.

Keywords: capping agents, ft-ir, nanoscale, surface plasmon resonance, zeta potential

Abbreviation: FT-IR – Fourier Transform Infrared spectroscopy, SEM – Scanning Electron Microscope, SPR – Surface Plasmon Resonance, Uv-Vis – Uv Visible spectrophotometer

Introduction

In recent years, appearance of nanometric scale technologies and biological techniques has given rise to a new field called nanotechnology, which focuses on creation and use of materials of nano scale (Goodsell, 2004; Shah *et al.,* 2015). Nanoparticles are of great interest due to their novel physicochemical, magnetic and optoelectronic properties that are governed by their shape and size distribution (Bogunia and Sugiaska, 2002; Kumar *et al.,* 2003). Nanotechnology concerns the size of matters in the range between one nm to 100 nm of size. The nano scale imparts ultra-small size, large surface to volume ratio, high reactivity (Dandapat *et al.,* 2014).

Biology of plant-mediated nanoparticles is gaining grounds. The green method of synthesis of nanoparticles has several important applications in the field of biolabelling sensors, drug delivery system. The nanoparticles formed with help of plant extracts exhibit new physico-chemical properties, which are not observed in polar or non-polar extracts of plants (Anil *et al.,* 2013).

Swertia chirayita is an important herb which is commonly available in India, Nepal and China. It is commonly known as Chireta (Hindi). The plant is found at an altitude of 1200-3,000 m and available throughout the year. It is generally consumed by the older people and people with type II diabetes. It is useful in lowering the blood glucose levels (Dutta *et al.,* 2012).

In this work, we report the synthesis of silver nanoparticles mediated by aqueous leaf extract of *Swertia chirayita*. This method yields faster and stable silver nanoparticles compared to other methods.

Materials and Methods

Preparation of plant extract

50 g of sieved leaf powder of *Swertia chirayita* was subjected to Soxhlet extraction using 350 ml of distilled water. The extract obtained was filtered, concentrated using rotary flash evaporator maintained at 45 °C. The dried extract was stored in airtight containers for further studies (Kumar *et al.,* 2014).

Synthesis of silver nanoparticles

1 mL of *Swertia chirayita* leaf extract was added 99 to mL of 1mM $AgNO_3$ (169.8 mg) solution. The mixture was allowed to stirr for two hrs at 90 °C, during which colour change was observed from light yellow to dark brown. The mixture was allowed to cool down and was centrifuged at

Received: 05 Jul 2017. Received in revised form: 28 Sep 2017. Accepted: 30 Sep 2017. Published online: 30 Sep 2017.

15000 RPM after washing three times with distilled water. A black powder was obtained which was dried overnight in an oven at 80 °C (Shankar *et al.,* 2004).

Characterization of nanoparticles

The characterization of silver nanoparticles was done by UV-vis spectrum analysis, SEM analysis, Fourier Transform Spectroscopy (FTIR) analysis and Light scattering & Zeta potential analysis.

a. UV Visible spectrophotometer (UV-Vis) analysis

The reduction of Ag^+ ions was monitored by measuring the UV-visible spectrum of the reaction medium after 5 hours by diluting a small aliquot of the sample into Millli-Q water. UV-visible spectral analysis was done by using Parkin Elmer Lambda 25 UV-visible spectrophotometer.

b. Scanning Electron Microscope (SEM) analysis

SEM (Scanning Electron Microscope) analysis was done using JEOL JSM-6390 LV (Japan) machine. Thin films of the sample were prepared on a carbon-coated copper grid by dropping a small amount of the sample on the grid, extra solution was removed using a blotting paper and then the film on the SEM grid was allowed to dry by putting it under mercury lamp for 5 min and was coated with gold using ion sputter.

c. Fourier transform infrared spectroscopy (FT-IR) analysis

FT-IR analysis was carried out on IP Resting - 21 (Shimadzu) in the diffuse reflectance mode operated at a resolution of 4 cm^{-1} in the range of 400 - 400 cm^{-1} to evaluate the functional groups that might be involved in nanoparticles formation.

d. Light scattering and Zeta Potential analysis

The light scattering and Zeta potential analysis of nanoparticles was carried on Malvern Nano ZS (U.K.).

Results

UV-vis spectrophotometer analysis

The aqueous solution changed from light yellow colour to dark brown, which is well known confirmation of nanoparticle formation (Shankar *et al.,* 2004; Dhanlakshmi *et al.,* 2012; Kumar *et al.,* 2014). As the aqueous leaf extract of *Swertia chirayita* was mixed with aqueous solution of $AgNO_3$, a colour change was seen from yellow to brown due to reduction of silver ion (Fig. 1), which indicated the formation of silver nanoparticles. In this study the silver nanoparticles formed showed a (surface Plasmon resonance) SPR peak at 450 nm. The result of UV-vis spectrophotometric analysis is shown in Fig. 2.

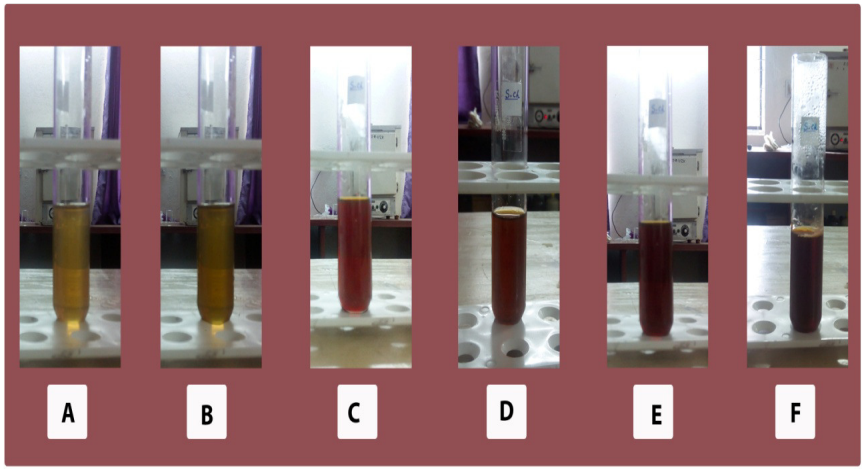

Fig. 1. Colour change from yellow to dark brown due to reduction of silver ions

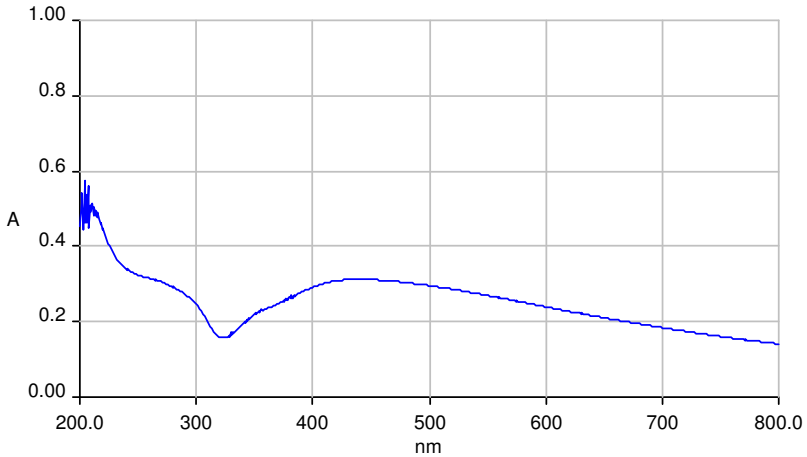

Fig. 2. result of UV-vis analysis showing SPR peak at 450 nm

Scanning Electron Microscope (SEM) analysis

The SEM analysis of silver nanoparticles synthesized using aqueous leaf extract of *Swertia chirayita* revealed the size and structure of the nanoparticles. The silver nanoparticles were spherical and cubical in shape and had diameter of 85 nm to 120 nm and average size was 101 nm. The Photographs of SEM is presented as Fig. 3.

Fourier Transform Infrared Spectroscopy (FT-IR) analysis

FT-IR analysis was done to determine the role of plant extract as capping agent and the functional groups (Kumar *et al.,* 2014) responsible for synthesis of silver nanoparticles by reducing Ag^{+} ions. The FT-IR absorption spectra of silver nanoparticles are represented as Fig. 4. The spectra showed broad transmission peak at 3610.74 cm^{-1}, 3089.96 cm^{-1}, 2125.56 cm^{-1}, 1666.50 cm^{-1} and 864.11 cm^{-1}.

Light scattering and Zeta potential

The light scattering (also known as static, Rayleigh or Multi-angle light scattering) provides a direct measure of particle size (Satinder and Verma, 2001). The results of light scattering analysis size distribution by number, intensity and volume is represented as Fig. 5(a), Fig. 5(b) and Fig. 5 (c) respectively. The result of Zeta potential analysis is presented as Fig. 6.

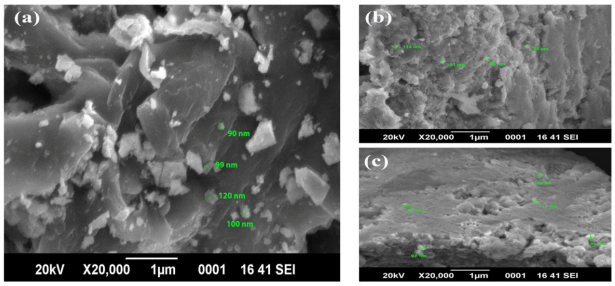

Fig. 3. SEM micrographs showing nanoparticles

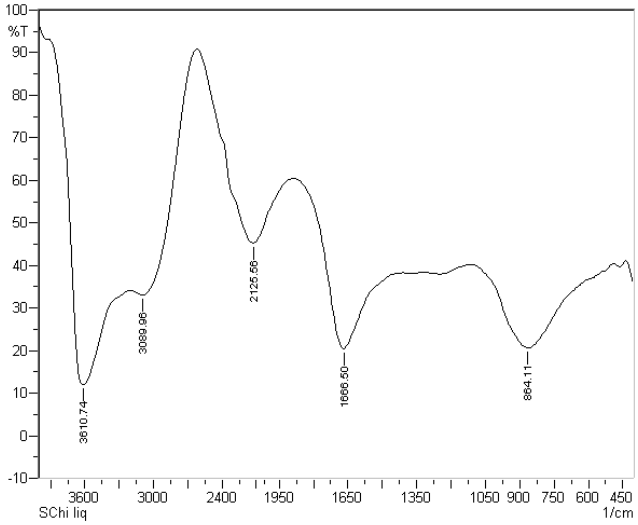

Fig. 4. FT-IR absorption spectra of Silver nanoparticles formed by reduction with aqueous leaf extract of *Swertia chirayita*

Discussion

UV Visible spectrophotometer (UV-Vis) analysis

After mixing the *Swertia chirayita* aqueous leaf extract with the $AgNO_3$ solution, the solution started to change colour from light yellow to brown colour due to reduction of silver ions (Dhanlakshmi *et al.,* 2012; Kumar *et al.,* 2014). The changing of solution from yellowish to dark brown colour has been reported by several authors (Dutta *et al.,* 2012; Anil *et al.,* 2013; Shah *et al.,* 2015) and is a well known confirmation of nanoparticle formation (Satinder *et al.,* 2001; Bindhu *et al.,* 2013, 2015). The brown colour exhibited is due to the formation of metallic nanoparticles and coherent excitation of all the free electrons within the conduction band, leading to a phase oscillation. The change in colour of solution is presented in Fig. 1. No significant change was observed after 240 min, indicating the end of the reaction (Prabhu and Johnson, 2015). Silver nanoparticles are known to exhibit a plasmon absorption band in the visible region just like gold nanoparticles (Prabhu and Johnson, 2015). From different literature it

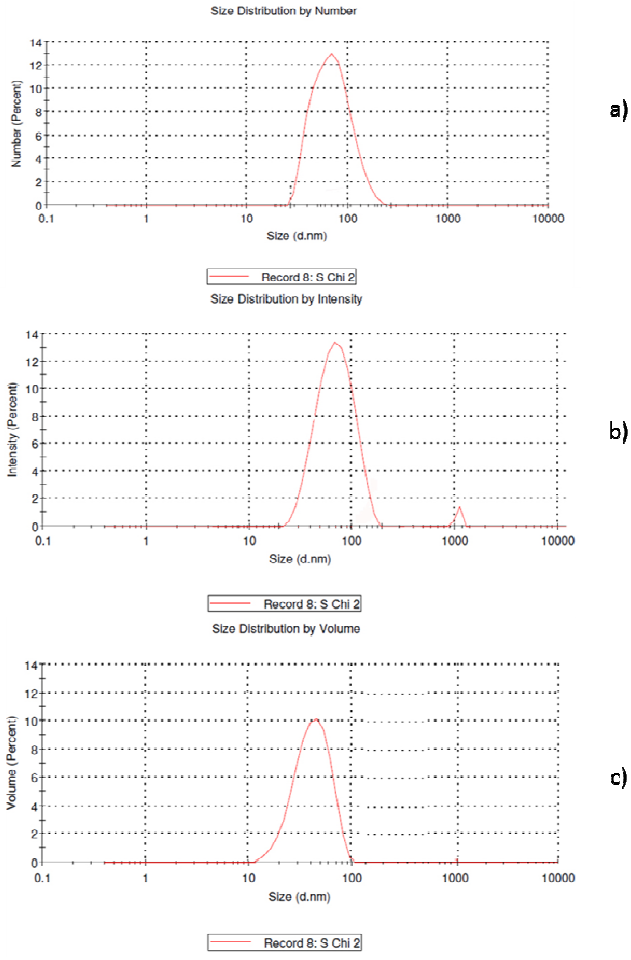

Fig. 5. a) Showing size distribution of nanoparticles by number; b) showing size distribution of nanoparticles by intensity; c) showing size distribution of nanoparticles by volume

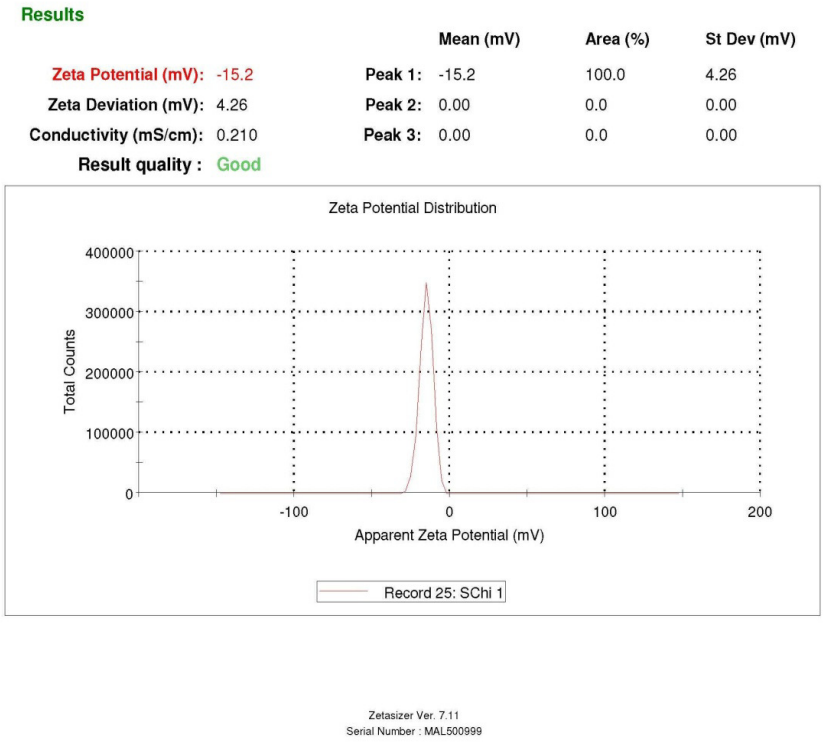

Fig. 6. Results of Zeta Potential analysis

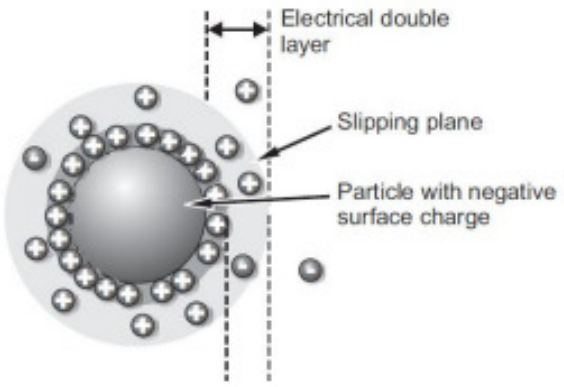

Fig. 7. Electric double layer surrounding nanoparticle [19]

was found that the silver nanoparticles show SPR peak from 415 - 490 nm (Kumar *et al.,* 2003; Anil *et al.,* 2013; Dandapat *et al.,* 2014; Prabhu and Johnson; 2015). A broad peak located at 463 nm was observed in this study. Prabu and Johnson (2015) reported SPR peak at around 463 nm for silver nanoparticles synthesized using different plant extracts.

Scanning Electron Microscope (SEM) analysis

The SEM analysis of silver nanoparticles were done with help of JEOL JSM-6390 LV (Japan) machine. The size of particles in the image can be measured with the help of software provided with the SEM set up on the display monitor. Kumar *et al.* (2003) reported size of alion mediated synthesized nanoparticles to be in range of 207-293 nm and the average size was 270 nm. Shah *et al.* (2015) reported the size of nanoparticles ranging from 20-150 nm in diameter.

Fourier transform infrared spectroscopy (FT-IR) analysis

The FT-IR absorption spectra of silver nanoparticles showed broad transmission peak at 3610.74 cm^{-1}, 3089.96 cm^{-1}, 2125.56 cm^{-1}, 1666.50 cm^{-1} and 864.11 cm^{-1}. FT-IR analysis is done to determine the role of plant extract as capping agent and the functional groups (Pawar and Kamble, 2017). The spectra observed were compared with reference values previously published. The fatty acid stretch was recorded at 1666.50 cm^{-1} (Pawar and Kamble, 2017). The present study confirms the presence of amines N-H (stretch) and C-N (stretch) characteristic peak at 864.11cm^{-1} (Pawar and Kamble, 2017). The detection of alkaloids is confirmed by the presence of primary and secondary amines (Corlet, 2017). Characteristic peak for hydroxyl compounds -OH (stretch) were obtained (3610.74 cm^{-1}). Detection of hydroxyl groups is an indication of presence of flavonoids, alcoholic and phenolic compounds (Corlet, 2017). The peak at 3089.96 cm^{-1} corresponds to the -CH stretching which represents the lipids (Starlin *et al.,* 2012). The peak at 2152.56 corresponds to the -C≡C- stretching.

Light scattering and Zeta Potential

The light scattering analysis of nanoparticles is an important tool for characterizing the size of nanoparticles in the solution. It measures the light scattered from a laser that passes through a colloidal solution and by analyzing the modulation of the scattered light intensity as a function of time. The results of light scattering is represented with size distributions of number, intensity and volume.

The result of number distribution is represented as Fig. 5(a) the figure shows one peak of 93.6 nm diameter of

nanoparticles with percentage distribution of 100%. The number distribution shows the number of particles in different size bins (NanoComposix's Guide, 2015). Thus, the result shows that the size of nanoparticles is below 100 nm, i.e. 93.6 nm. The number distribution graph thus shows that, almost 100% of particles formed had diameter of about 93.6 nm; this confirms the formation of nanoparticles.

The results of intensity distribution is represented as Fig. 5(b), which shows two peaks at 92.5 nm and 2120 nm with percentage intensity of 98.2% and 1.8% respectively. The intensity distribution describes how much light is scattered by the particles of different size bins (NanoComposix's Guide, 2015). It shows that 98.2% of light was dispersed by nanoparticles whose average size was 92.5 nm. This shows that about 98.2% of the particles in the suspension had average size of 92.5nm. Thus confirms the formation of nanoparticles (particles having diameter below 100 nm)

The results of volume distribution is represented as Fig. 5(c), two peaks of 78.2 nm and 2136 nm is visible with percentage volume of 71.1 and 28.9% respectively. The volume distribution shows the total volume of particles in different size bins (NanoComposix's Guide, 2015). The volume distribution shows that about 71.1% of total volume of nanoparticles formed had average diameter of 78.2 nm.

The Zeta Potential analysis is a technique for determining the surface charge of nanoparticles in solution (colloids). Nanoparticles have a surface charge that attracts a thin layer of ions of opposite charge to the nanoparticle surface. This double layer of ions travels with nanoparticle as it diffuses throughout the solution (NanoComposix's Guide, 2012).

The electric potential at the boundary of the double layer is known as Zeta potential of the particle. The Zeta potential of the particles has values ranging from +100mV to -100 mv (NanoComposix's Guide, 2012). The Zeta potential analysis of nanoparticles formed by aqueous leaf extract of *Swertia chirayita* showed peak of -15.2 mV with 100% area distribution (Fig. 6). Nanoparticles with Zeta potential values greater than +25 mV and less than -25 mV typically have high degrees of stability (NanoComposix's Guide, 2012). The value of Zeta potential of nanoparticles formed by aqueous leaf extract of *Swertia chirayita* was found to be in -ve side (-25 mV) which showed the efficiency of the capping material in stabilizing the nanoparticles providing intensive negative charges that keep all the particles away from each other. It implies that the Ag nanoparticle and the solution are stable (NanoComposix's Guide, 2012; Haider *et al.,* 2014).

There are several biological methods of synthesis of nanoparticles (Rajan *et a*l., 2015). The bioreduction potential of plant extracts is comparatively higher than the microbial and fungal methods; many researchers support this hypothesis (Iravani, 2011; Kanan *et al.,* 2011). Researchers have reported the supremacy of green nanoparticles over several applications of polar and non polar plant extracts such as antifungal activity (Dipankar and Murugan, 2012; Singh *et al*., 2013), antibacterial activity (Gopinath *et al.,* 2012; Satishkumar *et al*., 2012) and anticancer activities (Satyavani *et al*., 2011; Jacob *et al*., 2012).

Conclusions

On the basis of results obtained by characterization of nanoparticles formed by mediated by aqueous leaf extract of *Swertia chirayita* by Uv-Vis Spectrophotometer, Scanning Electron Microscopy, Fourier Transform Infrared Spectroscopy and Light Scattering & Zeta potential analysis; it is clear that nanoparticles (below 100 nm size) were formed. Thus, we report the formation of Ag nanoparticles mediated by aqueous leaf extract of *Swertia chirayita*.

Acknowledgements

The authors are thankful to the Central Instrumentation Facility (CIF), Birla Institute of Technology, Mesra for helping in FTIR spectroscopy, UV-Vis spectroscopy and Scanning Electron Microscope (SEM) analysis. The authors also acknowledge the facilities made available by the H.O.D., Department of Zoology, Ranchi University, Ranchi.

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