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Green synthesis of silver nanoparticles using Alternanthera dentata and its anti-bacterial activities

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Abstract : Silver nanoparticles (Ag-NPs) have been synthesized by the reduction of silver nitrate using the leaf extract of *Alternanthera dentata*. This plant is valued not only for its ornamental values but also contains a number of important compounds. It is rich in iron, vitamin C and is also an energy rich edible plant. Studies on the role of various concentrations of the extract on the size of Ag-NPs formed indicate that lower concentration of the extract leads to smaller Ag-NPs. Field emission scanning electron microscope (FESEM) was used to study the morphology and size range of the nanoparticles formed. The presence of silver was further confirmed using EDS, XRD and UV-Vis spectroscopy. Antibacterial activity studies were done by well diffusion method for E-Coli bacteria. Zone of inhibition was observed and this confirmed the antimicrobial properties of the silver nanoparticles. Green synthesis route using the leaves of seems to be a promising synthesis route to make silver nanoparticles with significant anti-bacterial activity.
Keywords: Silver nanoparticles, Alternanthera, Anti-bacterial activity, Green Synthesis.

Introduction:

Nanomaterials provide solutions to technological and environmental challenges in various domains as they evince numerous magnetic, electrical and optical properties. They are used for a wide range of applications, including drug delivery, antibacterial based products[1] and gene therapy[2]. Metal nanoparticles have proven to be highly beneficial in biomedical applications and have been an important topic of research around the world. Metal nanoparticles can be synthesized by using various physical and chemical methods like ball-milling, solid state, sol-gel, hydrothermal, co-precipitation etc. The chemical methods produce excellent nanoparticles of various shapes and size range but they also produce hazardous by-products and are notably quite expensive, which makes the product unfit for medical applications. Such hazardous remnants can cause innumerable side effects in human body. The rise in the use of metallic nanoparticles for medical applications has led to an increase in demand; that can be satisfied in particular by green synthesis method. They give out non toxic products, as the pytochelatin chelators favours heavy metal detoxification by preventing the toxic elements from getting adsorbed over the surface[1, 3]. The green route is economic and eco-friendly and is also effective in reducing the metal precursors to nanoparticles. They involve green elements like all parts of the plants that are readily available in our day to day life. Green synthesis based silver seems to be more useful in applications like topical both in cosmetics and with medicinal value, protein expression[4] and catalytic applications[5] so on. *Alternathera dentata* plant generally is used for ornamental purpose besides it posses number of unexploited medicinal values. Wide literature survey on the plant revealed that it is highly nutritious and very rich in iron and protein content. Implementing the role of peptides in the synthesis of nanoparticles, the leaf extract from *Alternanthera dentata* is utilized to synthesize silver nanoparticles of various sizes[6]. The general phenomenon involved in this is, $(Ag)^+$ ions in aqueous Silver nitrate solution is reduced by glucose and other polysaccharides[5] present in the leaf extract and yields colloidal silver with particle diameter of various nanometer ranges followed by the formation of Ag^0 stable silver atoms and oligomeric clusters[1,7].The presence of the Silver nanoparticles can be visually confirmed by the colour change from white color to different shades of yellow colour with an extraordinary band in the 380-400 nm range and low intense band at longer wavelength in the absorption spectrum[1]. Silver nanoparticles can be green reduced using various parts of the plant like roots, basil[8], latex[9], fruits, bark[10], flowers[11].

Here we report the synthesis of silver nanoparticles using leaf extract of Alternanthera dentata at five different concentrations, so as to find the concentration at which silver nitrate solution gets reduced effectively leading to the formation of silver nanoparticles. The change in the synthesized silver nanoparticles on addition of increasing amounts of the leaf extract was studied. Also the antibacterial activities of the synthesized silver nanoparticles were investigated.

Materials and methods:

Materials:

Silver nitrate was purchased from Rankem Private limited, Chennai, India (AR grade, Mol wt. 169.87) and was used without further purification. *Alternanthera dentata* (croton plant) was collected from S.R.M University, Kattankulathur, Tamilnadu, India. Deionized water (DI) was used for all the synthesis procedures.

Methods:

Preparation of Plant Extract:

5 grams of *Alternanthera dentata* leaf samples were collected and thoroughly washed three times with DI water for 10 minutes; air dried and cut into fine pieces. The leaves were then boiled in an Erlenmeyer flask with 100mL of sterile DI water for about 10 min at 80°C and were finally filtered to get the leaf extract.

Fig.1: ***Alternanthera dentata*** **leaves collected from the garden.**

Synthesis of Silver Nanoparticles:

Various concentrations (200µl, 400µl, 600µl, 800µl, 1000µl) of the plant extract were added to the five different beakers containing 10 ml of 1mM silver nitrate ($AgNO_3$) aqueous solution and marked as a, b, c, d, e respectively in an ice bath at below 5°C.Due to Surface Plasmon Resonance the silver nitrate solution colour changes from colourless to colour as shown in Fig.4. The difference in colour depends on size and shape of the nanoparticles formed. The solutions were centrifuged at 5000rpm for 30 minutes for washing purpose. Then the samples were re-suspended and stored below 5°C in dark to minimize agglomeration.

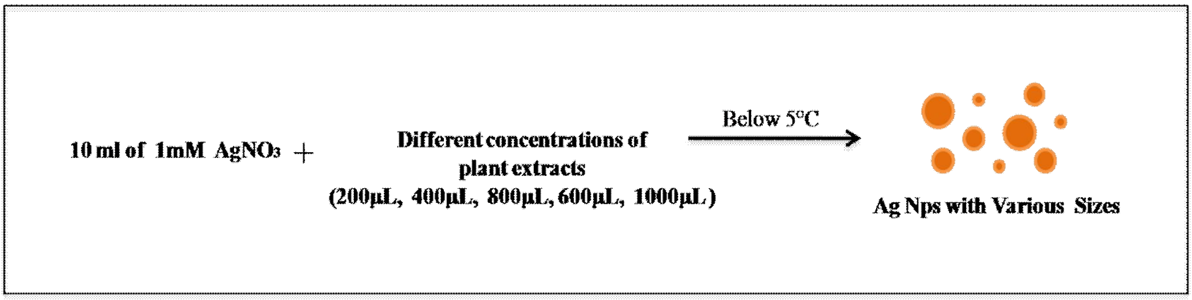

Fig 2: Schematic representation of Ag NPs synthesis using various concentrations of the plant extract.

Table.1: Tested conditions for the green synthesis of Ag Nps using Alternanthera dentata.

Samples	Concentration of $AgNO_3$	Aqueous solution of $AgNO_3$	Concentration of Plant Extract	Temperature
A	1mM	10ml	200µl	3-5°C
B	1mM	10ml	400µl	3-5°C
C	1mM	10ml	600µl	3-5°C
D	1mM	10ml	800µl	3-5°C
E	1mM	10ml	1000µl	3-5°C

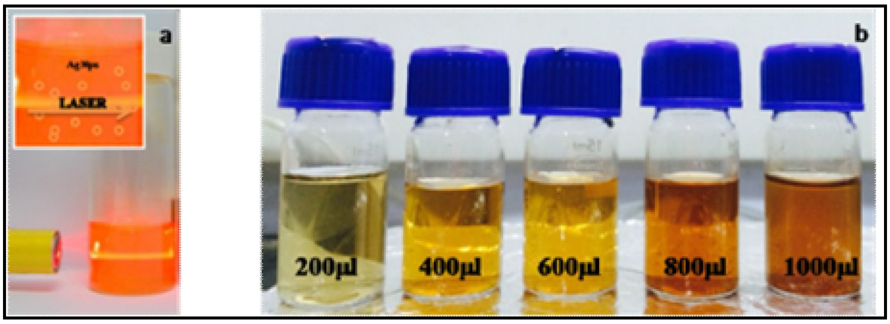

Fig.3a:1 mM AgNPs with plant extract showing confirmation of presence of nano colloids by Tyndall effect 3b: Ag NPs synthesized using various concentrations of plant extracts.

Characterization techniques:

All the samples were analyzed for their morphology, phase purity and for their antibacterial activity. The samples were dried in an aluminium foil for studying the size and the shape of the particles. Field emission scanning electron microscope (FESEM, FEI quanta 200G) attached with energy dispersive spectrometer (EDS, Bruker) was used to study the morphology and size changes of the Ag sample. The images were taken at a voltage of 20kv for different magnifications. EDS of all the samples were also taken at the same voltage. The samples were centrifuged at a higher rpm and the pellet was dried for x-ray diffraction studies. Powder X-Ray diffraction patterns were obtained using PANalytical, Xpert pro instrument with a copper source of wavelength (λ) kα=1.54Å. The scan range from 30 to 80º with a step size of 0.05 and time per step of 2s were carried.

UV-Vis absorption spectrum was analyzed using a Metrohm (Analytical Jena-Specord 200+) double beam instrument. The measurement was carried out from 250 nm to 800 nm wavelength for all the samples.

Antimicrobial activity by well diffusion method:

The anti-bacterial activity of silver nanoparticles (a-e) was studied using well diffusion method. The target strains used for screening antibacterial activity was procured from PCBS, Pondicherry. Mueller Hinton agar was prepared and *Enterococcus faecium* culture was swabbed onto the plate. *Enterococcus faecium* is a Gram positive bacteria which is common in the intestine which also cause disease like endocarditis. Sterile wells with diameter 6mm were punctured and 50 µl of the samples loaded. The test plates were incubated for

24h in a CO_2 incubator. The zone of inhibitions (diameter in mm) was measured for all the samples and the activity of the formed nanoparticles was studied.

Result and Discussion:

Spectroscopic Analysis of the samples:

UV-Vis spectrophotometric measurements were performed for the silver nanoparticles from 250 to 800nm, as shown in the figure 5. The absorbance peak was found to be at a wavelength of about 441nm, indicating the presence of silver nanoparticles. This result correlates with earlier literature reports, giving the average size of the nanoparticles to be around 50 nm[12]. The spherical nature of the synthesized silver nanoparticles was confirmed by the presence of a single Plasmon band[13]. It is further seen that increasing amounts of plant extract, leads to a higher absorption peak due to a corresponding increase in the reduction of silver precursor to silver nanoparticles.

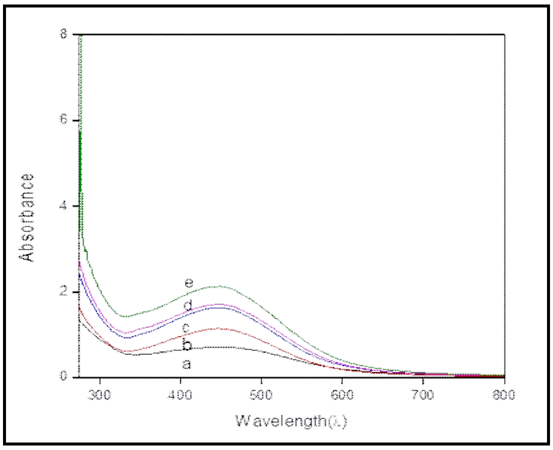

Fig.5: UV-Vis absorption spectra of the silver nanoparticles using various concentrations of plant extract.

X-ray diffraction studies:

The formation of silver nanoparticles was further confirmed using XRD. The peaks as shown in figure 6 matched with the standard JCPDS data (87-0718) of silver, confirming the absence of oxides.

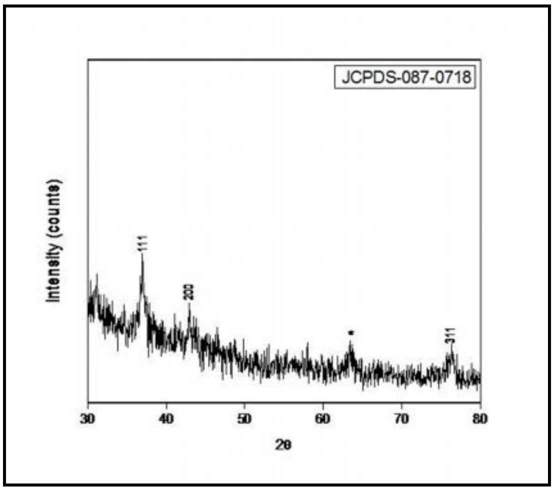

Fig.6: XRD peak of the formed Silver nanoparticles.

SEM Analysis of the samples:

The silver nanoparticles extracted from leaf extract were for their morphology and size using a field emission SEM. The particles dispersed in water were dried on a foil due to which they were agglomerated as seen in figure7. The particles were really small but as they were agglomerated their sizes were not measured.

From the 1μm images from below we can clearly confirm the smaller size of the particle and thus further characterization with a HRTEM is necessary to confirm the same. The particles are seems to be spherical in shape and the presence of silver was confirmed in all the samples through EDS attached to the SEM, Which is also shown in figure 7. This correlates well with our results from UV-Vis Spec and XRD data as discussed above.

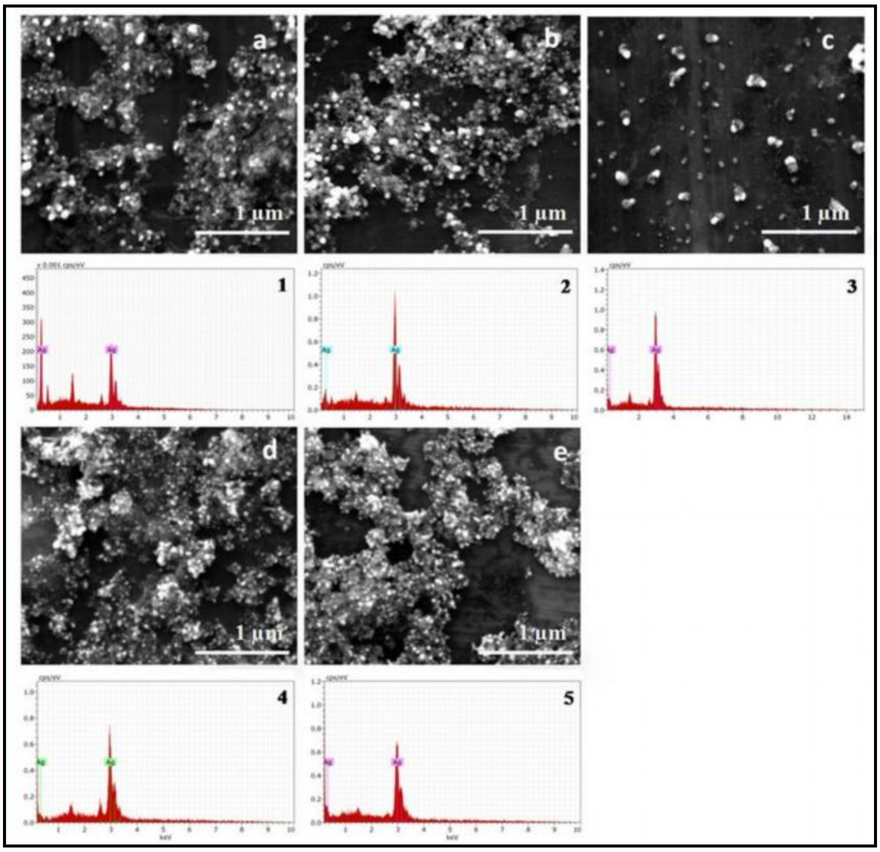

Fig.7: a,b,c,d,e are the SEM images of Ag NPs produced using various concentrations of plant extracts such as 200, 400, 600, 800 and 1000μl respectively, and 1,2,3,4,5 are the EDS spectrum of the corresponding samples.

Anti-Bacterial Studies:

The wells containing the silver nanoparticles showed zones of inhibition in pertriplates containg *Enterococcus faecium.* The various samples of silver nanoparticles showed varying zones of inhibition as seen in Table 2. It was observed that the silver nanoparticles synthesized using 1000μl concentration of plant extract showed the largest zone of inhibition. This confirmed the fact that the amount of stable silver nanoparticles synthesized is directly proportional to the volume of leaf extract added. The results indicated that silver nanoparticles have good antibacterial activity against *Enterococcus faecium* bacteria and hence has good potential for use in antibacterial applications.

Table 2: Zone of Inhibition exhibited by different samples of Ag NPs.

Concentration	Zone of inhibition (mm)
200	11
400	12
600	13
800	18
1000	21
Control	-

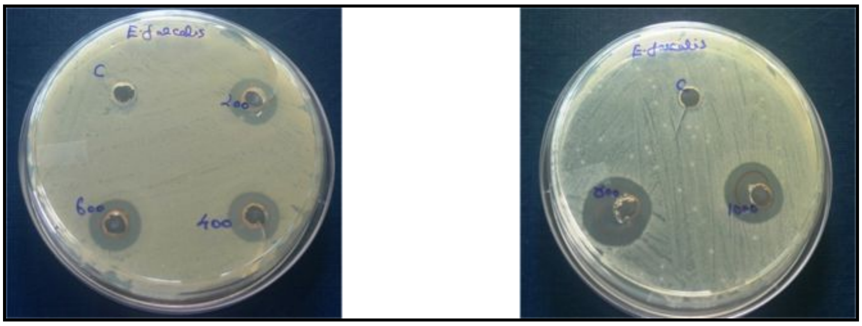

Fig 8: Zone of Inhibition exhibited by different samples of Ag NPs.

Conclusion:

The results indicate that the *Alternanthera dentata* plant which is grown as an ornamental plant in many gardens in India and other parts of the world can be beneficially used in the Nano-biotechnology based industries for Bio-inspired rapid synthesis of silver nanoparticles. The synthesized nanoparticles are found to have a size of approximately 50nm and they exhibit significant antibacterial activity.

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