



ISSN: 0974 - 0376

*The Ecoscan* : Special issue, Vol. V: 29-36: 2014  
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES  
[www.theecoscan.in](http://www.theecoscan.in)

## Plant mediated synthesis of silver nanoparticles using *Adhatoda vasica* aqueous leaf extract

Manoj Kumar *et al.*,



**Proceedings of National Conference on  
Harmony with Nature in Context of  
Environmental Issues and Challenges of the 21<sup>st</sup> Century  
(HARMONY - 2014)**

November 28 - 30, 2014, Udaipur, Rajasthan  
organized by

Department of Environmental Sciences,  
Faculty of Earth Sciences,  
M. L. Sukhadia University, Udaipur - 313 001 (Rajasthan)  
in association with  
NATIONAL ENVIRONMENTALISTS ASSOCIATION, INDIA  
[www.neaindia.org](http://www.neaindia.org)





The *Ecscan*  
AN INTERNATIONAL QUARTERLY JOURNAL OF ENVIRONMENTAL SCIENCES

# Plant mediated synthesis of silver nano-particles using *Adhatoda vasica* aqueous leaf extract

Manoj Kumar\*, Sukumar Dandapat and Manoranjan Prasad Sinha

Department of Zoology, Ranchi University, Ranchi – 834 008  
e-mail: dr17mk@gmail.com

**Abstract.** The leaves of *Adhatoda vasica* were collected and dried in shade and extracted in aqueous medium using Soxhlet extractor. The extract used for synthesis of silver nanoparticles. The characterization of silver nanoparticles was done. The formation of silver nanoparticles was confirmed by change in the colour of solution from yellow to dark brown. The silver nanoparticles thus formed were subjected to UV-vis spectrophotometric, Scanning Electron Microscop (SEM) and Fourier-Transform Infra-red (FTIR) analysis for characterization of the formed nanoparticles. Thus formation of nanoparticles in the range of 63.25 to 110 nm and average size of particles were 89.93 nm.

**Keywords:** *Adhatoda vasica*, Nanoparticles, SEM, FTIR

## 1. Introduction

Biology of plant mediated nanoparticles is an upcoming branch of nanotechnology which is gaining grounds. Nanobiotechnology refers broadly to a field of science whose theme is control of matter at the atomic and molecular scale [1]. The green method of synthesis of nano-particles have several important applications in the field of biolabelling sensors, drug delivery system, filters and also possess antimicrobial activity; these nanoparticles exhibit new physic-chemical properties, which are not observed in polar or non-polar extracts of plants [2].

Biological routes of synthesis of metal nanoparticles have been proposed by exploiting bacteria [3-6] yeast [7-9] fungi [10-13] actinomycetes [14] and virus [15, 16] involved synthesis, but the processes are not feasible industrially, for their pathogenicity and lab maintenance which require both money and skilled technicians.

In this work we report the synthesis of silver nanoparticles, reducing the silver ions present in the solution of silver nitrate by the aqueous extract of *Adhatoda vasica*, this method yields faster and stable silver nanoparticles compared to other methods. The quantitative formation of nanoparticles were monitored by UV-vis spectroscopy. Also the silver nanoparticles formation were confirmed by reddish brown colour formation.

## 2. Materials and Methods

*Adhatoda vasica* Nees (Acanthaceae) commonly known as Vasaka, distributed throughout

\*Corresponding author.

India up to an altitude of 1300m. The leaves, flowers, fruit and roots are extensively used for treating cold, cough, whooping cough, chronic bronchitis, asthma and sedative, expectorant and antispasmodic. It was also used by traditional midwives at the time of delivery. The leave, roots and flowers of *Adhatoda vasica* are extensively used in indigenous medicines [17].

### 2.1 Preparation of Plant Extract

Phenol was determined by Folin-Ciocalteu reagent following Ramamorthy and Bono [18]. Tannin was determined as standard published in the quality control methods for medicinal plant materials [19]. Aluminium Chloride colorimetric method was used with some modification to determine flavonoid content following Lin and Tang [20]. Alkaloids were determined by method used by Helrich [21]. Saponin content was determined following Obadoni and Ochuko[22]. The details have been described by Kumar *et al.* [23].

### 2.2 Synthesis of Silver Nanoparticles

For synthesis of nanoparticles, 1 mL of *Adhatoda vasica* leaf extract was added to 99 mL of 1mM AgNO<sub>3</sub> (169.8mg) solution. The mixture was allowed to stir for 2h at 90°C (Colour change was observed from light yellow to dark brown, and thereafter no further color change was observed even after 2h). The mixture was allowed to cool down after 2h and was centrifuged at room temperature at 9000 rpm after washing three times with distilled water, a black powder was obtained that was dried overnight in an oven at 80°C [24, 25].

### 2.3 Characterization of Nano-Particles

Characterization of silver nanoparticles was done UV-vis spectrum analysis, SEM analysis, Fourier Transform Infrared Spectroscopy (FTIR) analysis.

### 2.4 UV-visible spectrum analysis

The reduction of pure Ag<sup>+</sup> ions was monitored by measuring the UV-visible spectrum of the reaction medium after 5h after diluting a small aliquot of the sample into Milli-Q water. UV-visible spectral analysis was done by using Parkin Elmer Lambda25 UV-visible spectrophotometer.

### 2.5 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.

### 2.6 Fourier transform infrared spectroscopy (FT-IR) analysis

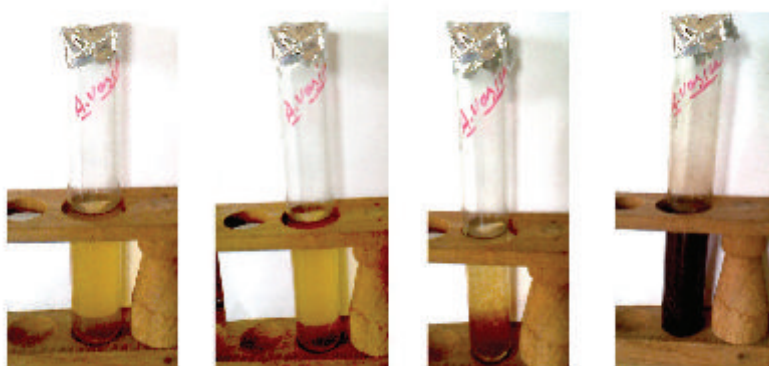
FT-IR analysis was carried out on IPResting-21 (Shimadzu) in the diffuse reflectance mode operated at a resolution of 4 cm<sup>-1</sup> in the range of 400 – 4000 cm<sup>-1</sup> to evaluate the functional groups that might be involved in nanoparticles formation.

## 3. Results and Discussion

### 3.1 UV-vis spectrophotometer analysis

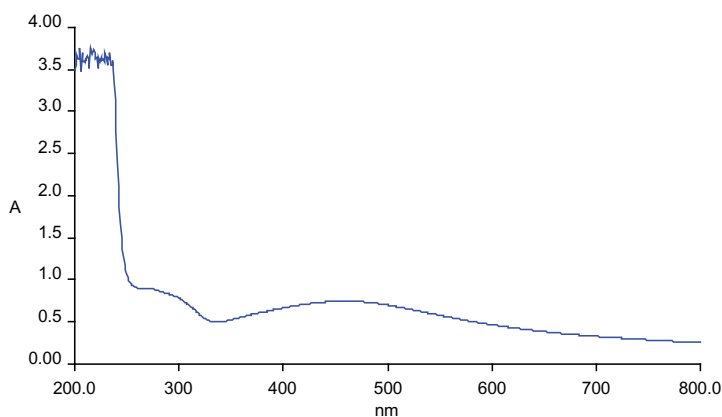
The aqueous solution changed from light yellow colour to dark brown, which is a well know confirmation of nanoparticle formation [26]. As the *Adhatoda vasica* fruit extract was mixed in the aqueous solution of the silver ion complex, it started to change the colour from yellow to brown due to reduction of silver ion (Fig. 1). Which indicated the formation of silver nanoparticles. The

colour change is due to the Surface Plasmon Resonance (SPR) phenomenon [27].



**Figure 1.** figure showing change in colour of aqueous solution from yellow to deep brown

From different literatures it was found that the silver nanoparticles show SPR peak at from 415-490 nm. In this study the *Adhatoda vasica* silver nanoparticles showed SPR peak at 458 nm. Dhinalakshmi [28] reported SPR peak for silver nanoparticles synthesized using *Tridax procumbens*. Several workers have reported absorbance peak from 450-500 nm for silver nanoparticles synthesizes using *Argemone Mexican* [29], Papaya fruit extract [30], *Trianthema decandra* [31].



**Figure 2.** Result of UV-vis spectrophotometric analysis, showing peak at 458 nm SEM analysis

The size and structure of silver nanoparticles synthesized with help of *Adhatoda vasica* leaf extract was further subjected to SEM analysis to examine the morphology of the nanoparticles. The *Adhatoda vasica* leaf extract mediated synthesized silver nanoparticles were spherical and cubical in shape and had diameter of nanoparticles ranged from 63.25 – 110 nm and average size of particles were 86.93 nm. Kumar [32] reported size of alion mediated synthesized nanoparticles to be in range of 287-293 nm and average size was 70 nm. Firdhouse [33] reported the size of nanoparticles ranging from 20 – 150 nm in diameter.

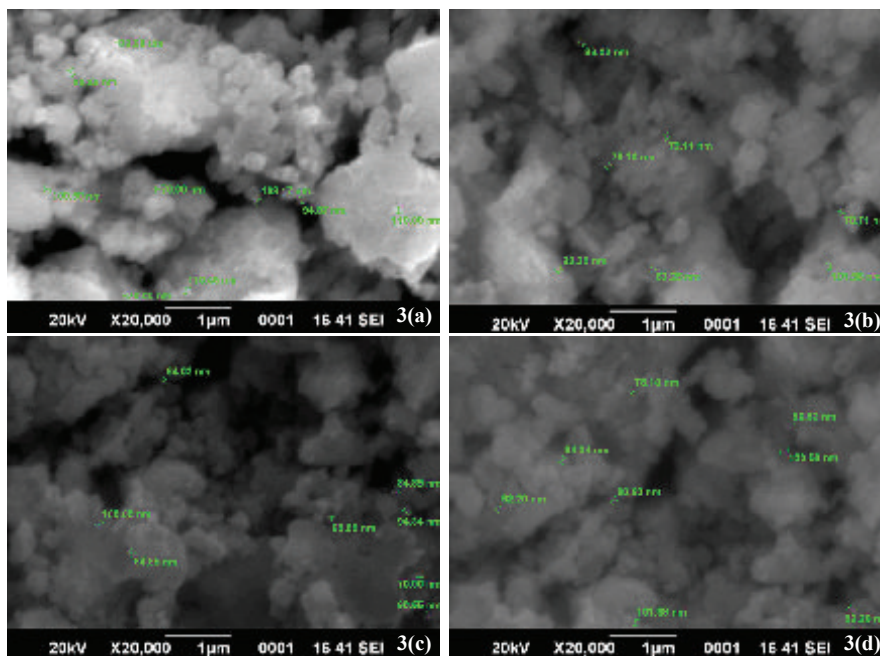


Figure 3(a, b, c, d). SEM images of *Adhatoda vasica* mediated synthesized Ag nanoparticles

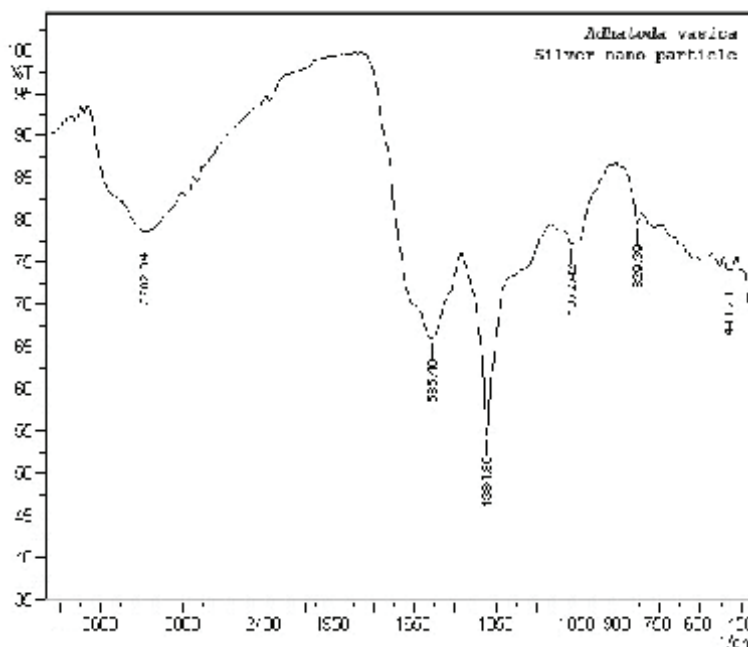


Figure 4. FT-IR spectrum of *Adhatoda vasica* mediated synthesized silver nano-particles

### 3.2 Fourier transform infrared spectroscopy (FT-IR) analysis

FT-IR analysis was carried out to analyze the role of plant extract as capping agent and highly bioreductant agent [34] and functional groups [35, 36]. FT-IR absorption spectra of green Ag nanoparticles is presented as Fig. 4.

The spectra observed by FT-IR analysis was compared with reference value previously published by Coates [37]. The spectra showed broad transmission peak at  $3282\text{ cm}^{-1}$ , which corresponds to hydrogen bonded hydroxyl group (O-H and H stretch) of alcohols and Phenols.  $1585\text{ cm}^{-1}$  corresponds to C=C stretch, which represents alkenes;  $1384\text{ cm}^{-1}$  corresponds to N=O bend as aliphatic nitro compounds;  $1072\text{ cm}^{-1}$  corresponds to C=N stretch that represents aliphatic amines;  $829\text{ cm}^{-1}$  corresponds to aliphatic phosphate symmetric P-O-C and  $493^{-1}$  corresponds to S-S stretch correspondings to Polysulfides.

The study included the synthesis of silver nanoparticles from the aqueous leaf extracts of *Adhatoda vasica* and their characterization. On the basis of observations of present study it is concluded that the aqueous silver ions exposed to the aqueous extract of *Adhatoda vasica* were reduced and nanoparticles were synthesized. The change in the colour of the solution containing the  $\text{AgNO}_3$  solution and aqueous leaf extract of *Adhatoda vasica* confirmed the formation of the silver nanoparticle. The formation of silver nanoparticles were further confirmed by UV-Vis spectrophotometric and SEM analysis.

## 4. Acknowledgements

Authors are thankful to Birla Institute of Technology for helping in the SEM, FTIR and UV-Vis analysis. Further we acknowledge the facilities made available by the Head, Department of Zoology, Ranchi University, Ranchi.

## References

- [1] Phanjom, P., Sultana, A., Sarma, H., Ramchiary, J., Goswami, K. and Baishya, P.: Plant Mediated Synthesis of Silver Nanoparticles using *Elaeagnus Latifolia* leaf extract. In *Digest Journal of Nanomaterials and Biostructures* 7(3), 1117-1123 (2012).
- [2] Avinash, I., Mahendra, R., Aniket G. and Manisha, B.: *Fusarium solani*: A novel biological agent for the extracellular synthesis of silver nanoparticles. *Journal of Nanoparticle Research* 11: 2079-2085 (2009).
- [3] Lengke, F.M., Fleet, E.M. and Southam, G.: Biosynthesis of silver nanoparticles by filamentous cyanobacteria from a silver (I) nitrate complex. In *Langmuir* 23: 2694-2699 (2007).
- [4] Kalishwaralal, K., Deepak, V., Ram, P.S. and Gurunathan, S.: Biosynthesis of gold nanocubes from *Bacillus Lichemiformis*. In *Bioresearch and technology* 100: 5356-5358 (2008).
- [5] Mokhtari, M., Deneshpojoh, S., Seyedbagheri, S., Atashdehghan, R., Abdi, K. and Sarkar, S.: Biological synthesis of very small silver nano particles by culture supernatant of *Klebsiella pneumoniae*. In *Materials Research Bulletin* 44: 1415-1421 (2009).
- [6] Anil, K.S., Abyaneh, M. K., Gosavi, S.W., Sulabha, K. K., Renu, P. and Absar.: Nitrate reductase-mediated synthesis of silver nanoparticles from  $\text{AgNO}_3$ . In *Biotechnology Letter* 29: 439-445. (2013).
- [7] Jha AK, and Prasad K.: Microbe mediated nano transformation: cadmium. *NANO*. In *Brief Rep. Rev.* 2, 239-242 (2007).
- [8] Prasad, K. and Jha A.K.: *Lactobacillus* assisted synthesis of titanium nanoparticles. In *Nanoscale Research Letter* 43, 248-250 (2007).
- [9] Jha, A.K. and Prasad, K.: A green low-cost biosynthesis of  $\text{Sb}_2\text{O}_3$  nanoparticles. In *Biochemical Engineering Journal* 43, 303-306 (2009).
- [10] Kathiresan, K., Manivannan, S., Nabeel, A.M. and Dhivya. B.: Studies on silver nanoparticles



- synthesized by a marine fungus *Penicillium fellutanum* isolated from coastal mangrove sediment. In *Biointerfaces* 71: 133-137 (2009).
- [11] Bansal, V., Rautray, D., Bharde, A., Ahire, K., Sanyal, A. and Ahmad, A.: Fungus-mediated biosynthesis of silica and titania particles. In *J. Mat. Chem.* 15: 2583-2589. (2005)
- [12] Fayaz, A.M., Balaji, K., Girilal, M., Kalaichelvan, P.T. and Venkatesan, R.: Mycobased synthesis of silver nanoparticles and their incorporation in to sodium alginate films for vegetable and fruit preparation. In *J. Agri. Food. Chem.* 57: 6246-6252 (2009).
- [13] Sastry, M., Ahmad, A., Khan, M.I. and Kumar, R.: Biosynthesis of metal nanoparticles using fungi and actinomycete. In *Current sciences* 85,162-170 (2013).
- [14] Ahmad, A., Senapati, S., Khan, M.I., Kumar, R. and Sastry M.: Extracellular biosynthesis of monodisperse gold nanoparticles by a novel extremophilic actinomycete *Thermonospora* sp. In *Langmuir*, 19, 3550-3553.
- [15] Douglas, T., and Young, M.: Host-guest encapsulation of materials by assembled virus protein cages. In *Nature* 393: 152-155 (1998).
- [16] Dujardin, E., Peet, C., Stubbs, G., Culver, J.N. and Mann, S.: Organization of metallic nanoparticles using tobacco mosaic virus templates. In *Nano Lett* 3: 413-417 (2003).
- [17] Kumar, M., Dandapat, S., Kumar, A. and Sinha, M.P.: Anti-typhoid activity of *Adhatoda vasica* and *Vitex negundo*. In *Persian Gulf Crop Protection* 2(3), 64-75 (2013).
- [18] Ramamoorthy, P.K. and Bono. A.: Antioxidant activity, total phenolic and flavonoids content of *Morinda citrifolia* fruit extracts from various extraction process. In *Journal of Engineering Science and technology* 2(1),70-80 (2007).
- [19] Obadoni, B.O., and Ochuko P.O.: "Phytochemical studies and comparative efficacy of the crude extract of some homeostatic plants in Edo and Delta states of Nigeria. In *Global Journal of Pure and Applied Sciences* (2003-2008)
- [20] Kumar, M., Dandapat, S., Kumar, A. and Sinha, M.P.: Determination of Nutritive value and Mineral elements of Five-leaf chaste tree (*Vitex negundo* L.) and Malabar nut (*Adhatoda vasica* Nees). In *Academic Journal of PLant Sciences* 6(3), 103-108 (2013).
- [21] Helrich, K.: *Methods of analysis of the Association of Official analytical chemists.* 15. Virginia: AOAC, Inc (1990).
- [22] Stuart, B.: *Infrared spectroscopy: Fundamentals and Applications.* John Wiley & Sons (2004).
- [23] Silverstein, R.M., Bassler, G.C., and Morrill, T. C.: *Spectrometric identification of organic compounds.* 4. New York: John Wiley & Sons (1981)
- [24] Shankar, S.S., Rai, A., Ankamwar, B., Singh, A., Ahmad, A. and Sastry, M.: Biological synthesis of triangular gold nanoparticles. In *Natural material:* 482-488 (2004).
- [25] Panigrahi and Tamasa.: Synthesis and Characterization of Silver Nanoparticles using leaf extract of *Azadirachta indica*. Ph.D. thesis, Rourkela: Department of Life Science, National institute of Technology, Orissa (2013).
- [26] Dhanlakshmi, T., and Rajendran, S.: Synthesis of silver nanoparticles using *Tridax procumbens* and its antimicrobial activity. In *Archives of applied science research.* 4(3) :1289-1293 (2012)
- [27] Parashar, V.R., Parashar, Sharma, B. and Pandey A.C.: Parthenium leaf extract mediated synthesis of silver nanoparticles: a novel approach towards weed utilization. In *Digest Journal of Silver Nanoparticles and biostructure* 4(1), 45-50. (2009).
- [28] Jain, D., Daima, H. K., Kachhwaha, S., and Kothari, L.: Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobials. In *Digest Journal of Nanomaterials and Biostructures* 4(3), 557-563 (2009).
- [29] Geethalakshmi, R., and Sarada, D.V.L.: Synthesis of plant-mediated silver nanoparticles using *Trianthema decandra* extract and evaluation of their anti-microbial activities. In *International Journal of Engineering Science and Technology* 2(5): 970-975 (2010).
- [30] Kumar, Chaitanya, T.V., Tollamadugu N. V. K. V., Adilaxamma K., Mekapogu A., Yagireddy



- M. and Pagadala E.P.: Novel synthesis of nanosilver particles using plant active principle and evaluation of their cytotoxicity effect against *Staphylococcus aureus*. In *Asian Pacific journal of Tropical Diseases* 4(1), 92-96 (2014).
- [31] Firdhouse, M., Jannathul, P., Lalitha, K., Shubashini and Sripathi, K.: Novel synthesis of silver nanoparticles using leaf ethanol extract of *Pisonia grandis* (R. Br). In *Der Pharma Chemica* 4(6), 2320-2326 (2012).