



Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Disease

journal homepage: www.elsevier.com/locate/apjtd

Document heading

Rapid synthesis of silver nanoparticles from *Polyalthia longifolia* leaves

Tollamadugu Nagavenkata Krishina Vara Prasad^{1*}, Erusan Kuppan Elumalai², Shaik Khateerja³¹Institute of Frontier Technology, Regional Agricultural Research Station, Acharya N.G.Ranga Agricultural University, Tirupati, India²P.G. and Research Department of Zoology, Voorhees College, Vellore, India.³College of Home Science, Acharya N.G. Ranga Agricultural University, Hyderabad, India

ARTICLE INFO

Article history:

Received 5 August 2012

Received in revised form 18 September 2012

Accepted 19 November 2012

Available online 28 December 2012

Keywords:

Silver nanoparticles

P. longifolia

Eco-friendly

Non toxic

ABSTRACT

Objective: Metallic nanoparticles are traditionally synthesized by wet chemical techniques, where the chemicals used are quite often toxic and flammable. In this research article we present a simple and eco-friendly biosynthesis of silver nanoparticles using *P. longifolia* leaf extract as reducing agent. **Methods:** Characterization using UV-Vis spectrophotometry, Transmission Electron Microscopy (TEM) was performed. **Results:** TEM showed the formation of silver nanoparticles with an average size of 57 nm. **Conclusions:** *P. longifolia* demonstrated strong potential for synthesis of silver nanoparticles by rapid reduction of silver ions (Ag^+ to Ag^0). Biological methods are a good competent for the chemical procedures, which are enviro-friendly and convenient.

1. Introduction

Nanoparticles have a wide range of applications in the field of environmental pollution control, drug delivery system, material chemistry and so on. There has been extensive research in chemical synthesis of nanoparticles. The chemical synthesis of nanoparticles has several occupational exposure hazards like carcinogenicity, genotoxicity, cytotoxicity and general toxicity. So there is a pressing need to develop clean non-toxic and eco friendly procedures for synthesis and assembly of nanoparticles^[1]. Plants in contrast with chemical synthetic methods can be a good alternative for nanoparticles synthesis, since it does not employ toxic chemicals. Recently several authors have accomplished the biosynthesis of metal nanoparticles using biomass obtained from unicellular organisms like bacterial^[2] and fungi^[3], as well as extracts of plants, e.g. *E.hirta*^[4], *C. roseus*^[5], *S.tumbuggaia*^[6], *Diopyros kaki*^[7]. The rate of synthesis of nanoparticles by plant extracts is comparable to those of chemical methods and faster than green synthesis by microorganisms.

Polyalthia longifolia (Annonaceae) is a tall handsome evergreen tree and it's cultivated all over India. The plant has been used as traditional systems of medicine for treatment of various diseases. The plant extracts and isolated compounds were studied for various biological activities like antibacterial, cytotoxicity, antiulcer activity^[8,9]. In this study, we explored for the first time the potential of the *P. longifolia* to enlarge the scope of non-toxic biological systems for the biosynthesis of metallic nanomaterials.

2. Materials and Methods

2.1. Materials

All chemicals used in this experiment were of highest purity and obtained from Sigma (Bangalore, India) and Merck (Mumbai, India). *P. longifolia* leaves were collected from Regional Agriculture Research Station, Tirupathi, Andhra Pradesh, India.

2.2. Plant material and Synthesis of silver nanoparticles

Plant leaf extract was prepared by mixing 10 g of dried powder with 100ml deionized water in 500 mL of Erlenmeyer

*Corresponding author: Dr. Tollamadugu Nagavenkata Krishina Vara Prasad Institute of Frontier Technology, Regional Agricultural Research Station, Acharya N.G.Ranga Agricultural University, Tirupati-517 502, (A.P.), India.

Tel: +91-9440342151

E-mail: tnkvprasad@gmail.com

flask and boiled for 10 min. For the reduction of Ag^+ ions, 10ml of leaf extract was mixed to 90 mL of 1mM aqueous of AgNO_3 was heated at 80°C for 15 min. A change from brown to reddish color was observed.

2.3. UV–Vis Spectra analysis

The reduction of pure Ag^+ ions was monitored by measuring the UV–Vis spectrum of the reaction medium at 5 hours after diluting a small aliquot of the sample into distilled water. UV–VIS spectral analysis was done by using UV–VIS spectrophotometer UV–2450 (Shimadzu).

2.4. Transmission Electron Microscopy (TEM)

Transmission electron microscopy TEM (HITACHI, H–7500) is a microscopy technique whereby a beam of electrons is transmitted through an ultra–thin specimen, interacting with the specimen as it passes through. An image is formed from the interaction of the electrons transmitted through the specimen; the image is magnified and focused onto an imaging device.

2.4. Antimicrobial activity study

Antimicrobial activities of the synthesized Ag nanoparticles were determined, using the agar well diffusion assay method[10]. Approximately 20 mL of molten and cooled media (NA/SDA) was poured in sterilized petridishes. The plates were left overnight at room temperature to check for any contamination to appear. The test organisms were grown in selected broth for 24 h. A 100 mL broth culture of each test organism (1×10^5 cfu/ml) was used to prepare lawns. Agar wells of 5 mm diameter were prepared with the help of a sterilized stainless steel cork borer. Two wells were prepared in the agar plates. The wells were labeled as A,B. 'A' well was loaded with 30 μL of Ag nanoparticles suspended 'hydrosol' and 'B' well loaded with 30 μL of positive control drugs(Chloromphenical/Ketoconazole)(Table.1) were used as positive controls. The plates containing the test organism and Ag nanoparticles were incubated at 37°C . The plates were examined for evidence of zones of inhibition, which appear as a clear area around the wells. The diameter of such zones of inhibition was measured using a meter ruler and the mean value for each organism was recorded and expressed in millimeter.

3. Results

3.1. UV–Vis Spectra analysis

The color change showed the presence of Ag nanoparticles in the *P. longifolia* leaf extract and it was characterized by UV–Visible spectrophotometer and monitored by taking readings at regular time intervals in UV–Visible spectrophotometer UV–2450 (Shimadzu). The strong broad peak located at 430 nm was observed for Ag nanoparticles (Fig 1).

Table 1. The antimicrobial activity of silver nanoparticle synthesis from *P. longifolia*.

Tested organisms	Zone of inhibition (mm)	
	Ag nanoparticle	Reference drugs
<i>S.aureus</i>	13.05 \pm 0.12	19.02 \pm 0.01
<i>E.coli</i>	14.01 \pm 0.03	20.01 \pm 0.40
<i>K.pneumoniae</i>	7.02 \pm 0.01	21.05 \pm 0.12
<i>B.cereus</i>	7.02 \pm 0.01	18.04 \pm 0.10
<i>C.albicans</i>	16.01 \pm 0.50	23.03 \pm 0.01
<i>C.tropicalis</i>	11.03 \pm 1.00	21.04 \pm 0.20
<i>C.krusei</i>	6.01 \pm 0.41	19.07 \pm 0.01

Reference drug: Chloromphenical/Ketoconazole.

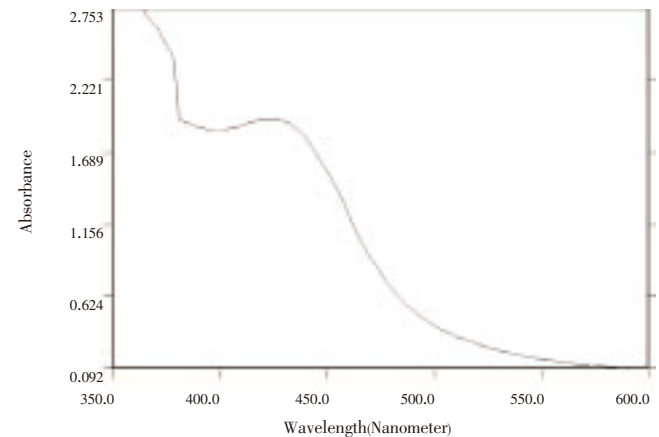


Figure 1. UV–VIS absorption spectra of silver nanoparticle synthesized from *P. longifolia* leaves at 1mM silver nitrate.

3.2. TEM analysis of Silver nanoparticles

The silver nanoparticles synthesized by the help of *P. longifolia* extract were scanned using TEM (HITACHI, H–7500) from which we can conclude that the average mean size of silver nanoparticles was 57.53 nm and seems to be spherical in morphology as shown in figure 2.

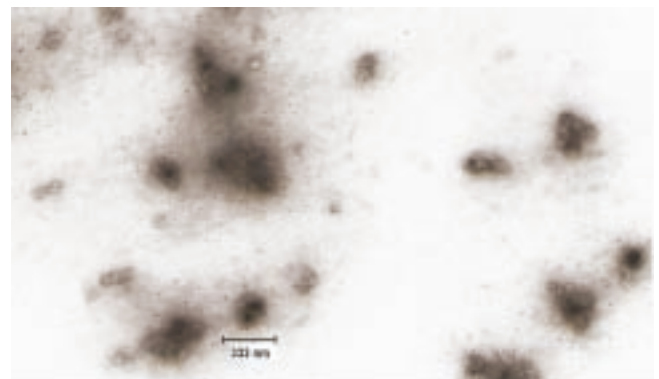


Figure 2. TEM image of the silver nanoparticles synthesised from *P. longifolia* leaf.

3.3. Antimicrobial activity studies

Biosynthesis of silver nanoparticles were studied for antimicrobial activity against pathogenic microorganisms

(clinical isolate) by using standard zone of inhibition (ZOI) microbiology assay, with a well size of 5 mm diameter and 30 μ L of samples. Chloromphenical/Ketoconazole of 10mg/ml concentration was used as a control antimicrobial agent. The silver nanoparticles synthesized showed inhibition zone against all the test organisms. Maximum zone of inhibition was found to *C.albicans* (16 mm), *E.coli* (14 mm), *S.aureus*(13 mm) and minimum of zone of inhibition was found to *B.cereus* (7 mm); *K.pneumoniae* (7 mm) and *C.krusei* (6 mm) in all the test organisms(Table.1, Fig,3).

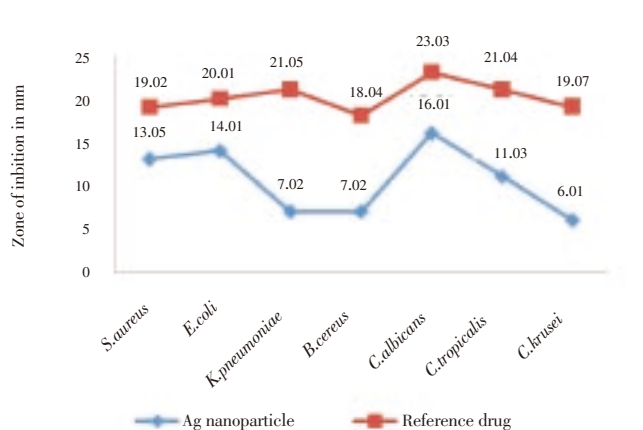


Figure 3. The antimicrobial activity of silver nanoparticle synthesis from *P. longifolia*.

4. Discussion

The development of biologically inspired experimental processes for the synthesis of nanoparticles is evolving into an important branch of nanotechnology. The present study deals with the synthesis of silver nanoparticles using leaves extract of *P. longifolia* and aqueous Ag^{++} ions. Comparative experiments were carried out to study the effect of biomass dosage and the concentration of silver nitrate on the rate of bioreduction of silver ions. The approach appears to be cost effective alternative to conventional methods of assembling silver nanoparticles.

Formation and stability of silver nanoparticles in aqueous colloidal solution are confirmed using UV–Vis spectral analysis. It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of surface plasmon vibrations in silver nanoparticles[11,12]. As the *P. longifolia* leaf extract was mixed with aqueous solution of the silver nitrate, it started to change the color from watery to reddish brown due to reduction of silver ion; which indicated the formation of silver nanoparticles. It is generally recognized that UV–Vis spectroscopy could be used to examine size and shape–controlled nanoparticles in aqueous suspensions[13]. Absorption spectra of silver nanoparticles formed in the reaction media has absorbance peak at 430 nm and broadening of peak indicated that the particles are polydispersed.

4.1. Antimicrobial activity

Silver has been widely utilized for thousands of years in

human history, it's applications include jewels, utensils, currency, dental alloy, photography and explosives. Among silver's many applications, its disinfectant property is being exploited for hygienic and medicinal purposes, such as treatment of mental illness, nicotine addiction and infectious disease like syphilis and gonorrhea[14]. Silver nanoparticles have been demonstrated to exhibit antimicrobial properties against bacteria with close attachment of the nanoparticles themselves with the microbial cell and the activity being size dependent[15].

Shrivastava *et al*[16] studied antibacterial activity against *E. coli* (ampicillin resistant), *E. coli*, *S. aureus*, and *S. typhi* (multi–drug resistant). They reported that the effect was dose dependent and was more pronounced against gram–negative organisms than gram–positive ones. They found that the major mechanism through which silver nanoparticles manifest antibacterial properties was either by anchoring or penetrating the bacterial cell wall, and modulating cellular signaling by dephosphorylating putative key peptide substrates on tyrosine residues[16]. The antibacterial efficacy of the biogenic silver nanoparticles reported in the present study may be ascribed to the mechanism described above but it still remains to clarify the exact effect of the nanoparticles on important cellular metabolism like DNA, RNA and protein synthesis.

It has been demonstrated that the extract of *P. longifolia* leaf are capable of producing a nanoparticles extracellularly and the Ag nanoparticles are quite stable in solution. The formed silver nanoparticles should considerable antimicrobial activity compared to the respective antibiotics. This biosynthesis silver nanoparticles and prove to be potential candidates for medical applications.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgement

The author thankful to Regional Agricultural Research Station for providing facilities to carry out the experiment of this work.

References

- [1] Mukherjee P, Roy M, Mandal BP, Dey GK, Mukherjee PK, Ghatak J, Tyagi AK, Kale SP. Green synthesis of highly stabilized nanocrystalline Ag particles by a non–pathogenic and agriculturally important fungus *T. asperellum*. *Nanotechnology* 2008;**19**: 075103.
- [2] Shahverdi AR, Minaeian S, Shaverdi HR, Jamalifar H, Nohi AA. *Process Biochem* 2007; **2**:919.
- [3] Varshney R, Mishra AN, Bhadauria S, Gaura MS. Novel microbial route to synthesize silver nanoparticles using fungus *Hormoconis resinae*. *D. J of Nanomat And Biostru* 2009; **4**(2): 349 –355.
- [4] Elumalai EK, Prasad TNVKV, Hemachandran J, Vijiyan

- Therasa S. Thirumalai T, David E. Extracellular synthesis of silver nanoparticles using leaves of *Euphorbia hirta* and their antibacterial activities. *J. Pharm. Sci. & Res.* 2010; **2** (9): 549–554.
- [5] Mukunthan KS, Elumalai EK, Trupti N Patel, Ramachandra Murty V. *Catharanthus roseus*: A natural source for the synthesis of silver nanoparticles. *As Paci .J .of Trop. Biomed* 2011;**4**:270–274.
- [6] Venkateswarlu P, Ankanna S, Prasad TNVKV, Elumalai EK, Nagajyothi PC, Savithamma N. Green synthesis of Silver Nanoparticle Using *Shorea tumbuggaia* Stem Bark. *Int. J. of Drug Develop. & Res* 2010; **2** (4):720–23.
- [7] Song JY, Jang HK, Kim BS, *Process Biochem* 2009; **44**: 1133.
- [8] Malairajan P, Geetha G Narashiman S, K.J. Jessi kala Veni S. Evaluation of Antulcer activity of *polyathia logifloia* in experimental animals. *Indian J.Pharmac* 2008; **4**(3):126–128.
- [9] Ghosh G, Subudhi BB, Badajena LD, Ray J, Mishra MK, Mishra S.K. Antibacterial activity of *P. longifolia* var. *angustifolia* stem bark extract. *Int. J of Pharm Tech Res* 2011; **3**(1): 256–260.
- [10] Perez C, Paul M, Bazerque P. Antibiotic assay by agar well diffusion method. *Acta Biol Med Exp* 1990; **15**:113.
- [11] Krishnaraj C, Jagan EG, Rajasekar S, Selvakumar P, Kalaichelvan PT, Mohan N. *Colloids and Surfaces B: Biointerfaces* 2010; **76**:50–56.
- [12] Noginov MA, Zhu G, Bahoura M, Adegoke J, Small C et al. The effect of gain and absorption on surface plasmon in metal nanoparticles. *Ap Phys B* 2006;**86**:455–460.
- [13] Shrivastava S, Dash D. *J Nanotechnol* 2009; **12**: 240–243.
- [14] Huang J, LiQ, Sun D, Lu Y, Su Y, Yang X, et al. Biosynthesis of silver and gold nanoparticles by novel sundried *C.camphora* leaf. *Nanotec* 2007;**18**:105104–14.
- [15] Sondi I, Salopek–Sondi B. Silver nanoparticles as antimicrobial agent: A case study on *E. coli* as a model for gram–negative bacteria. *J Colloid Interface Sci* 2004; **275**: 177–182
- [16] Shrivastava S, Bera T, Roy A, Singh G, Ramachandrarao P, Dash D. *Nanotec* 2007; **18**:225103.