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Phytofabrication and Characterization of Silver Nanoparticles from *Piper betle* Broth

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ABSTRACT

Nanotechnology can be defined as a research for the design, synthesis and manipulation of structure of particles with dimension smaller than 100 nm. Nanotechnology emerges from the physical, chemical, biological and engineering sciences where novel techniques are being developed to probe and manipulate single atoms and molecules. The biomimetic approaches of silver nanoparticles were reduced by a simple and eco-friendly process. The advantage of using plants for the synthesis of nanoparticles is that they are easily available, safe to handle and possess a broad variability of metabolites that may aid in reduction. We report a biomolecules hosting, rapid, environmentally benign, bio-degradable, non-toxic and green synthesis of silver nanoparticles by using *Piper betle* leaf broth as a reducing and stabilizing agent. The sizes of synthesized silver nanoparticles were formed on the treatment of aqueous $AgNO_3$ solution with *Piper betle* broth, in the range of 3-37 nm. A UV visible spectrum of the aqueous medium containing silver ions demonstrated a peak at 440 nm corresponding to the surface plasmon resonance of silver nanoparticles; An XRD analysis reveals the crystalline nature of silver nanoparticles. The FTIR spectrum suggests that the proteins act as capping agents around the nanoparticles. The size and shape of the nanoparticles were employed by the Transmission Electron Microscope (TEM).

Key words: *Piper betle* leaves, green synthesis, transmission electron microscopy, x-ray diffraction, fourier transform infrared spectroscopy

INTRODUCTION

The use of the green chemistry is an increasing interest of the synthetic procedure for nanoproducts. Which are targeted as potential applications in the fields of catalysis in chemical reactions (Crooks *et al.*, 2001), medicinal (Warisnoicharoen *et al.*, 2011; Bhumkar *et al.*, 2007; Poovi *et al.*, 2011), biolabelling (Hayat, 1989), microelectronic (Gittins *et al.*, 2000), information storage (Dai and Bruening, 2002) and opteolectronic devices (Li *et al.*, 2010). The broad spectrum of silver nanoparticles was produced by different physical and chemical methods (Singh *et al.*, 2011). For environmental concerns, there is a need to develop benign nanoparticles using non toxic chemicals in the synthesis protocols in order to avoid adverse effects in medical applications. At present, several groups of researchers concentrate on biomimetic approaches such as plant or plant leaf extracts, Nuts, microorganisms and yeast to synthesize the metal nanoparticles called as "green chemical or phytochemical" approach (Savitramma *et al.*, 2011; Ramgopal *et al.*, 2011; Sinha *et al.*, 2009; Kannan and Subbalaxmi, 2011). One of the synthesizes procedure such as leaf extracts of *geranium* lemon grass, neem and several others which have been reported (Shankar *et al.*, 2003, 2004a, b; Satyavathi *et al.*, 2010; Dubey *et al.*, 2010; Song *et al.*, 2009; Satyavani *et al.*, 2011; Rajesh *et al.*, 2009). The *Piper betle* is a traditional medicinal plant of India which is a source of bioreductant and stabilizer but so far, there has been no report on the development of silver nanoparticles by using *piper betle* leaves. It is also well known for its phenolic content (Jamal *et al.*, 2010), as also for its antibacterial (Fathilah *et al.*, 2009; Nalina and Rahim, 2006; Vaghasiya *et al.*, 2007) and antioxidant (Rathee *et al.*, 2006) activities as well. So far, there have been no reports on the synthesis of nanoparticles by using *Piper betle* leaf extract. In this study, we report on the synthesis of silver nanoparticles using *Piper betle* leaf extracts as a simple, low cost and reproducible method.

MATERIALS AND METHODS

Preparation of leaf extract: The authors have carried out the experimental work during 14th February, 2011 to 31st August; 2011. The fresh leaves of *Piper betle* were collected from a retail shop in Tirupati, Andhra Pradesh, India. Silver nitrate ($AgNO_3$, 99.99%) was purchased from Sigma-Aldrich chemicals, 10 g of fresh leaves were washed thoroughly under the running tap water, while finely cut leaves were added with 50 mL of distilled water in a 250 mL Erlenmeyer flask and then boiled for 10 min before decanting it. The extract was filtered and stored at 4°C for further experiments.

Synthesis of silver nanoparticles: The leaf broth with various concentration levels, ranging from 50 to 150 μL was added to 3 mL of 1 mM aqueous $AgNO_3$ solution kept at room temperature. The bioreduced silver nitrate solution was monitored by periodic sampling of aliquots (0.3 mL). It was diluted to the ratio of 1:10 with distilled water, to avoid errors due to high optical density of the solution for measuring UV-Vis spectra.

RESULTS AND DISCUSSION

UV-Visible absorbance spectroscopy: The concentration variation with bioreduced Ag^+ ions, in aqueous component were measured with an UV-Vis spectrometer, (Perkin-Elmer lambda 25) which operated at a resolution of 1 nm in the range of 370-800 nm. The progress of the reaction between the *betle* leaf broth and the metal ions were observed by UV-Vis spectra of silver nanoparticles which are shown in Fig. 1. A bathochromatic shift in the surface plasmon resonance band of silver nanocolloid, with an increasing concentration of leaf extract and consequent color change was observed. From the spectrum, we observed that the peak blue shift was at 477 to 440 nm while the amount of leaf extract was constantly increased. The reduction of silver ions and the synthesis of stable nanoparticles occurred with a concentration variation reaction, making it one of the smart phytofabrication methods, in order to produce Ag nanoparticles reported nowadays (Dwivedi and Gopal, 2010; Konwarh *et al.*, 2011; Gils *et al.*, 2010; Philip and Unni, 2011).

X-ray diffraction spectral analysis: An X-Ray Diffraction (XRD) measurements of a thin film of the bioreduced silver ions aqueous solution were drop coated onto a glass slide and carried out

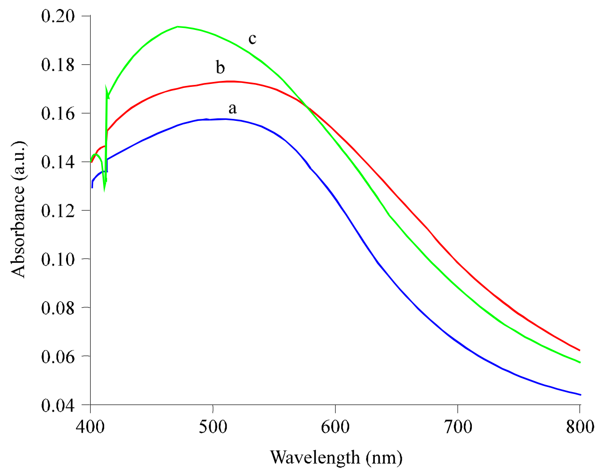

Fig. 1: UV-Vis spectra of silver nitrate with *Piper betle* leaf extract at different concentrations. (a) 50 µL, (b) 100 µL and (c) 150 µL

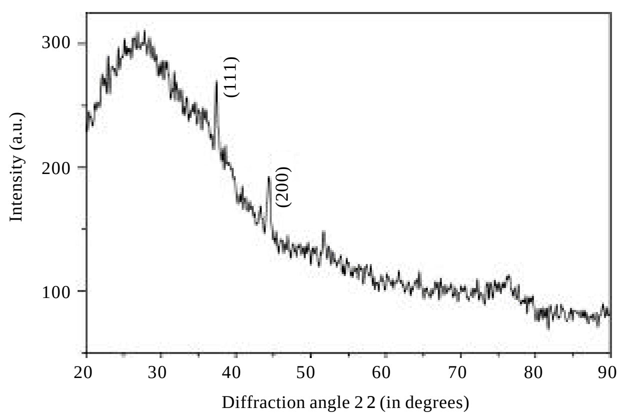

Fig. 2: X-Ray diffraction spectrum of synthesized silver nanoparticles

on an INEL X-ray diffractometer. The diffraction pattern was recorded by Co-kα_1 radiation with λ of 1.78A° in the region of 2θ from 20 to 90° at 0.02° min^{-1} and the time constant was 2 sec. The size of the nanoparticles was calculated through the Scherer's equation (Mulvaney, 1996). The Crystalline nature of Ag nanoparticles was studied with the aid of an X-ray diffraction (Fig. 2). The diffracted peaks were observed at 37.6 and 44.4° corresponding to the (111) and (200) facets of the face centered cubic crystalline in nature and the data was matched with the Joint Committee on Powder Diffraction Standards (JCPDS) file No.03-0921. The domain size of the phytofabricated silver nanoparticles is found to be 5.4 nm, by using the width of the (111) Bragg's reflection which was in consonance with the size of the particle, calculated from the TEM image.

Transmission electron microscopy studies: The morphology and size of the silver nanoparticles were studied by the Transmission Electron Microscopy (TEM) image, by using the PHILLIPS TECHNAI FE 12 instrument. The TEM grids were prepared by placing a drop of the bio reduced diluted solution, on a carbon-coated copper grid and by later drying it under a lamp.

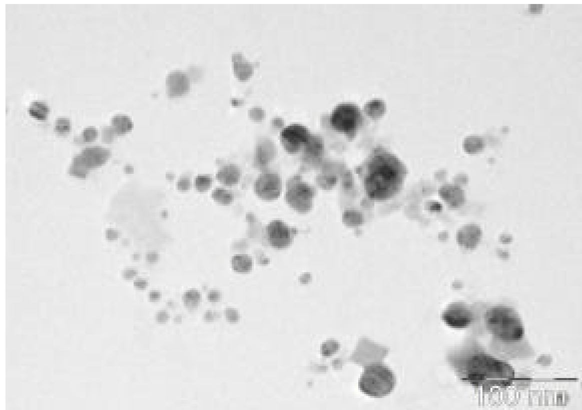

Fig. 3: Transmission electron microscopy image of silver nanoparticles

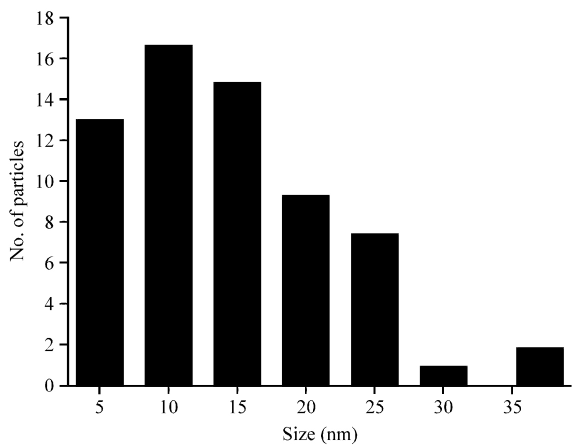

Fig. 4: Histogram of synthesized silver nanoparticles

The TEM image (Fig. 3) was employed, so that the bio synthesized nanoparticles were in the size of 3-37 nm. The small sized nanoparticles were able to easily penetrate across the membrane and similar results have been reported on literature (Morones *et al*., 2005; Pal *et al*., 2007; Jaidev and Narasimha, 2010). It was spherical in shape and few nanoparticles were also agglomerated. Under careful observation, it is evident that the silver nanoparticles are surrounded by a faint thin layer of other materials. The histogram of fabricated silver nanoparticles is shown in Fig. 4.

Fourier transforms infra-red spectroscopy: For Fourier Transformed Infrared (FTIR) measurements; the bio reduced Ag^+ ion aqueous component was centrifuged at 10,000 rpm for 15 min. The dried sample was grinded with KBr pellets and analyzed on Thermo Nicolet Nexus 670 IR spectrometer which was operated at a resolution 4 cm^{-1} in the region of 4000-400 cm^{-1}. The FTIR spectrum o f synthesized silver nanoparticles by using *Piper betle* leaf extract is shown in Fig. 5. It confirmed the fact that to identify the biomolecules for reducing and efficient stabilization of the metal nanoparticles, the band at 3419 cm^{-1} corresponds to O-H, as also the H-bonded alcohols and phenols. The peak at 2920 cm^{-1} indicates carboxylic acid. The band at 1640 cm^{-1} states

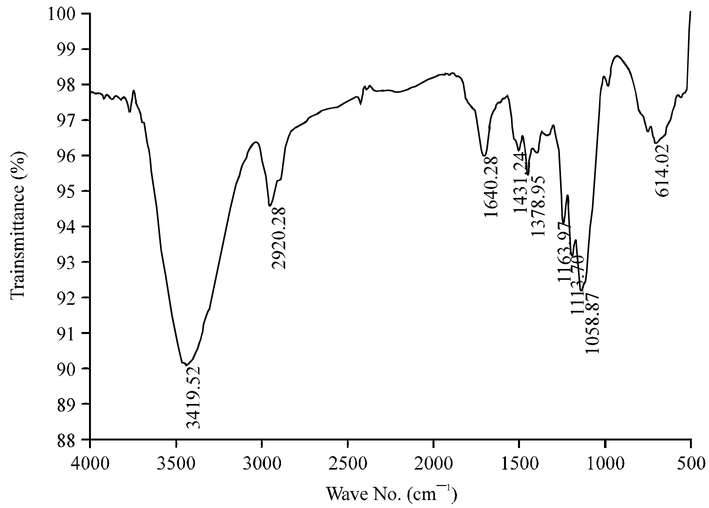

Fig. 5: FTIR spectrum of biologically synthesized silver nanoparticles

primary amines. The band at 1431 cm^{-1} corresponds to C-C stretching aromatics, while the peak at 1378 cm^{-1} states C-H rock alkenes and 1163, 1113 and 1058 cm^{-1} indicates that C-O stretching alcohols, carboxylic acids, esters and ethers. Therefore, the synthesized nanoparticles were encapsulated by some proteins and metabolites such as terpenoids having functional groups of alcohols, ketons, aldehydes and carboxylic acids.

CONCLUSIONS

The synthesis of silver nanoparticles using leaf broth of *Piper betle* provides a natural, simple, less time consuming, cost effective and efficient route for benign nanoparticles. The spherical size of the silver nanoparticles was estimated 3-37 nm from TEM image. From FTIR results we conclude that the reduced silver nanoparticles were stabilized by proteins and metabolites such as terpenoids having functional groups of amines, alcohols, ketons, aldehydes and carboxylic acids. From a technological point of view, these obtained silver nanoparticles have potential applications in the biomedical field and this simple procedure has several advantages such as the compatibility for medical and pharmaceutical applications and large scale commercial productions as well.

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