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ORIGINAL ARTICLE

Phytochemical fabrication and characterization of silver nanoparticles by using *Pepper* leaf broth

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Abstract The *Pepper* leaves extract acts as a reducing and capping agent in the formation of silver nanoparticles. A UV–Vis spectrum of the aqueous medium containing silver nanoparticles demonstrated a peak at 458 nm corresponding to the plasmon absorbance of rapidly synthesized silver nanoparticles that was characterized by UV–Vis spectrophotometer. The morphology and size of the benign silver nanoparticles were carried out by the transmission electron microscope (TEM) and field emission scanning electron microscope (FE-SEM). The sizes of the synthesized silver nanoparticles were found to be in the range of 5–60 nm. The structural characteristics of biomolecules hosted silver nanoparticles were studied by X-ray diffraction. The chemical composition of elements present in the solution was determined by energy dispersive spectrum. The FTIR analysis of the nanoparticles indicated the presence of proteins, which may be acting as capping agents around the nanoparticles. This study reports that synthesis is useful to avoid toxic chemicals with adverse effects in medical applications rather than physical and chemical methods.

1. Introduction

Over the past decade Nanoscience and Nanotechnology is a sprouting interdisciplinary field of research interspersing material science, bionanoscience and technology. Remarkable advances are made in the field of biotechnology and nanotechnology to harness the benefit of life sciences (Huang et al., 2008), healthcare (Ahmad et al., 2010) and industrial biotechnology (Elechiguerra et al., 2005). Nanomaterials may provide solutions to technological and environmental challenges in the

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areas of solar energy conversion (Arango et al., 2000), catalysis (Tsujino and Matsumura, 2007), medicine (Jun et al., 2005) and water treatment (Bao et al., 2007). This increasing demand must be accompanied by ''green'' synthesis procedures. There is a constant interest in the synthesis of noble metal nanoparticles for their applications such as electronics (Ravel et al., 2002), optics (Zhang et al., 2006), and environmental biotechnology (Gardea-Torresdey et al., 2002, 2003; Chandran et al., 2006). In environmental sustenance, there is a need to develop the environmental friendly procedures to avoid the toxic chemicals in the synthesis protocols in order to avoid adverse effects in medical applications. From recent results, the researchers were inspired to develop benign nanoparticles using microorganisms, yeast and plant or plant extracts on biological systems that was termed as the ''green chemistry approach'' (Sinha et al., 2009). A group of researchers developed silver nanoparticles being extensively synthesized using various plant extracts (Shankar et al., 2003, 2004a,b; Satyavathi et al., 2010; Dubey et al., 2010; Song et al., 2009; Rajesh et al., 2009; Dwivedi and Gopal, 2010; Konwarh et al., 2011; Gils et al., 2010; Philip and Unni, 2011; Khalil et al., in press). *Pepper* is a medicinal plant, it is described as a drug which increases digestive power, improves appetite, cures cold, cough, dyspnoea, diseases of the throat, intermittent fever, colic, dysentery, worms and piles (Ravindran, 2001). So far, there has been no report on the synthesis of nanoparticles by using *Pepper* leaves extract. In the present investigation, we synthesized silver nanoparticles from the *Pepper* leaf extract and ascertained their characterization.

2. Material and methods

2.1. Preparation of ***Pepper*** leaf extract

AR grade silver nitrate ($AgNO_3$) was purchased from Sigma–Aldrich chemicals and fresh *Pepper* leaves were collected from the Sri Venkateswara University Campus, Tirupati, Andhra Pradesh, India. The fresh *Pepper* leaf extract used for the reduction of Ag^+ ions to Ag° was prepared by taking 20 g of thoroughly washed finely cut leaves in a 500 ml Erlenmeyer flask along with 100 ml of distilled water and then boiling the mixture for 2 min before decanting it. Further, the extract was filtered with Whatman No. 1 filter paper and stored at 4 °C and used for further experiments.

2.2. Synthesis of silver nanoparticles

In a typical experiment, the leaves extract various concentration levels (0.3–0.6 ml) was added to 10 ml of 1 mM $AgNO_3$ aqueous solution. The bioreduced aqueous component (0.5 ml) was used to measure UV–Vis spectra of the solution. The particle suspension was diluted to 1:10, with distilled water, to avoid the errors due to the high optical density of the solution.

3. Results and discussion

3.1. UV–Vis spectral analysis

The bioreduction of pure Ag^+ ions was monitored by a periodic sampling of the aliquots (0.5 ml) of the suspension, then diluting the samples with 5 ml deionized water and subsequently measuring UV–Vis spectra of the resulting diluents. UV–Vis spectroscopic analyses of the silver nanoparticles produced were carried out as a function of bioreduction at room temperature on UV–Vis spectrometer (Perkin-Elmer lambda 25). Reduction of silver ions was visually evident from the color change and was completed within 10 min with a stable brown color indicating the formation of the silver nanoparticles in aqueous solution as shown in Fig. 1. The progress of the reaction between metal ions and the leaf extracts were monitored by UV–Vis spectra of Ag nanoparticles in aqueous solution with different concentration reactions as shown in Fig. 2. The UV–Vis spectra showed the appearance of single and strong absorption peaks centered at 458 nm respectively. This band is called the surface plasmon resonance (SPR).

3.2. Transmission electron microscopy

The morphology and size of the silver nanoparticles were studied by the transmission electron microscopy (TEM) images, 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. The TEM image (Fig. 3) was employed so

Figure 1 Aqueous solution of 0.001 M $AgNO_3$ with *Pepper* leaf extract.

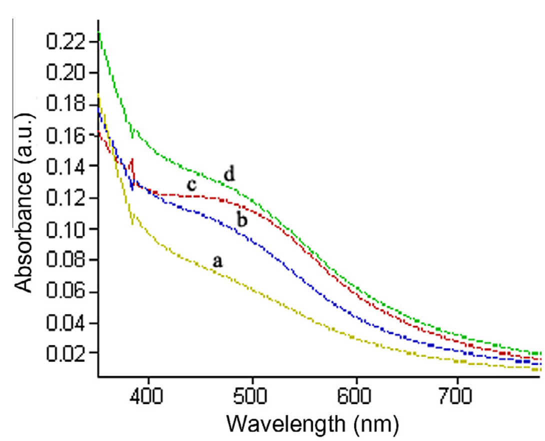

Figure 2 UV–Vis absorption spectra of silver nanoparticles by exposure of *Pepper* with 0.001 M silver nitrate. (a) 0.3 ml; (b) 0.4 ml; (c) 0.5 ml; (d) 0.6 ml.

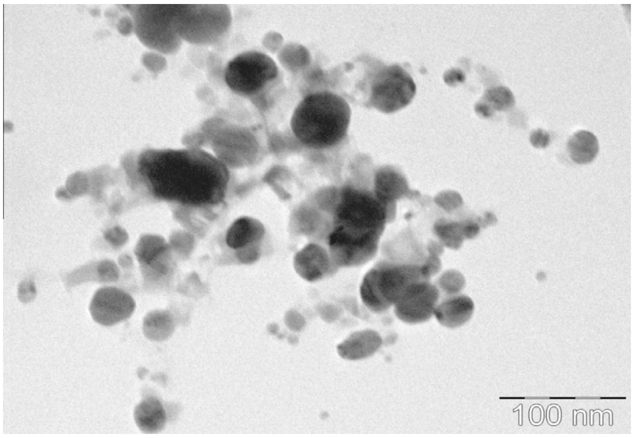

Figure 3 Transmission electron microscopy image of silver nanoparticles.

that the bio synthesized nanoparticles were in the size of 5–60 nm. It was spherical in shape and few nanoparticles were agglomerated. Under careful observation, it is evident that the silver nanoparticles are surrounded by a faint thin layer of other materials. The small-sized nanoparticles were able to easily penetrate across the membrane (Morones et al., 2005; Pal et al., 2007; Jaidev and Narasimha, 2010).

3.3. Field emission scanning electron microscopy

The morphology observations were carried out on a FE-SEM SUPRA-55, CARL ZEISS, and GERMANY instrument. The FE-SEM images of the silver nanoparticles are shown in Fig. 4. The morphology of the bio-reduced nanoparticles was spherical in shape. Bio-reduced silver nanoparticles were utilized to characterize the morphology, sizes and distribution in aqueous suspension and were prepared by dropping the suspension onto a clean glass plate and allowing water to completely evaporate. It was evident that the edges of silver nanoparticles are brighter than the center of the nanoparticles, suggesting the particles are encapsulated by biomolecules such as proteins in the *Pepper* leaf extract.

3.4. Energy dispersive X-ray spectroscopy (EDS)

The energy dispersive spectrum (Fig. 5) revealed the clear identification of the elemental composition profile of the synthesized

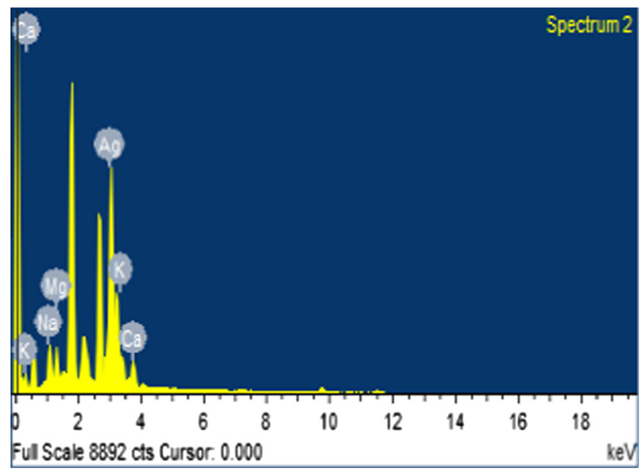

Figure 5 Energy dispersive X-ray spectrum of nanoparticles.

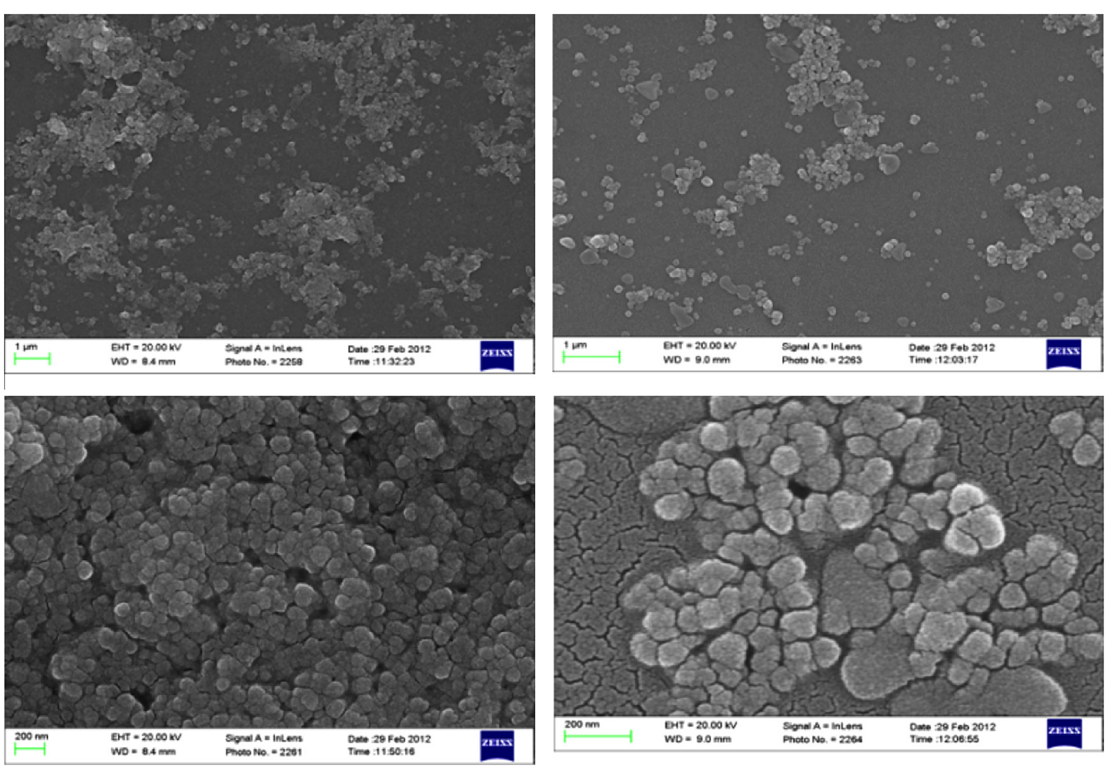

Figure 4 Field emission scanning electron microscopy images of bio-reduced silver nanoparticles.

nanoparticles, which suggests the presence of silver as the ingredient element. Metallic silver nanoparticles typically show an optical absorption peak at 3 keV due to the surface plasmon resonance (Kaviya et al., 2011). However, other elemental signals along with silver nanoparticles were also recorded, which were not observed for the biosynthesis of many other nanoparticles.

3.5. XRD analysis

An X-ray diffraction (XRD) measurement of a thin film prepared by aqueous solution was drop coated onto a glass slide and studied with XRD-SMART lab – Rikagu, JAPAN diffractometer. The diffraction pattern was recorded by Cu-Kα_1 radiation with λ of 1.54A° in the region of 2θ from 20° to 80° at 0.02°/min. and the time constant was 2 s. The size of the nanoparticles was calculated through the Scherer's equation. The crystalline nature of silver nanoparticles was studied with the aid of X-ray diffraction as shown in Fig. 6. A number of strong Bragg's diffracted peaks observed at 38.19°, 44.36°, 64.70° and 77.50° corresponding to the (1 1 1), (2 0 0), (2 2 0) and (3 1 1) facets of the face centered cubic lattice of silver were obtained. It suggests that the synthesized silver nanoparticles are biphase in nature. The slight shift in the peak positions indicated the presence of strain in the crystal structure which is a characteristic of nanocrystallites (Rajakumar and Abdul Rahuman, 2011). The broadening of the Bragg reflection peaks indicates the formation of nanoparticles. The average domain size of the silver nanoparticles was found to be 12.6 nm; and it was done by using the width of the (1 1 1) Bragg's reflection. In addition, yet some unassigned peaks were also observed suggesting the crystallization of bio-organic phase occurs on the surface of silver nanoparticles (Satyavathi et al., 2010).

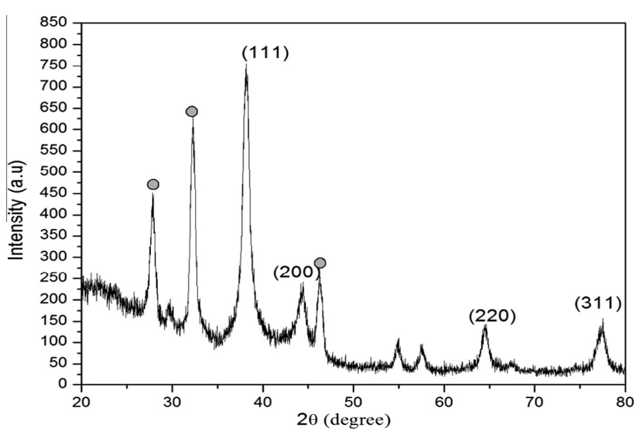

Figure 6 X-ray diffraction spectrum of stabilized silver nanoparticles.

3.6. Fourier transform infra-red spectroscopy

For Fourier transformed infrared (FTIR) measurements, the bio reduced Ag^+ ion aqueous component was centrifuged at 10,000 rpm for 20 min. The dried sample was ground with KBr pellets and analyzed on a Thermo Nicolet Nexus 670 IR spectrometer which was operated at a resolution of 4 cm^{-1} in the region of 4000–400 cm^{-1}. The FTIR spectrum of synthesized silver nanoparticles by using *Pepper* leaf extract is shown in Fig. 7. It is confirmed the fact that to identify the biomolecules for reduction and efficient stabilization of the metal nanoparticles, the band at 3421 cm^{-1} corresponds to O–H, as also the H-bonded alcohols and phenols. The peak at 2923 cm^{-1} indicates carboxylic acid. The band at 1629 cm^{-1} states primary amines. The band at 1431 cm^{-1} corresponds to C–C stretching aromatics, while the peak at 1379 cm^{-1} states C–H rock alkenes and bands at 1162 and 1058 cm^{-1} indicate the presence of C–O stretching alcohols, carboxylic acids, esters and ethers. An immediate reduction of silver ions in the present investigation might have resulted due to water soluble phytochemicals like flavones, quinones, and organic acids present in the leaf *Pepper*, silver reduction and fabrication accomplished due to phytochemicals (flavonoids or other polyphenols), some proteins and metabolites such as terpenoids

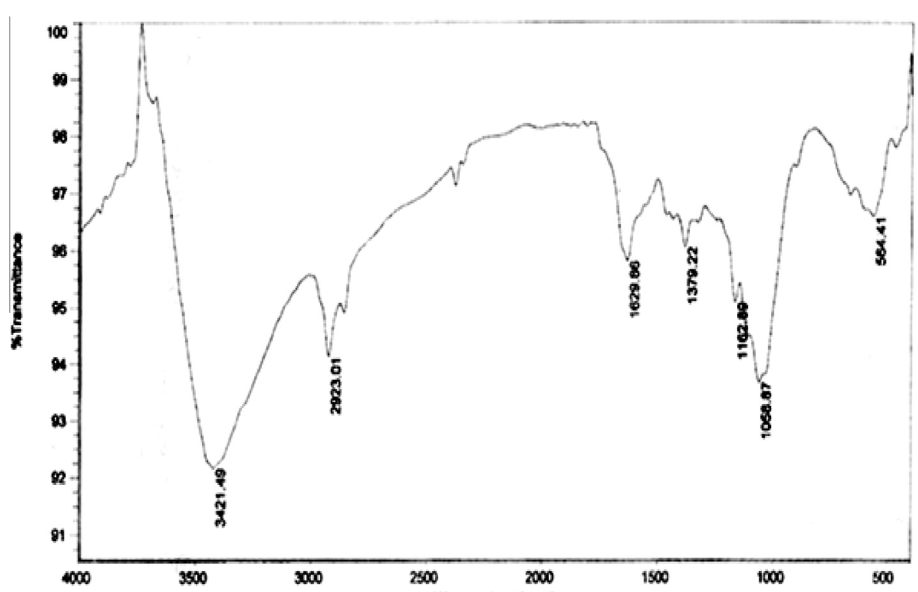

Figure 7 FTIR spectra of the silver nanoparticles synthesized by the reduction of silver nitrate with the *Pepper* leaf extract.

having functional groups of alcohols, ketones, aldehydes and carboxylic acids present in *Pepper* leaves may be considered as a significant advance in this direction.

4. Conclusions

In conclusion, silver nanoparticles have been synthesized from the *Pepper* leaf extract. Structural analysis by XRD together with the chemical composition by EDS, strongly suggests the formation of elemental silver nanoparticles instead of their oxides. From the TEM analysis, the average sizes of the nanoparticles are found to be 5–60 nm. FTIR and FE-SEM measurements provided strong evidence for proteins to form a coat covering the silver nanoparticles to stabilize and prevent the agglomeration of the particles. This simple procedure for the bio synthesis of silver nanoparticles has several advantages such as cost-effectiveness, compatibility and eco-friendliness for biomedical and pharmaceutical applications. In addition, the eco-friendly method will be a competitive alternative to the existing methods for producing nano scale inorganic materials.

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