Aparna Mani. *et al*. / *International Journal of Biological & Pharmaceutical Research.* 2012; 3(4): 631-633.

e- ISSN 0976 - 3651
Print ISSN 2229 - 7480

International Journal of Biological & Pharmaceutical Research
Journal homepage: www.ijbpr.com

IJBPR

BIO-MIMETIC SYNTHESIS OF SILVER NANOPARTICLES AND EVALUATION OF ITS FREE RADICAL SCAVENGING ACTIVITY

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ABSTRACT

Nanocrystalline silver particles have found tremendous applications in the field of diagnostics and therapeutics. In recent years plant mediated biological synthesis of nanoparticles is gaining importance due to its simplicity and eco-friendliness. In this study, the biosynthesis of silver nanoparticles was carried out using *Piper nigrum* fruit extract as reducing agent. Quantification of silver nanoparticle synthesis was done using U-V Spectrophotometer. The synthesized silver nanoparticles were characterized with Scanning electron microscopy (SEM). The *in vitro* antioxidant properties of the biosynthesized silver nanoparticles have been evaluated using the DPPH assay, and these nanoparticles were found to have significant antioxidant capacity and thus can be used as potential radical scavenger against deleterious damages caused by the free radicals.

Key Words: *Piper nigrum* fruit extract, Nanocrystalline silver particles.

INTRODUCTION

Nanotechnology finds extensive applications in nanomedicine, an emerging new field. Nanotechnology is mainly concerned with synthesis of nanoparticles of variable sizes, shapes, chemical composition and controlled dispersity and their potential use for human benefits. Nanoparticles can be synthesised by chemical and physical methods but these methods are quite expensive and toxic (Putheti *et al*., 2008). Use of biological organisms, plant extracts could be an alternative method for production of nanoparticles (Wang *et al*., 2008).

In recent years plant mediated biological synthesis of nano particles is gaining importance due to its simplicity and eco-friendliness (Farooqui *et al*., 2010). Several plants serve as potential biological materials for the synthesis of nanoparticles (Kaushik Thakkar *et al*., 2010). Among the various inorganic metal nanoparticles, silver (Ag) nanoparticles have received substantial attention for various reasons (Song *et al*., 2009). Silver is an effective antimicrobial agent, exhibits low toxicity and has diverse *in vitro* and *in vivo* applications (Jain *et al*., 2009). Black pepper (*Piper nigrum*) is a flowering vine in the family Piperaceae, cultivated for its fruit, which is usually dried and used as a spice and seasoning. Black peppers are native to India and are extensively cultivated here and elsewhere in tropical regions. Pepper gets its spicy heat mostly from the piperine compound, which is found both in the outer fruit and in the seed. Black pepper contains between 4.6% and 9.7% piperine by mass.

Black Pepper was believed to cure illness such as constipation, diarrhoea, earache, gangrene, heart disease, hernia, hoarseness, indigestion, insect bites, insomnia, joint pain, liver problems, lung disease, oral abscesses, sunburn, tooth decay, and toothaches (Karthikeyan and Rani, 2003). Piperine present in black pepper acts as a thermogenic compound and enhances the thermogenesis of lipid and

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accelerates energy metabolism in the body and also increases the serotonin and beta-endorphin production in the brain (Wu *et al*., 2003). Piperine and other components from black pepper may also be helpful in treating vitiligo (Zhixiu Lin *et al*., 2007), various bioactivity studies describe that *Piper nigrum* was a potent medicinal plant and established its analgesic, antiseptic, antispasmodic, anti-inflammatory, larvicidal and anti-oxidant properties (Gulcin, 2005). In this present study, silver nanoparticles were synthesized using the unripe fruits of *Piper nigrum* , and the free radical scavenging property of the synthesized silver nanoparticles was evaluated.

MATERIALS AND METHODS

Chemicals

DPPH (1, 1-diphenyl, 2-picrylhydrazyl), TCA (trichloroacetic acid) and ferric chloride were obtained from Sigma Chemical Co. CRC,bangalore. Ascorbic acid was obtained from SD Fine Chem. Ltd., Biosar, India.

Preparation of extract

10gm of *Piper nigrum* fruits were washed thoroughly with distilled water and air dried. The fruits were powdered using a hand grinding mill. The dried powder was boiled in 50 ml of water for 10min and cooled at room temperature. The supernatant was then filtered using a Whatman filter paper1. The pH was adjusted to 12 by adding 1% NaOH.

Phyto-assisted synthesis of silver nanoparticles

To 100 ml of 1mM $AgNO_3$ solution, 1 ml of the extract was added drop by drop and the mixture was kept in a magnetic stirrer for 2 hrs. The mixture was then centrifuged at 3000 rpm for 10 min and the residue was collected and used for characterization studies.

DPPH free radical scavenging activity (Ebrahimzadeh *et al*., 1995)

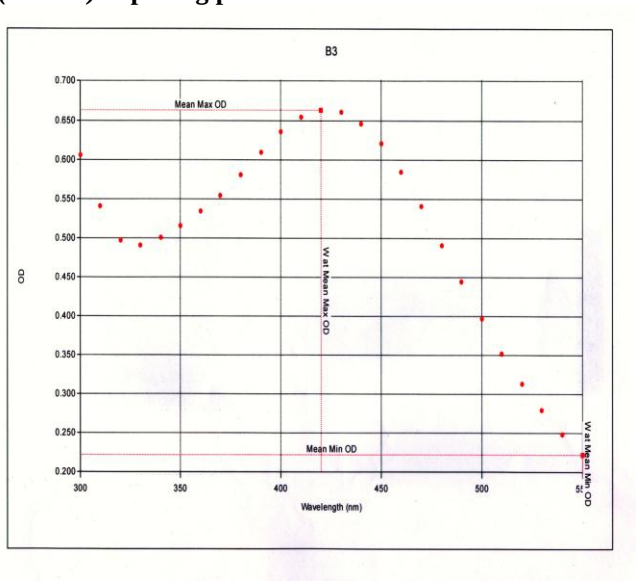
The free radical scavenging capacity of the extract and synthesised silver nanoparticles was determined using DPPH (Pham-Huy *et al*., 2008). DPPH solution (0.004% w/v) was prepared in 95% methanol. Sample was mixed with 95 % methanol to prepare the stock solution (5 mg/ml). Freshly prepared DPPH solution (0.004% w/v) was taken in test tubes and synthesised silver nanoparticles *(PnSNP)* was added in serial dilutions (20 µg to 120 µg) to every test tube so that the final volume was 3 ml and after 10 min, the absorbance was read at 515 nm using a spectrophotometer (HACH 4000 DU UV – visible spectrophotometer). Ascorbic acid was used as a reference standard and dissolve in distilled water to make the stock solution with the same concentration (5 mg/ml). Control sample was prepared containing the same volume. 95 %methanol served as blank. % scavenging of the DPPH free radical was measured. IC50 values were obtained by probit analysis (Viturro *et al*., 1999).

RESULTS AND DISCUSSION

UV- Vis- Spectroscopy

The reduction of silver metal ions to silver nanoparticles was preliminarily analysed using UV-Vis Spectrophotometer between 300-700nm. This analysis showed an absorbance peak at 420 nm which was specific for Ag nanoparticles.

Figure 1. UV-Visible absorption spectra of biosynthesized silver Nanoparticle from *Piper nigrum (PnSNP)* depicting peak at 420 nm



Scanning Electron Microscope

The synthesised nanoparticles morphology were characterised by scanning electron microscopy. The silver nanoparticles formed were predominantly spherical with uniform shape. The SEM image exposed that the formed nanoparticles were within the size range of 40- 60nm.

Figure 2. The SEM images of silver Nanoparticle synthesized from *Piper nigrum* fruit extract

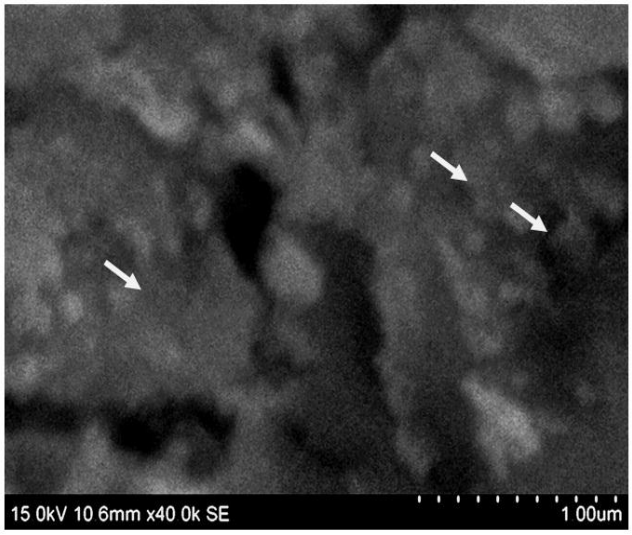
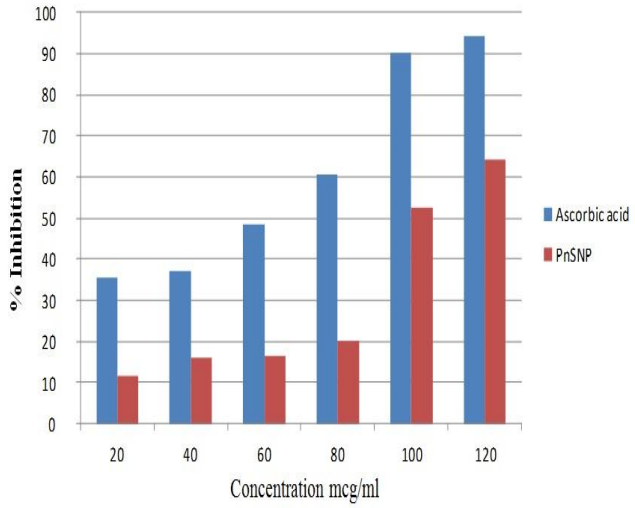


Figure 3. DPPH free radical scavenging assay of PnSNP and AA (Ascorbic acid)



DPPH free radical scavenging activity

The DPPH free radical scavenging assay showed potent inhibitory capacity of synthesised silver nanoparticles (PnSNP) when compared with ascorbic acid (AA)at higher concentrations. The percentage of inhibition of free radicals increased with increase in concentration of substrates. The IC 50 value was 183.24 ± 0.93mcg/ml. These results suggest that at concentartions above 120 mcg/ml, the synthesised AgNPs may serve as potent antioxidants.

CONCLUSION

From this study, the free radical scavenging property as measured by DPPH method showed that percentage of inhibition increases with increasing concentrations of synthesised silver nanoparticles. Thus the synthesised silver nanoparticles could play the role of a neoadjuvant antioxidant offering effective protection from free radicals in a wide range of conditions.

ACKNOWLEDGEMENTS

Authors gratefully acknowledge the TSS sponsored programme, Indian Council of Medical Research, New Delhi, India for funding the research development (No. PhD (Integrated)/-17-F.T./2011) to carry out this work.

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