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J. Nat. Prod. Plant Resour., 2013, 3 (5):36-40
(http://scholarsresearchlibrary.com/archive.html)

ISSN : 2231 – 3184
CODEN (USA): JNPPB7

A facile method for green synthesis of stabilized silver nanoparticles and its *in vitro* antagonistic applications

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ABSTRACT

A facile method for synthesis of biologically stabilized silver nanoparticles was developed using the weed Parthenium hysterophorus L. The bio fabricated nanoparticles were characterized by UV-Vis spectrophotometry, X-Ray Diffraction and FTIR. The nanoparticles demonstrated potent antibacterial and antifungal activity against Staphylococcus aureus, Bacillus subtilis, Aspergillus niger and Escherichia coli. The silver nanoparticles synthesized thus demonstrated antagonistic activity against bacteria and fungi and hence the nanoparticles possess potential applications in medicine and pharmaceutical fields.

Key words: Green synthesis, Silver nanoparticles, antagonistic activity.

INTRODUCTION

Nanotechnology is an emerging field which deals with synthesis of nanoparticles and also their applications. Metallic nanoparticles like gold and silver nanoparticles have various applications in fields of medicine, defense, drug synthesis etc. These nanoparticles find applications also in fields dealing with optics and magnetism due to their unique properties. Nanoparticles synthesized by biological means (from plants or microbes) find potent applications as anti bacterial and anti fungal agents. Synthesis of silver nanoparticles from *Euphorbia hirta* L [1] and ginger [2] have already been reported. As synthesized from plants, these nanoparticles seem to incorporate the plant peptides as capping agents as mentioned by Devendra Jain *et a*l. [3] during synthesis, which stabilizes the nanoparticles as well as enhances their antagonistic properties. In this study we test the antagonistic activity of silver nanoparticles against various gram positive and gram negative bacteria.

MATERIALS AND METHODS

1.1 Plant material

Parthenium hysterophorus was collected from Modern College campus, Shivajinagar Pune-05. Healthy leaves of *Parthenium hysterophorus* were collected and authenticated by Botany Department, Modern College, Shivajinagar,

Pune-05. The leaves were thoroughly washed with autoclaved distilled water and cut into small pieces and crushed to make 10gm/L aqueous extract. This extract was filtered and stored at 4^{o}C until further use.

1.2 Chemicals

Silver nitrate ($AgNO_3$) was purchased from Fisher Scientific; USA. Nutrient agar was purchased from Himedia, India. Autoclaved distilled water was used throughout the experiment.

1.3 Synthesis of nanoparticles

A modified modification of the method described by Parashar *et al*. (2009) was used for synthesis of nanoparticles. 10ml of freshly prepared 10mM $AgNO_3$ solution was added to 10ml of fresh plant extract. The reaction mixture was incubated for 30min or till color change to dark brown was observed. The nanoparticles were then synthesized by drying at 80°C.

1.4 Characterization of nanoparticles

The synthesized nanoparticles were characterized using UV-Vis Spectroscopy (Schimadzu UV -1600) over a range of 250-700 nm. The topography of the nanoparticles was studied by subjecting them to SEM (Scanning Electron Microscope) analysis. FTIR (Fourier Transform Infrared Spectroscopy) was performed to obtain wide spectrum of nanoparticles over narrow range. This method gives us information about plant peptides that have coated the particles during synthesis procedure. The XRD (X-Ray Diffraction Analysis) was performed to note the size of the obtained nanoparticles.

1.5 Antagonistic activity against bacteria and fungi

The antagonistic activity of silver nanoparticles was studied against bacteria and fungi by using the agar well diffusion method. Sterile nutrient agar plates were prepared and incubated to check overnight sterility. 0.1ml of test bacterial and fungal cultures was spread on the Nutrient agar plates. Using a cork borer 6mm wells were prepared on agar plates. To these wells 20µl of nanoparticles solution of (40mg/ml) concentration was added [4]. The plates were incubated at 37^{o}C overnight. After 24 h the zone of inhibition was measured.

RESULTS AND DISCUSSION

1.6 Synthesis and characterization of silver nanoparticles

Aqueous plant extract of *Parthenium hysterophorus* acts as a reducing agent as stated by Balaprasad [5] which reduces metallic silver to nanosilver and hence the color change was obtained (Fig.1).

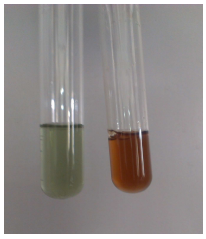

Fig.1- Extract of *Parthenium hysterophorus* showing color change after incubation with Silver nitrate solution

The synthesized nanoparticles was characterized using SEM, XRD, FTIR, EDS and UV Vis spectroscopy analysis. The reduction of silver ions to nanosilver was monitored and confirmed using UV spectra. After color change was obtained a small aliquot of sample was diluted with distilled water and subjected to UV analysis. The characteristic peak value for silver nanoparticles is between 400-570nm. (Fig. 2)

The spectrum of the sample was obtained for wavelength range from 270nm to 570nm. The λ_{max} of the nanoparticles was observed at 470nm. This is because of a phenomenon called Surface Plasmon Resonance (SPR) exhibited by silver nanoparticles. The silver nanoparticles oscillate when exposed to electromagnetic radiation and this oscillation gives a typical peak value. The SEM image of the nanoparticles represents the topography of the particles is shown in Fig.3.

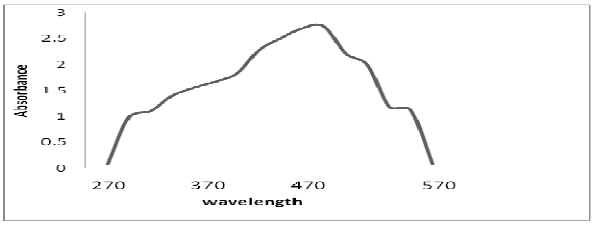

Fig.2-UV Vis spectra analysis of nanoparticles produced by extract of *P. hysterophorus* extract

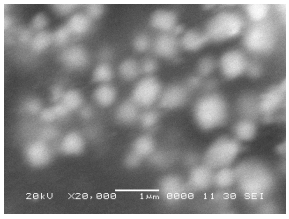

Fig.3- SEM of silver nanoparticles produced by extract of *P. hysterophorus*

The SEM image suggests the presence of roughly spherical silver. The incidence of X-rays on the powdered nanoparticles gives a particular pattern which helps to characterize the nanoparticles as shown in the XRD graph (Fig. 4).

Fig.4-XRD of silver nanoparticles produced by extract of *P. hysterophorus*

XRD (manual mode) was used to characterize the AgNp. The 2d angle is converted into the diameter using Scherrer formula. The size of silver nanoparticles synthesized by green synthesis was found to be around 20- 50 nm [4]. FTIR analysis is used to confirm the presence of the plant peptides visible due to the bending produced by amide bonds (Fig.5).

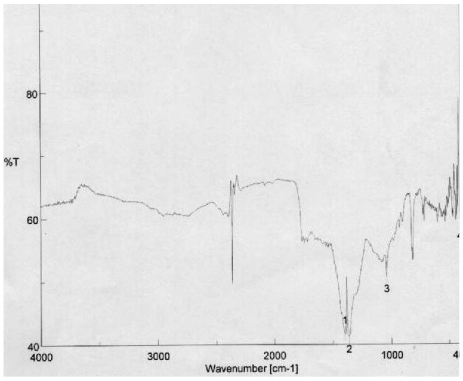

Fig.5 FTIR spectra of nanoparticles produced by extract of *P. hysterophorus*

The FTIR image clearly indicates the presence of proteins as evidenced by the above figure 5.
Synthesis of metallic nanoparticles using green synthesis have been reported earlier [5-11], however we have emphasized the antagonistic application of the nanoparticles stabilized by plant proteins .

2.2 Antagonistic activity against fungi and bacteria

The antimicrobial and antifungal activity of silver nanoparticles were studied against gram positive as well as gram negative organisms such as *Staphylococcus aureus*, *Escherichia coli*, *Bacillus subtilis* and antifungal activity was studied using *Aspergillus niger* .

The zones of inhibition showed by these bacteria and fungi is represented in table 1

Table 1: Antagonistic Action of silver nanoparticles against bacteria and fungi

Organism	Zone Of Inhibition (mm)
Escherichia coli	**9**
Staphylococcus aureus	**10**
Bacillus subtilis	**12**
Aspergillus niger	**10**

Green synthesis of silver nanoparticles using weeds has been reported previously [10]. We have produced silver nanoparticles by the method described by Parashar *et al.* (2009). The antimicrobial activity against gram positive and negative bacteria as well as fungi was studied. The phytochemicals and peptides act as stabilizing agents for the nanoparticles and may enhance the antagonistic activity.

CONCLUSION

In this present study the synthesis of silver nanoparticles was synthesized by biological method using *P. hysterophorus* L leaf extract which acts as a reducing agent to reduce silver metal to nanosize. The synthesized silver nanoparticles were subjected to analysis such as SEM, UV Vis Spectroscopy, XRD and FTIR in order to characterize them. The antagonistic activity of silver nanoparticles was studied and they showed effective activity against gram negative, gram positive bacteria as well as against fungi. Thus this study proves to be an effective and economical method to produce silver nanoparticles that show in vitro antagonistic action.

Acknowledgements
We thank Board of College and University Development (BCUD), University of Pune for financial support. We are grateful to the Principal, Modern College for providing facilities for the research work. We thank Physics Department, University of Pune for SEM and XRD of samples.

REFERENCES

[1] Elumalai,E ;Prasad, T; Kambala,V ; Nagajyothi,P ; David,E. *Archives of Applied Science Research.* **2010**, 2 (6),76-81.
[2] Singh,C; Sharma,V; Naik,P; Vikas, K; Harvinder, S. *Digest Journal of Nanomaterials and Biostructures*. **2011**, 6,(2), 535-542.
[3] Jain, D; Daima,H; Kacchwaha ,S; Kothari, S. *Digest Journal of Nanomaterials and Biostructures*. **2009**, (4) ,4, 723-727
[4] Thombre ,R; Mehta, S; Mohite,J; Jainsinghani,P; *International journal of Pharma and Biosciences*.**2013**,4(1), 184-192.
[5] Balaprasad Ankamwar. *e-Journal of Chemistry*. **2010**,7(4), 1334-1339
[6] Jae Yong Song ; Beom Soo Kim. *Bioprocess Biosyst Eng*.**2009**, 32,79–84.
[7] Ganesh Kumar, V; Inbakandan, D; Radhika Rajasree, S.R ; Stanley Abraham, L; Manoharan, N; Govindaraju, K; Singaravelu, G. *International Journal of Biological Technology*. **2010**,1(1),75-77
[8] Thirumurugan,A; Tomy,N; Kumar,H; Prakash,P. *International Journal of Nanomaterials and Biostructures* **2011**,1(2), 22-24
[9] Lal,S; Nayak,P. *International journal of science innovations and discoveries*. **2012**, 2, (3) 3,325-350.
[10] Parashar,V; Parashar,R; Sharma,B; Pandey**,**A. *Digest Journal of Nanomaterials and Biostructures*.**2009**,4, No.1, 45 – 50
[11] Amir Tabrizi; Fatma Ayhan; Hakan Ayhan. *Hacettepe journal of biology and chemistry*. **2009**,37 (3), 217-226.