International Journal of Basic and Applied Biology
p-ISSN: 2394-5820, e-ISSN: 2349-2539, Volume 5, Issue 2; April-June, 2018, pp. 109-113

http://www.krishisanskriti.org/Publication.html

Antibacterial Potency of Green Synthesized Silver Nanoparticles

Anshu Singh[1] and J.N. Shrivastava[2]

[1]Department of Botany, Dayalbagh Educational Institute, Dayalbagh, Agra- 282005
[2]Department of Botany, Dayalbagh Educational Institute, Dayalbagh, Agra- 282005
E-mail: [1]ambitious.57@rediffmail.com, [2]janendra.srivastava@gmail.com

Abstract—*In few recent years, the field of nanotechnology has aroused a huge interest as it is found to show a great impact on distinct areas including agriculture, electronics, chemicals and medicine. Metal nanoparticles, especially Silver nanoparticles synthesized using green materials has been a growing concern in past few days as it is a rapid, low-cost, eco-friendly and single-step method. In this concern, this study investigates green synthesis of AgNPs from silver nitrate using Cucurbitapepo L. seed extract as a novel bio-resource of cost effective non-hazardous reducing and stabilizing compounds. Different concentrations of silver nitrate (1, 2 & 3 mM) were prepared and equal amount of C.pepo L. seed extract were added to each molar concentration solution of above prepared silver nitrate solutions. The formation of silver nanoparticles was screened by UV-Vis Spectroscopy, XRD and FTIR, and then subjected to antibacterial assay against selected pathogens. The results showedstrong potential of C.pepo seed extract with great antibacterial effectivity against the targeted pathogens.*

1. INTRODUCTION

Nanobiotechnology is establishing itself as a fast growing field employing several microorganisms, such as bacteria, fungi and yeast coming up as nanofactories synthesizing different metal nanoparticles. However, fabrication of nanoparticles using plants is gaining its importance because of its rapid, economical, eco-friendly protocol, and it provides a single step technique for the biosynthesis process (Huang *et al*. 2007). Biological approaches using microorganisms and plants or plant extracts for metal nanoparticle synthesis have been suggested as valuable alternatives to chemical methods. Most commonly silver nanoparticles are being synthesized using plant extracts. Researchers are focusing mainly on biological applications of nanosilvermaterials.Inrecent times, efficient nanosilver materials are progressively used in diverse fields of biotechnology and medicine; having major focus of researchers over their biological applications. Different types of nanoparticles known to available but silver nanoparticles have proved to be most effective as it has good antimicrobial efficacy. Plants containing a variety of secondary metabolites are responsible for the reduction of silver nitrate to silver ions thus leading to the production of silver nanoparticles. A number of plant parts including root, leaf, stem, bark, fruit, seed etc. have been used for silver nanoparticle synthesis. Some of the workers are reported to carry out the synthesis of silver nanoparticles using various plants extract including use of Alfalfa leaves (Gardea et al. 2002), Geranium leaves (Shankaret al. 2003), Aloe vera leaves (Chandran et al. 2006), Cinnamomum camphora leaves (Huang et al. 2007), Persimmon leaves (Song et al. 2008), Magnolia leaves (Song et al. 2009), Cycas leaves (Jha et al. 2010), Holarrhenaanti dysenterica leaves (Song et al. 2011), Olea europea seeds (Khadri et. al 2013), Brassica niger seeds (Manisha et al. 2014), Benincasa hispida seeds (Singh et al. 2013), Cucurbita pepo leaves (Gonelli et al. 2015), Jatropha curcas seeds (Bose et al. 2012) etc.

In this paper *C. pepo* seeds have been targeted for green synthesis of silver nanoparticles since seeds of *C. pepo*are potent in acting as nano-medicine due to the presence of various phytochemicals or secondary metabolites which act as reducing and capping agent. With the increasing use of antibiotics in the present era, microorganisms are gaining resistance against the antibiotics employed for targeted diseases thus leading to multi-drug resistance against a wide range of bacteria so there is a great need of replacing the use of those antibiotics with the natural products with almost negligible chances of side effects and drug resistance gaining property by the micro-organisms. Thus silver nanoparticles synthesized using *C. pepo* seed extract were further used as nano-drug against some selected pathogens.

2. MATERIALS AND METHODOLOGY

2.1. Extraction of plant material

The fresh and healthy seeds of *C. pepo* were collected from the local market of Dayalbagh, Agra. Seeds were then completely dried in shed and ground to powder form. *C. pepo* seeds powder extract was prepared by boiling a mixture of 2 g of dried seeds powder into 50 ml of sterile distill water in an Erlenmeyer flask for 30 minutes in water bath and then filtered through Whatman filter paper No. 1 (pore size 125 mm). Further the solution was centrifuged at 10,000 rpm for 10 minutes to obtain the pure plant extract and stored at 4˚C. It was then used within a week after its preparation.

2.1.1 Synthesis of silver nanoparticles

C. pepo seeds powder extract was prepared by boiling a mixture of 2 g of dried seeds powder into 50 ml of sterile distill water in an Erlenmeyer flask for 30 minutes in water bath and then filtered through Whatman filter paper No. 1 (pore size 125 mm). Further the solution was centrifuged at 10,000 rpm for 10 minutes to obtain the pure plant extract and stored at 4˚C. It was then used within a week after its preparation. 1 mM, 2 mM and 3 mM aqueous solutions of silver nitrate were prepared respectively for the silver nanoparticles synthesis. 1:10 ratio of plant extract with silver nitrate solution was prepared by adding 2.5 ml of plant extract with 25 ml of each of the three concentrations of silver nitrate solutions in separate flasks for bioreduction of Ag+ ions in the solution and kept at room temperature for 24 hours.

2.2. Characterization

2.2.1. UV-Visible spectroscopy

The bioreduction of pure Ag+ ions in aqueous solution was monitored after 24 hours of by diluting a small aliquot of the sample into distilled water. UV-Vis spectral analysis of silver was done at room temperature on UV-Vis double beam spectrophotometer Thermo Systeronics- 2201.

2.2.2 XRD measurement

About 10-12 drops of silver nanoparticle solution obtained were oven dried on a clean slide at increasing temperature for about 4-5 hours. The dried drops were subjected to XRD analysis foranalyzing their structure and composition. The crystalline domain size was calculated from the width of XRD peaks using Debye Scherer'sequation.

Debye Scherer's equation

$d = 0.9\lambda / \beta \cos\theta$

Where, $\beta = \sqrt{\beta M^2 - \beta S^2}$,

βM = FWHM (FWHM = Full Width Half Maximum)

$\beta S = 0.025$, $\lambda = 1.540598$ A˚, $\cos\theta = \theta$ of maximum intensity peak / 2

2.2.3. FTIR analysis

FTIR analysis was used to understand the existence of surface functional groups in metallic interactions. Different functional groups absorb characteristic frequencies of IR radiation. Thus, IR spectroscopy is an important and popular tool for structural elucidation and compound identification. Our observation confirms the presence of such compounds in the sample which coat covering the silver nanoparticles known as capping agents.

3. ANTIBACTERIAL ASSAYS

AgNPs synthesized from *C. pepo* seeds extract were used for evaluation of antibacterial effect on *Bacillus subtilis*, *Bacillus fusiformis* and *Pseudomonas aeruginosa*by paper disc diffusion method (Mukherjee *et al.* 1995). AgNPs solution was further diluted to five and ten times respectively and also the combination of AgNPs and antibiotic (Chloramphenicol) was prepared by adding 0.5 ml each of AgNPs solution and antibiotic solution (from 1mM chloramphenicol solution in 25 ml distill water). The bacterial culture was spreaded evenly on the nutrient agar plate using sterile glass rods. Five sterile discs were dipped with each of silver nanoparticles solution, silver nitrate solution, pure plant extract, AgNPs+Chloramphenicol solution and standard antibiotic chloramphenicol respectively were separately placed on the agar plate and for each bacteria experiment was set up in duplicate to obtain more accurate results.After incubation at 37 ℃ for 24 hr, the diameter of inhibition zones around AgNPs were measured and compared with the diameter of inhibition zone around commercial standard antibiotic chloramphenicol, AgNPs+Chloramphenicol solution, silver nitrate solution and pure plant extract.

4. RESULTS AND DISCUSSION

4.1 Visual observations and UV-Visible spectroscopy

The color of $AgNO_3$ solution and seed exudate of *C. pepo* reaction was changed from yellow to brown color after an hour of incubation. The formation and stability of silver nanoparticles formed from the plant extract was confirmed by this technique (Bankar *et al.* 2010). The change in color was observed due to the absorption of light by the AgNPs solution in the UV range of 400-500 nm.The UV–Vis spectra of silver nanoparticles formation using a constant AgNPs concentration (1×10^{-3} M) with different concentrations (1mM, 2mM and 3mM) of the pure $AgNO_3$ solution showed the sharp peak at 439 nm. Here,the sharp peak indicated the presence of particles with a broad size distribution. The absorption of UV light by the silver nanoparticles solution is due to a phenomenon called surface plasmon resonance. UV-vis absorption spectrum of silver nanoparticles in the presence of *C. pepo* seedextract is shown in Fig. 1. The absorption peak is centered around 400–500 nm for *C. pepo* synthesized nanoparticles.

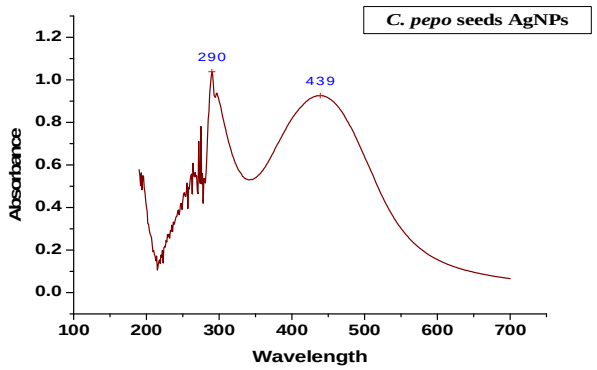

Fig. 1: UV-visible absorption spectra of silver nanoparticles synthesized by incubating 1mM silver nitrate solution with *C. pepo* seed extract and incubated at room temperature

4.2 XRD

XRD analysis was performed with the liquid state solution of AgNPs itself. XRD data showed diffraction peaks at 2h= 32.2, 38,42.4, 46.3, 54.65, 57.7 and can be indexed to (111), (200), (220), (311), (222) and (400) planes of pure silver ions indicating the biosynthesis of silver nanoparticles. Figure 2 reveals several intense peaks in the whole spectrum of 2θ value ranging from 30° to 70°, corresponding tothe diffraction facets of silver.

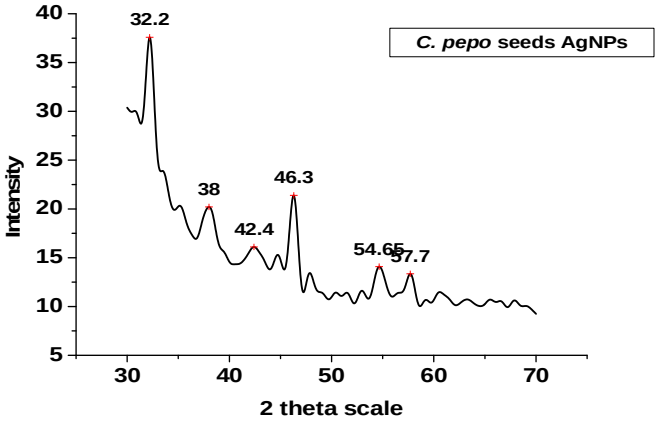

Fig. 2: XRD analysis graph of *C.pepo*AgNPs showing the peaks of silver

4.3 FTIR study

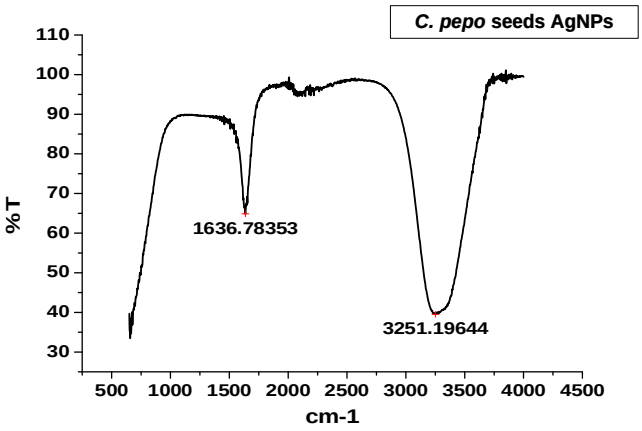

Fig. 3: FTIR spectra of *C.pepo* seeds powder extract silver nanoparticles

Our observation confirms the presence of such compounds in the sample which coat covering the silvernanoparticles known as capping agents. The FTIR (Perkin-Elmer FTIR-1600, USA) analysis in the range of 500-4500 cm^{-1} was performed to determine the presence of capping agent and role of molecules involved in the synthesis of AgNPs. FTIR analysis of AgNPs from seeds of *C.pepo*has been shown on figure 3. FTIR showed the peaks at 1636.78 cm^{-1}that corresponds to strong aromatic groups and is due to the presence of C=C stretching that have medium bond strength and band at 3261.18 cm^{-1} is due to the presence of strong O-H bonds and it is the broadest band among all which correpsonds to the reduction of $AgNO_3$ into Ag, thus acting as the main capping agent in the production of silver nanoparticles.

4.4 Antibacterial susceptibility assay

The *C. pepo* seed extract was subjected to antibacterial assay against *Bacillus subtilis, Bacillus fusifomis*and *Pseudomonas aeruginosa*respectively. Antimicrobial activity of silver nanoparticles has also reported against *Vibrio cholerae*, *Proteus vulgaris, Staphylococcus aureus* and *Pseudomonas aeruginosa* (Prabhu *et al.* 2010).In the present investigation, silver nanoparticles have exhibited antibacterial activity against *Bacillus subtilis* and *Bacillus fusifomis.* Both of the pathogens were found to be susceptible for antibiotic and AgNPs+Chloramphenicol. Plant extract, $AgNO_3$ and AgNPs alone were found ineffective against both of the pathogens. *C.pepo* seed AgNPs were found effective in combination with the antibiotic only. The zones of the inhibitions were measured in mm.

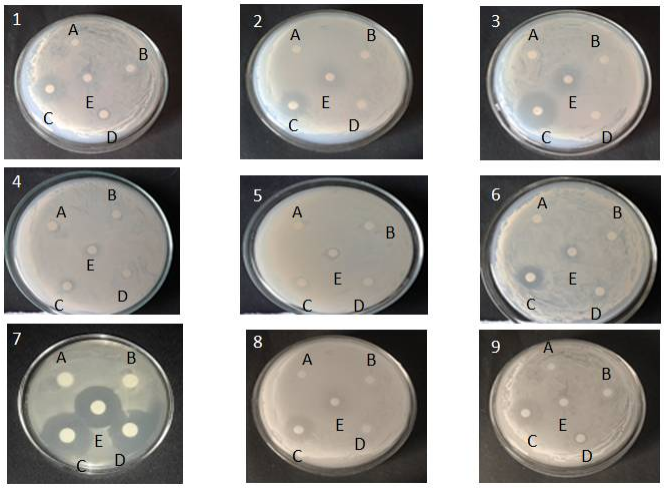

Fig. 3: Antibacterial activity of synthesized silver nanoparticles from *C. pepo* seeds extract by paper disc diffusion assay (1), (2) and (3) are of *Bacillus subtilis*, (4), (5), (6) are of *Bacillus fusiformis and* (7), (8), (9) are of *Pseudomonas aeroginosa*. A= Plant seed extract, B= $AgNO_3$, C= Chloramphenicol, D= *C. pepo* AgNPs and E= AgNPs+Chloramphenicol.

Table 1: Zones of inhibition (in mm) of *Bacillus subtilis* marked by *C. pepo* synthesized silver nanoparticles

Zone of inhibition (in mm) with different dilutions of AgNPs solution giving Mean +S.D values			
Sterile discs with different solutions	**Without diluted**	**1:5 times diluted**	**1:10 times diluted**
A	-	-	-
B	-	-	-
C	8.5+0.57	8.75+1.5	12.5+1.29
D	4+0	-	-
E	6.5+0.57	4.5+0.57	7.5+1

Table 2: Zones of inhibition (in mm) of *Bacillus fusiformis* marked by *C. pepo* synthesized silver nanoparticles

Zone of inhibition (in mm) with different dilutions of AgNPs solution giving Mean+S.D values			
Sterile discs with different solutions	**Without diluted**	**1:5 times diluted**	**1:10 times diluted**
A	-	-	-
B	-	-	-
C	5+0	7.25+0.5	8.5+0.57
D	3.75+0.5	-	-
E	6.5+0.57	7+0	4.25+0.5

Table 3: Zones of inhibition (in mm) of *Pseudomonas aeruginosa* marked by *C.pepo* synthesized silver nanoparticles

Zone of inhibition (in mm) with different dilutions of AgNPs solution giving Mean+S.D values			
Sterile discs with different solutions	**Without diluted**	**1:5 times diluted**	**1:10 times diluted**
A	-	-	-
B	0	-	-
C	20+2	11.66+0.57	15.33+1.52
D	19+1	16+1	11.66+0.57
E	20.66+2.08	13+2	13+1

Where A= Plant seed extract, B= $AgNO_3$, C= Chloramphenicol, D= *C. pepo* AgNPs and E= AgNPs+Chloramphenicol.

The antibiotic sensitivity testing of *C. pepo* seeds extract mediated AgNPs with*P. aeruginosa* showed the maximum inhibition zone of 20.66$\pm$2.08 by combination of AgNPs andChloramphenicol solution while *B. fusiformis* showed minimum inhibition zone of 3.75$\pm$0.5 in PureAgNPs solution i.e. without diluting the solution. Thus it can be concluded that *C. pepo* seeds AgNPs can proved to be a potential nano-drug against the selected bacterial species and even the side effects of antibiotic can be minimized upto a large extent by using the combination of AgNPs and Antibiotic.

REFERENCES

1. Bankara A, Joshi B, Ravi Kumara A, Zinjardea S. 2010. Banana peel extract mediated novel route for the synthesis of silver nanoparticles**.** *J Colloids Surf. A: Physicochem. Eng* 368: 58–63.
2. Bose Anjali, KehariaHaresh and Deshpande MP, 2012. Eco-friendly phyto-synthesis of silver nanoparticles using *Jatropha* seedcake extract. *Industrial Crops and Products.* 30(12).
3. Chandran, SP, Ahmad, A, Chaudhary, M, Pasricha, R, Sastry, M, 2006. Synthesis of gold nanotriangles and silver nanoparticles using *Aloe vera* plant extract. *Biotechnol. Prog*. **22** (2): 577–583.
4. Gardea, JL, Gombez, E, Parsons, JG, 2002. Formation and growth of Au nanoparticles inside live *Alfalfa* plants. *Nano Lett*. **2** (4): 397–401.
5. Gonnellia Cristina, Cacioppoa Federica, Giordanobc Cristiana, Capozzolib Laura, Salvaticibc Cristiana, Salvaticib Maria Cristina, ColziaIlaria, Bubbad Del Massimo, Ancillottid Claudia & Brett DW, 2006. A Discussion of Silver as an Antimicrobial Agent: Alleviating the Confusion. *Ostomy Wound Manage.***1**(52): 34-41.
6. Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, Wang H, Wang Y, Shao W, He N, Hong J and Chen C, 2007. Biosynthesis of silver and gold nanoparticles by novel sundried *Cinnamomumcamphora*leaf. *Nanotech.* **18**: 105104–105115.

7. Jha, AK, Prasad, K, 2010. Green synthesis of silver nanoparticles using *Cycas*leaf. *Int. J. Green Nanotechnol.Phys. Chem*. **1**:110–117.
8. KhadriHabeeb, Alzohairy Mohammad, JanardhanAvilala, Arthala Kumar Praveen, NarsimhaGolla, 2013.Green Synthesis of Silver Nano-particles with High Fungicidal Activity from Olive Seed Extract. *Advan. InNanopar*. **2:** 241-246.
9. Manisha DR, MeruguRamchander, PratapRudraMP, 2014. Bio-fabrication of antibacterial silver nanoparticles from black mustard seed powder and their characterization. *Inter. J. Nanomat. Biostruc*. **4**(3): 58-62.
10. Shankar, SS, Ahmad, A, Pasricha, R, Sastry, M, 2003. Bioreduction of chloroaurate ions by *Geranium* leaves and its endophytic fungus yields gold nanoparticles of different shapes. *J. Mater. Chem.***13**: 1822–1826.
11. Singh Kumar Pankaj and SubirKundu, 2013. Biosynthesis of elixir of life (gold nanoparticles) from plants. *J. Environ. Nanotechn*. **1**(2): 52-62.
12. Song, JY, Jang, HK, Kim, BS, 2009. Biological synthesis of gold nanoparticles using *Magnolia kobus*and*Diopyrus kaki* leaf extracts. *Process Biochem*. **44**: 1133–1138.
13. Song, JY, Kim, BS, 2008. Rapid biological synthesis of silver nanoparticles using plant leaf extracts. *Bioprocess Biosyst. Eng*. **44**: 1133–1138.
14. Yadav A, Rai, M, 2011. Bioreduction and mechanistic aspects involved in the synthesis of silver nanoparticles using *Holarrhenaantidysenterica.J. Bionanosci*. **5**: 70–73.
15. Mukherjee, PK, Balasubramanian P, Saha K, Saha BP and Pal M, 1995. Antibacterial efficiency of *Nelumbonucifera* (Nymphaeaceae) rhizomes extract. *Indian Drugs*; 32: 274-276.
16. Prabhu N, Divya TR, YamunaG (2010). Synthesis of silver phyto nanoparticles and their antibacterial efficacy. *Digest J. Nanomater. Biostruct.* **5(1)**: 185-189.