

In Vitro antibacterial studies of phytosynthesized silver nanoparticles using *Dianthus Caryophyllus* L. (Carnation)

Rachna^{1*}, Baljeet S. Saharan², Mahender S. Yadav¹, Nisha Sharma²

¹Department of Physics, Kurukshetra University, Kurukshetra, 136119, India

²Department of Microbiology, Kurukshetra University, Kurukshetra, 136119, India.

DOI: 10.5185/amp.2018/712

www.vbripress.com/amp

Abstract

A simple and efficient synthesis of silver nanoparticles (AgNPs) is reported here using red *Dianthus caryophyllus* flower, acting both as reducing and capping agent. The resultant silver colloids were characterized using UV-visible spectrophotometer, X-ray Diffractometer (XRD) and Transmission electron microscope (TEM). The surface absorption plasmon response and kinematics of reduction of silver ions were observed by UV-visible spectroscopy. The crystalline fcc structure of AgNPs was confirmed by its XRD pattern. Their morphological study was done with TEM, showing spherically shaped AgNPs in the range 10-20 nm. The antibacterial action was also studied using Agar well diffusion method against pathogenic bacteria cultures (*Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*). AgNPs showed better antimicrobial activity against *S. aureus* culture. Copyright © 2018 VBRI Press.

Keywords: Nanobiotechnology, green synthesis, *Dianthus caryophyllus*, silver nanoparticles, antibacterial activity.

Introduction

Nanotechnology is the basis of many researches with growing field of producing and utilizing metal nanoparticles in 21st century. Nanosized metals have been gaining more and more attention due to the unusually unique size dependent properties. Among these metal nanoparticles, silver nanoparticles (AgNPs) have a wide set of applications in optics, electronics, chemistry, medicine and many other biomedical fields [1, 2]. Many approaches are available for the synthesis of AgNPs like thermal reduction [3], electrochemical reduction [4], microwave assisted processes [5] and green synthesis [6]. The recent researches have shifted their focus from chemically toxic and physically expensive processes to their biological and eco friendly synthesis due to its cost effectiveness and easy methodology [7].

Over the past years, plants, fungi, bacteria and viruses are found efficient in the AgNPs production mechanism [8] but among them living plants are mostly preferred due to their vast availability and medicinal values. The fact is supported by many plants assisted synthesis process of AgNPs using *Aloe vera* [9], *curcuma longa* [10], green tea [11], henna leaf [12], stems of *Jatropha curcus* plants [13] etc. However the stability and morphology largely depends on the specificity and methodology of experimental process. Thus a specific methodology is needed to be followed, keeping its optimum conditions constant, in order to obtain desired shape and size AgNPs [14]. In present work AgNPs was

synthesized using *D. caryophyllus* flowers, which have various medicinal and therapeutic values. About 4 mM AgNO₃ and 2% (v/v) diluted aqueous flower extract (pH 9) under the incubation of 40 °C and 180 rpm for 24 hours. Appearance of deep yellow colour showed the presence of AgNPs which was further confirmed through UV-visible spectrometer, TEM and XRD techniques. The result showed stable and spherically shaped AgNPs in the range 10-20 nm.

AgNPs have unique physio-chemical properties like catalytic action, chemical stability, high electrical and chemical conductivity, optical behaviour and surface plasmon raman scattering [15]. Besides these properties, they have a wide window of antimicrobial and disinfectant properties making them more reliable and promising against new emerging threat of bacteria resistance and infections. Sometimes plant assisted AgNPs are more advantageous to other microorganism assisted synthesis and here an attempt is done to evaluate the antimicrobial strength of flower assisted AgNPs. The inhibitory and antimicrobial activity of AgNPs was tested using Agar well diffusion assay. An inhibition zone was measured against test cultures (*Staphylococcus aureus*, *Bacillus cereus* and *Escherichia coli*). The findings suggest that there is vast potential of AgNPs to improve human health and ecology and further large scale in vivo and in vitro researches need to be done to maximize its potential.

Experimental

Materials and methods

Silver nitrate (99.8%) from Qualigens Fine Chemicals, Mumbai, Nutrient agar media (Peptone 5g/L; NaCl 5g/L; Beef extract 3g/L; pH 7.0±0.2) and Sodium hydroxide from HiMedia Laboratories pvt. Ltd., Mumbai, *D. caryophyllus* from D S flower shop, Faridabad (Haryana).

Synthesis of AgNPs

Properly washed and sundried red *D. caryophyllus* flowers were used for the bioreduction of AgNO₃. About one gram of these dry flower petals was boiled with 10 ml distilled water for 20-30 min to release their cellular content. The flower extract was then filtered using Whatmann filter paper 1 and was stored at 4°C for its further use.

It is well known fact that the stability and methodology of AgNPs is process dependent, thus the synthesis route of AgNPs was optimized with different silver ion concentrations and extract dilutions. As a result of such optimization, 4 mM AgNO₃ and 2% (v/v) diluted aqueous flower extract was found suitable to synthesis stable mono-dispersed AgNPs. This reaction mixture was kept under incubation (40 °C, 180 rpm; pH 9) for 24 hours and the appearance of deep yellow colour indicated the formation of AgNPs.

Characterizations

The absorption spectrum and optical properties colloidal AgNPs were recorded in UV-visible spectrophotometer (PC based double beam Spectrophotometer 2202 Systronics) to confirm the nanoparticle production. The resultant colloidal solution was centrifuged and repeatedly washed with distilled water to get purified AgNPs which was subjected to further characterization.

AgNPs were characterized using XRD (X-ray Diffraction System X'Pert PRO Diffractometer) to observe its crystalline structure. And their morphology, shape and size were analysed with Transmission electron microscope (TEM, Hitachi H-7500) images.

Antibacterial study

Agar well diffusion assay was utilized to study their antibacterial action against pathogenic bacterial strains viz., *E. coli*, *S. aureus* and *B. cereus*, by measuring their inhibition zone.

Result and discussion

Optimization and formation of AgNPs

UV-visible spectroscopy is a simple and reliable technique for confirming the formation of AgNPs and checking their stability. During optimization, a sharp and narrow UV-visible absorption spectra peak appeared at 404 nm, **Fig. 1(a)** corresponding to 2% (v/v) extract

dilution and at 406 nm, **Fig. 1(b)** for 4 mM AgNO₃ solution, thus confirming the formation of AgNPs. In further experiments, 4 mM AgNO₃ and 2% (v/v) flower extract was used to synthesize the desired shape and size AgNPs, as shown by UV-visible absorption spectra peak at 409 nm, **Fig. 1(c)**.

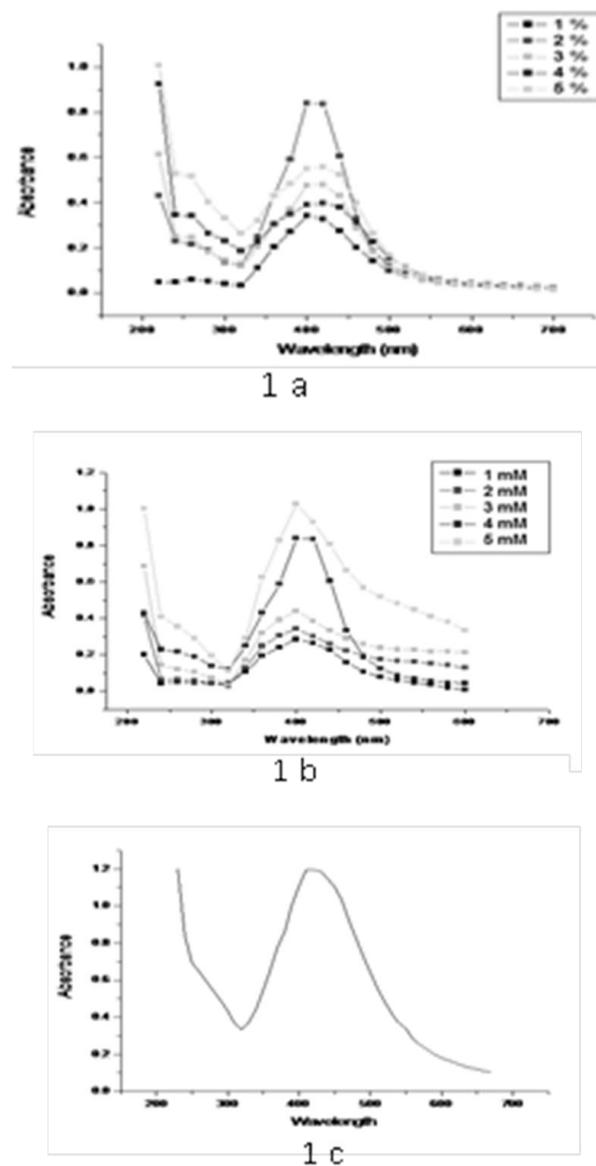


Fig. 1. UV-visible spectrum (a) 4 mM AgNO₃ with varying extract dilutions, (b) 2% (v/v) extract dilution with varying AgNO₃ concentrations, (c) 4mM AgNO₃ against 2% (v/v) extract dilution.

Characterization

The detailed morphological study was carried out using XRD and TEM techniques. The XRD pattern (**Fig. 2**) showed the Bragg's Diffraction peaks, characteristic of face centered cubic (fcc) silver phase as indexed in JCPDS data 04-0783. There is corresponding diffraction peaks at 37.1°, 44.3° and 77.1° indicating the presence of (111), (200) and (311) crystalline planes in AgNPs. The TEM images (**Fig. 3**) of purified sample showed well

separated spherically shaped nanosized AgNPs in the range 10-20 nm which is in confirmation of the size calculated from XRD pattern. The test sample showed no sign of agglomeration, thus indicating the presence of stable AgNPs at room temperature.

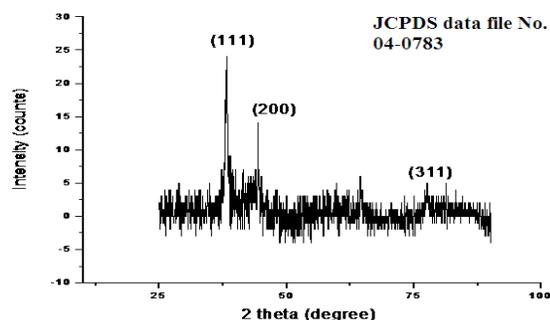


Fig. 2. XRD pattern of AgNPs.

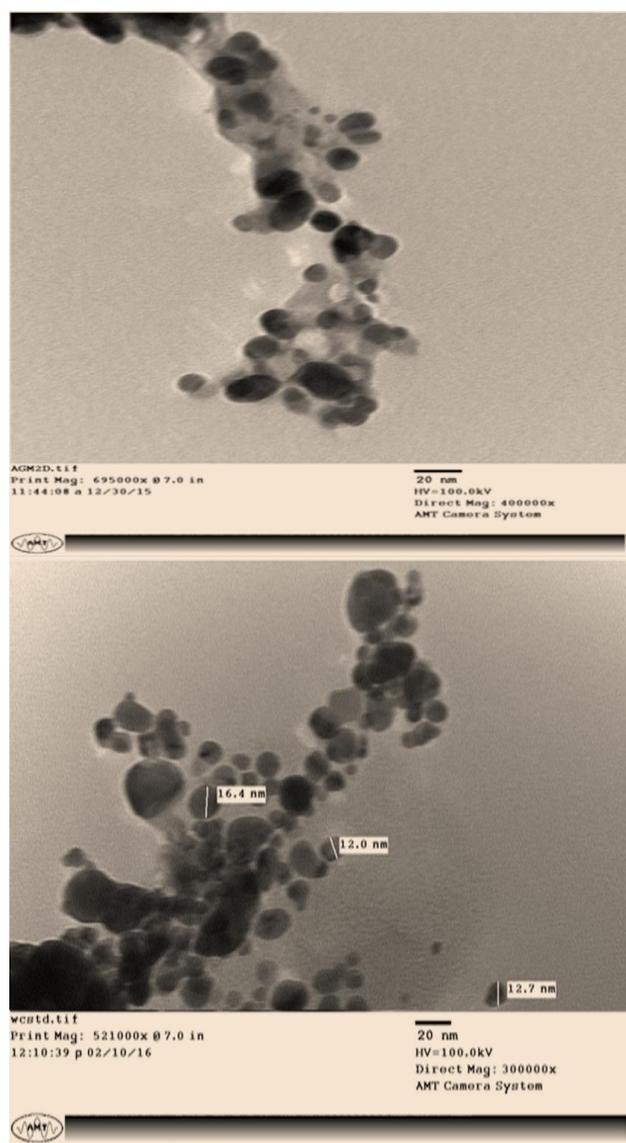


Fig. 3. TEM images of AgNPs.

Antibacterial Action

The antibacterial action of phytosynthesised AgNPs was studied using Agar well diffusion method. Their inhibition zone was measured against pathogenic bacteria strains. The zone of inhibition of AgNPs against *S. aureus*, *B. cereus* and *E. coli* was found to be 24 mm, 20 mm and 13 mm, shown in Fig. 4.

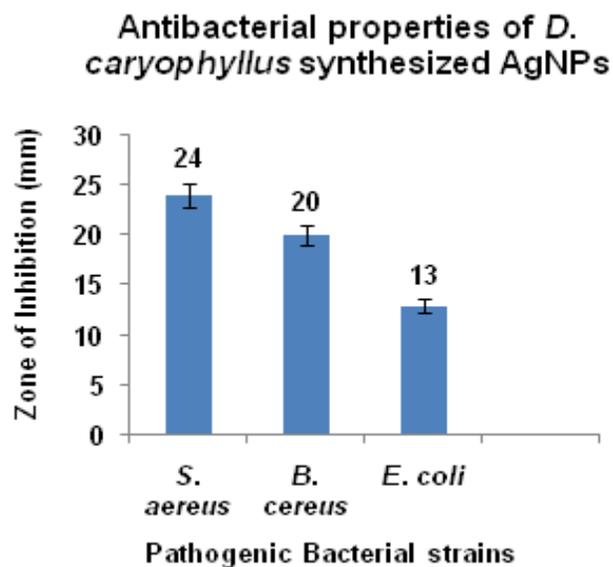


Fig. 4. Inhibition zone of AgNPs.

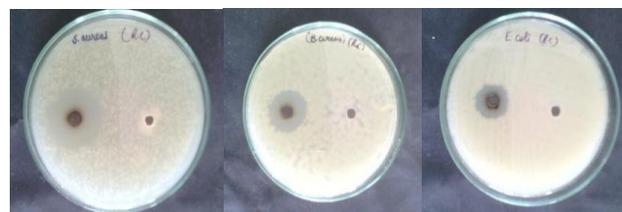


Fig. 5. (Left to Right) Antibacterial action of AgNPs against *S. aureus*, *B. cereus* and *E. coli*.

Conclusion

The biological reduction of 4 mM AgNO₃ was done successfully using 2% (v/v) aqueous flower extract under the incubation of 40°C at 180 rpm for 24 hours. The resultant colloidal solution reported to have an absorption peak at 409 nm, confirming the formation of AgNPs using UV-visible spectroscopy. TEM and XRD techniques were used to study their detailed structural analysis. AgNPs were found nano-sized spherically shape, mono-dispersed and stable at room temperature. They also showed their effectiveness against *S. aureus*, *B. cereus* and *E. coli* pathogenic bacteria cultures, thus making them valuable for many biomedical applications.

Acknowledgement

This work was financial supported under BSR scholarship by University Grants Commission (UGC), Government of India.

Reference

1. Sonvico, F.; Dubemet, C.; Colombo, P.; *Curr. Pharm. Des.*, **2005**, 11, 2091-2105.
DOI: [10.2174/1381612054065738](https://doi.org/10.2174/1381612054065738)
2. Shipway, A. N.; Katz, E.; Willner, I., *J. Phys. Chem.*, **2000**, 1, 18-52.
DOI: [10.1002/1439-7641\(20000804\)1:1<18::AIDCPHC18>3.0.CO;2-L](https://doi.org/10.1002/1439-7641(20000804)1:1<18::AIDCPHC18>3.0.CO;2-L)
3. Navaladian, S.; Viswanathan, B.; Viswanath, R. P.; Varadarajan, T. K.; *Nanoscale Res. Lett.*, **2007**, 2(1), 44-48.
DOI: [10.1007/s11671-006-9028-2](https://doi.org/10.1007/s11671-006-9028-2)
4. Starowicz, M.; Stypula, B.; Banas, J.; *Electrochem. Commun.*, **2006**, 8(2), 227-230
DOI: [10.1016/j.elecom.2015.11.018](https://doi.org/10.1016/j.elecom.2015.11.018)
5. Sreeram, K. S.; Nidhi, M.; Nair, B. U.; *Bull. Mater. Sci.*, **2008**, 31(7), 937-942.
DOI: [10.1007/s12034-008-0149-3](https://doi.org/10.1007/s12034-008-0149-3)
6. Begum, N. A.; Mondal, S.; Basu, S.; Laskar, R. A.; Mandal, D.; *Colloids surf., B*, **2009**, 71(1), 113-118.
DOI: [10.1016/j.colsurfb.2009.01.012](https://doi.org/10.1016/j.colsurfb.2009.01.012)
7. Iravani, S.; *Green Chem.*, **2011**, 13, 2638-2650.
DOI: [10.1039/C1GC15386B](https://doi.org/10.1039/C1GC15386B)
8. Thakkar, N. K.; Mhatre, S. S.; Parikh, Y. R.; *Nanomed. Nanotechnol. Biol. Med.*, **2009**, 6(2), 257-262.
DOI: [10.1016/j.nano.2009.07.002](https://doi.org/10.1016/j.nano.2009.07.002)
9. Yongqiang, Z.; Xinfeng, Cheng.; Yucang, Z.; Xinghua, Xue.; Yunzhi, F. *Colloid Surf., A*, **2013**, 423, 63.
DOI: [10.1016/j.colsurfa.2013.01.059](https://doi.org/10.1016/j.colsurfa.2013.01.059)
10. Muthuswamy, S.; Krishnamurthy, S.; Yeoung, S.Y. *Bioresour. Technol.*, **2010**, 101, 7958.
DOI: [10.1016/j.biortech.2010.05.051](https://doi.org/10.1016/j.biortech.2010.05.051)
11. Nestor, A. R. V.; Mendieta, V. S.; Lopez, M. A. C.; Espinosa, R. M. G.; Alatorre, J. A. A.; *Mater. Lett.*, **2008**, 62(17), 3103-3105.
DOI: [10.1016/j.matlet.2008.01.0138](https://doi.org/10.1016/j.matlet.2008.01.0138)
12. Kasthuri, J.; Veerapandian, S.; Rajendiran, N.; *Colloids Surf., B*, **2009**, 68(1), 55-60.
DOI: [10.1016/j.colsurfb.2008.09.021](https://doi.org/10.1016/j.colsurfb.2008.09.021)
13. Bar, H.; Bhui, D. K.; Sahoo, G. P.; Sarkar, P.; De, S. P.; Misra, A.; *Colloids Surf., A*, **2009**, 339(1-3), 134-139.
DOI: [10.1016/j.colsurfa.2009.02.008](https://doi.org/10.1016/j.colsurfa.2009.02.008)
14. (a) Sanguesa, C. D.; Urbina, H. R.; Figlarz, M.; *J. Solid State Chem.*, **1992**, 100, 272-280.
DOI: [10.1016/0022-4596\(92\)90101-Z](https://doi.org/10.1016/0022-4596(92)90101-Z)
(b) Burshtain, D.; Zeiri, L.; Efrima, S.; *Langmuir*, **1999**, 15, 3050-3055.
DOI: [10.1021/1a980543a](https://doi.org/10.1021/1a980543a)
(c) Shon, S. Y.; Colorado, Jr. R.; Williams, T. C.; Brain, D. C.; Lee, R. T.; *Langmuir*, **2000**, 16, 541-548.
DOI: [10.1021/1a9816981](https://doi.org/10.1021/1a9816981)
15. Krutyakov, Y. A.; Kudrynskiy, A. A.; Olenin, A. Y.; Lisichkin, G. V.; *Russ. Chem. Rev.*, **2008**, 77, 233
DOI: [10.1070/RC2008v077n03ABEH003751](https://doi.org/10.1070/RC2008v077n03ABEH003751)