Innovare

Academic Sciences

International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491

Vol 7, Issue 3, 2015

Short Communication

GREEN SYNTHESIS AND CHARACTERIZATIONS OF SILVER NANOPARTICLES USING FRESH LEAF EXTRACT OF *MORINDA CITRIFOLIA* AND ITS ANTI-MICROBIAL ACTIVITY STUDIES

ASHA R PAI[a*], KAVITHA S[a], SHWETA RAJ S.[a], PRIYANKA P[a], VRINDA A[a], VIVIN T. S.[a], SILPA SASIDHARAN[a]

[a]Amrita School of Biotechnology, Amrita Vishwa Vidyapeetham, Amritapuri, Kollam 690525
Email: asharp@am.amrita.edu

Received: 22 Oct 2014 Revised and Accepted: 15 Nov 2014

ABSTRACT

Objective: To develop a rapid method of synthesis of silver nanoparticles (Ag NPs) using the fresh aqueous leaf extract of *Morinda Citrifolia* from 0.1 M $AgNO_3$ solution, to characterize the resulting Ag NPs and also compare their antimicrobial activity with those of standard drugs against human pathogenic bacteria.

Methods: 25 ml of the aqueous extract was added to 25 ml of 0.1 M aqueous $AgNO_3$ at room temperature. The mixture was stirred continuously for 5-10 minutes. The reduction was completed with the appearance of brownish-black colored dispersion. The resulting nanoparticles were characterized using UV-Vis absorption spectra and Particle size analysis (DLS method). Further the Antimicrobial activity was compared with the drugs against *S. aureus* and *P. aureginosa* strains using the disk diffusion method.

Results: The formation of Silver nanoparticles was confirmed with the help of UV-Vis absorption spectra at $\approx$ 425 nm and particle size as approximately 100 nm using Particle size analysis (DLS method). The anti-microbial activity of the Ag NPs so synthesized was studied against human pathogens in wound infections such as *S. aureus* and *P. aureginosa* strains. The inhibitory activity for Ag NPs was compared with those of known drugs such as tetracycline, Ceftazidime and Amikacin at 30 mcg. The inhibitory activity of the Synthesized Ag NPs was found pronounced against *P. aureginosa* strains.

Conclusion: A rapid method of synthesizing Ag NPs has been developed by using the fresh leaf extract of *Morinda Citrifolia* and it was found that the extract is a potential reducing agent to produce stable Ag NPs. The research provides a new input to the development of anti-microbial agent.

Keywords: Nanomaterial, Green synthesis, Silver nanoparticles, *Morinda Citrifolia*, Anti microbial activity.

Nanomaterials are synthesized from the novel, eco-friendly and sustainable techniques of physical, chemical, biological and engineering processes. In this 21st century, the nanotechnology has emerged as an interdisciplinary field with the biosynthesis of metal nanoparticles. Nanotechnology is gaining importance in various fields such as health care, food and feed, cosmetics, environmental health, biomedical science, chemical industries, drug and gene delivery, energy science, electronics, mechanics and space industries. There are many ways to synthesis nanoparticles such as solid reaction, co-precipitation, chemical reaction and sol gel method etc, [1]. Bio-based approaches for the synthesis of nanoparticles are rapidly gaining importance due to their ease of synthesis, eco-friendliness and formation of stable and biocompatible nanoparticles. Bacteria, fungi, plants and seaweeds are the potential sources utilized for the synthesis of nanoparticles [2]. The green synthesis with plant extracts are simpler and advantageous over other biological processes as they are safe to handle.

In biological method, the plant extracts has been used as reducing agent and capping agent for the synthesis of nanoparticles [3] due to their reducing properties [4]. Some properties such as size, distribution, and morphology of the particles are clearly obtained from the nanoparticles [5]. Silver nanoparticle acts as antimicrobial agent which finds applications in medical field such as Ag NPs coated blood collecting vessels, coated capsules, band aids etc., [3 - 6]. The silver is non toxic to animal cells and highly toxic to bacteria and other microorganisms such *E coli*, *P. aeroginosa*, *S. aureus* etc. Due to these phenomena it is considered to be a safe and effective bactericidal metal [7 - 9] and therefore can be incorporated with several materials such as cloths, ointments etc. The band aids so developed are highly sterile and therefore can be useful in the hospitals to prevent or to minimize the infections with pathogenic bacteria such as *E coli*, *S. aureus* etc,.[10].

Synthesis of silver nanoparticles using plant extracts have been reported in *Boswellia ovalifolilata*, *Shorea tumbuggaia Svensoina hyderobadensis, Thespesia populnea, Vinca rosea* [11], *Cassia auriculata* [12]. *Morinda citrifolia* (Noni) has been extensively used in folk medicine for over 2,000 years. It has been reported to have broad therapeutic effects, including cancer activity, in both clinical practice and laboratory animal models [13]. Noni has traditionally been used for colds, flu, diabetes, anxiety, and high blood pressure, as well as for depression and anxiety. The green fruit, leaves, and root/rhizomes were traditionally used in Polynesian cultures to treat menstrual cramps, bowel irregularities, diabetes, liver diseases, and urinary tract infections [14]. The mechanism for these effects remains unknown [15 - 19]. Hence, in the present work, a very rapid green synthesis of silver nanoparticles using the leaf extract of *Morinda citrifolia* is investigated and their anti microbial activities are studied against human pathogenic bacteria such as *S. aureus* and *P. aeroginosa* are compared with the standard drugs in the market. 5 grams of fresh leaves of the plants were collected and washed with distilled water 3-4 times to remove the dust particles. Leaves were chopped into small pieces and mixed into 150 ml distilled water separately. Mixture was stirred at 800 rpm for 3 hrs in magnetic stirrer. The aqueous extract was separated by filtration with whatmann No. 1 filter paper. The filtrate was collected and stored at 4°C.

The extract stored was used for biosynthesis of silver nanoparticles from silver nitrate. 25 ml of the prepared extract was added to 25 ml of aqueous $AgNO_3$ (0.1M in 100 ml) at room temperature. The mixture was stirred continuously for 5-10 minutes.

$$Ag\ NO_3 \rightarrow Ag^{+} + NO^{3-}$$

$$Ag^{+} + 2H_2O \rightarrow Ag^{0} + 4H^{+} + O_2$$

The reduction was completed with the appearance of brownish-black color which confirms the formation of silver nanoparticles. The contents were centrifuged at 8, 000 rpm for 10 minutes. The supernatant was used for the characterization of Silver nanoparticles. The bioreduction of Silver ions was studied the UV-VIS spectrophotometer.

UV-Vis spectroscopy measurements of synthesized Silver nanoparticles were characterized by Perkin-Elmer UV-Vis spectrophotometer. The Scanning range for the samples was 200 - 700 nm. In order to find out the particle size distribution the Ag nanoparticle dispersed in water was studied and the data on particle size were extracted using a Zetasizer Nicomp, 380ZLS (Nicomp, USA) using the Dynamic Light scattering method.

Antimicrobial activity of the synthesized silver nanoparticles was done using the agar well diffusion assay method and agar disk diffusion method. Approximately 25 ml of the Muller Hinton Agar was poured in sterilized Petri dishes. The test organisms were grown from the broth for 24 hrs and the OD was measured to confirm the lag phase stage while inoculation.

Agar plates were prepared and wells bored into it. The wells were labeled and loaded with 5 µl of the Silver nano liquid and incubated for 24 hrs at 37 °C. The organisms studied were *S. aureus* and *P. aureginosa*. The plates were examined for confirmation of zone of inhibitions (ZOI) which became visible as the clear area around the wells. The diameters of these ZOI were measured using a meter scale and the mean value for each organism was recorded in millimeters. The antimicrobial activity of the synthesized Ag NPs were also compared with the standard drugs such as Tetracycline, Ceftazidime and Amikacin (30mcg) using the agar disk diffusion method. Circular disk were prepared and were soaked in 30 µl of Silver nano liquid and these were bored into the Agar plates of Muller Hington Agar along with the desired antibiotics and the diameters of the ZOI so formed are measured and recorded in millimeters.

During the process of biosynthesis the Silver nanoparticles were formed giving a brownish black color to the aqueous solution which is due to the excitation of Surface Plasmon Resonance. In course of time, the mixture grew darker and darker but remained to be highly stable within a few hours. This color change clearly indicated the reduction of Ag+ ions confirming qualitatively the formation of Ag-Nanoparticles.

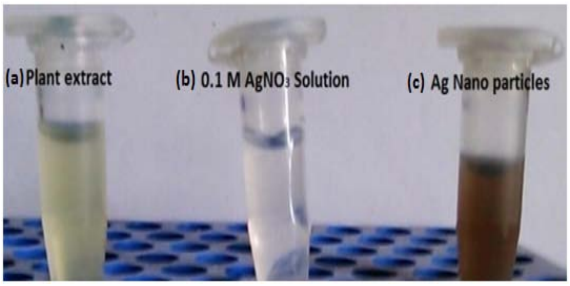
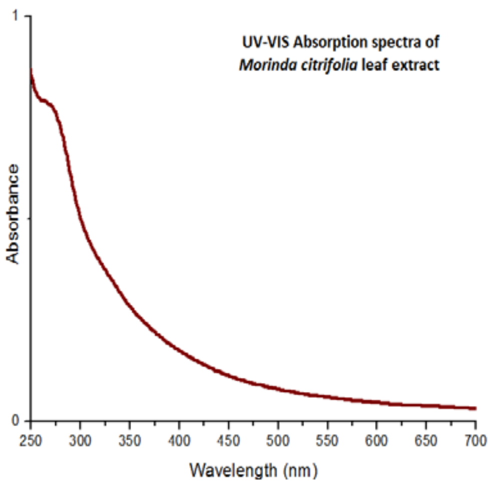


Fig. 1: Visual observations of (a) Plant extract (b) 0.1 M AgNO3 solution and (c) Silver nanoparticles



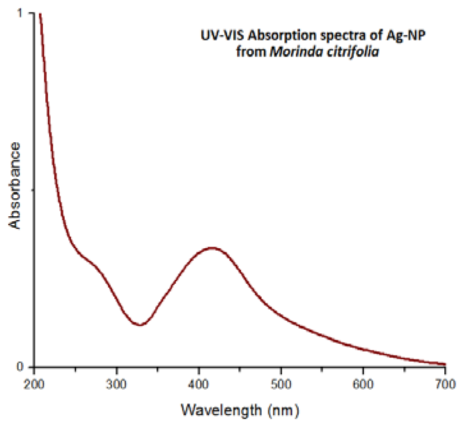


Fig. 2: UV-Vis spectra of (a) Morinda Citrifolia leaf extract and (b) Silver nanoparticles synthesized from the leaf extract

From the visual observations it was clear that the plant extract of *M. Citrifolia* was a good reducing agent for Ag+ ions to Silver nanoparticles. The UV-Vis absorption spectra of the Silver nanoparticles formed is shown in the fig. 2 (b). Absorption spectra of both the extract and the nanoparticles are shown which shows the formation of Silver nanoparticles with an absorption maximum at ≈ 425 nm.

The particle size distribution exposed to particle size diameter in the nano silver liquid was given by the Figure.3. The maximum size of the nanosilver was 220 dnm and the minimum size was 8 dnm

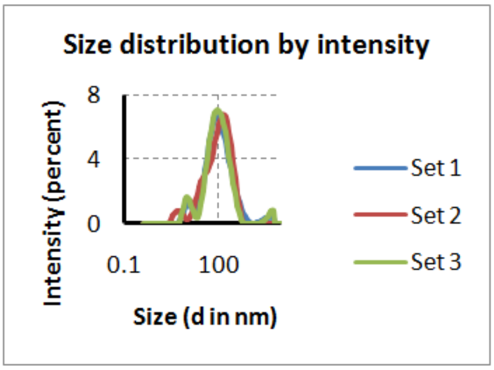


Fig. 3: Particle size analysis of Silver nanoparticles synthesized from *Morinda Citrifolia* leaf extract

Both *M. Citrifolia* leaf extract and the Ag-NPs synthesized with the leaf extract were tested for their antimicrobial activities against Human pathogens such as *S. aureus* and *P. Aureginosa*. The Ag NPs showed a better inhibitory activity for *P. Aureginosa* compared to the standard drugs such as tetracycline, Ceftazidime and Amikacin and negligible activity for *S. aureus*. The green extract was taken as the control which did not have any inhibitory activity either on *S. Aureus* or *P. Aureginosa.*

In this study, the Silver nanoparticles were synthesized using a much simpler and fast means using the leaf extract of *Morinda Citrifolia*. The Silver ions readily get reduced to Silver nanoparticles hence the leaf extract of *Morinda Citrifolia* can be considered as potential reducing agent. The primary confirmation was done qualitatively with the color change within 5 to 10 minutes from pale yellow to brownish black. The bioreduction was observed by the change in the color. The color change of the dispersion is attributed to the Surface Plasmon resonance vibration which is shown by the electrons of the nanoparticles synthesized. This was confirmed by the absorbance maximum found around 425 nm using UV-Vis spectral analysis. The DLS method clearly indicates the presence of evenly sized particles around 100 nm. Also a comparative study of the antimicrobial activity of the 30 µL of Ag NPs with the standard drugs like tetracycline, Ceftazidime, Amikacin etc at 30 mcg showed a potential use of synthesized Ag NPs as antimicrobial agents.

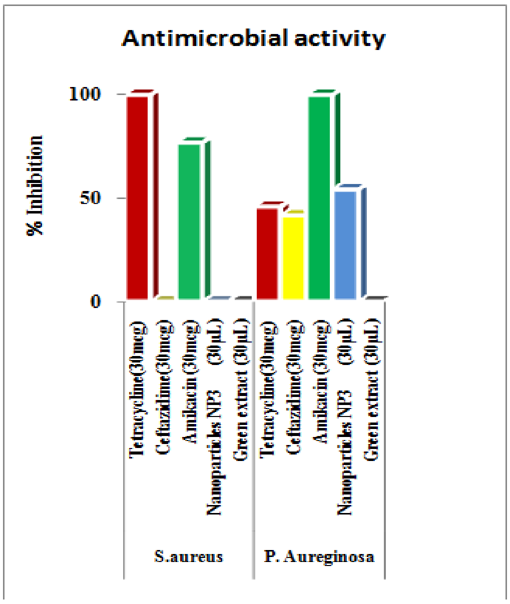


Fig. 4: A comparative study of antimicrobial activity of Silver nanoparticles synthesized from Silver nanoparticle (30 µL) with the drugs such as tetracycline, Ceftazidime and Amikacin at 30 mcg

A novel and rapid green biogenesis production of sliver nanoparticles by using leaf extract of *Morinda Citrifolia* has been developed and presented in this work. The developed process was fast and better from previously known processes in terms of time taken and cost effectiveness. The size of the synthesized nanoparticles was much smaller than other previously known methods of synthesizing nanoparticles. The reduction of silver nanoparticles was also confirmed by UV-Vis spectra with an absorbance at around ~425 nm. The average size of the synthesized nanoparticles was approximately 100 nm. The anti microbial inhibitory activity study of the environmentally benign Silver nanoparticles against the human pathogens such as *S. aureus* and *P. aureginosa* strains clearly indicated the development of pathogenic resistant bioactive drug delivery compounds for wound healing treatments.

ACKNOWLEDGMENT

The authors would like to thank the operators and staff of Amrita Centre for Nanoscience and Nanomedicine, Kochi and Amrita School of Biomedical engineering, Ettimadai for the characterisation facility provided there. The authors also like to thank Dr. Bipin Nair, Dean and the staff of Amrita school of Biotechnology, Amritapuri and also would like to mention about the BRITE initiatives at Amrita School of Biotechnology, Amritapuri for which this project was considered.

CONFLICT OF INTEREST

Declared None

REFERENCES

1. Saranyadevi K, Subha V, Ernest Ravindran RS, Renganathan S. Green synthesis and characterization of silver nanoparticle using leaf extract of *Capparis zeylanica*. Asian J Pharm Clin Res 2014;7 Suppl 2:44-8.
2. Dhas SP, Mukherjee A, Chandrasekaran N. Synergistic effect of biogenic silver nanocolloid in combination with antibiotics: a potent therapeutic agent. Int J Pharm Pharm Sci 2013;5(1):292-5.
3. Akl M Awwad1, Nidà M. Green synthesis of silver nanoparticles by mulberry leaves extract. Nanosci Nanotechnol 2012;2(4):125-8.
4. Umesh B Jagtap, Vishwas A Bapat. Green synthesis of silver nanoparticles using Artocarpus heterophyllus Lam. Seed extract and its antibacterial activity. Ind Crops Prod 2013;46:132–7.
5. Nethradevi C, Sivakumar P, Renganathan S. Green synthesis of silver nanoparticles using *Datura metel* flower extract and evaluation of their antimicrobial activity. Int J Nanomat Biostruct 2012;2(2):16–21.
6. Geoprincy G, Saravanan P, Nagendragandhi N, Renganathan S. A novel approach for studying the combined antimicrobial effects of silver nano particles and antibiotics through agar over layer method and disk diffusion method. Digest J Nanomat Biostruct 2011;6(4):1557-65.
7. Kalimuthu K, Babu RS, Venkataraman D, Bilal M, Gurunathan S. Biosynthesis of silver nanocrystals by *Bacillus licheniformis*. Colloids Surf B 2008;65(1):150-3.
8. Wijnhoven SWP, Peijnenburg WJGM, Herberts CA, Hagens WI, Oomen AG, Heugens EHW, *et al*. Nano–silver: a review of available data and knowledge gaps in human and environmental risk assessment. Nano Toxicol 2009;3:109.
9. Klueh U, Wagner V, Kelly S, Johnson A, Bryers JD. Efficacy of silver-coated fabric to prevent bacterial colonization and subsequent device-based biofilm formation. J Biomed Mater Res 2000;53(6):621–31.
10. Karthick S, Avimanyu. Silver nanoparticle synthesis from *lecanicillium lecanii* and evalutionary treatment on cotton fabrics by measuring their improved antibacterial activity with antibiotics against staphylococcus *aureus* (atcc 29213) and e Coli (atcc 25922) strains. Int J Pharm Pharm Sci 2011;3(4):190-5.
11. Savithramma N, Linga Rao M, Ankanna S, Venkateswarlu P. Screening of medicinal plants for effective biogenesis of silver nano particles and efficient anti-microbial activity. IJPSR 2012;3(4):1141-8.
12. Velavan S, Arivoli P, Mahadevan K. Biological reduction of silver nanoparticles using cassia auriculata flower extract and evaluation of their *in vitro* antioxidant activities. Nanosci Nanotec An Int J 2012;2(4):30-5.
13. Wang MY, Su C. Cancer preventive effect of Morinda citrifolia (Noni). Ann N Y Acad Sci 2001;952:161-8.
14. Wang MY, West BJ, Jensen CJ, Nowicki D, Su C, Palu AK, *et al*. Morinda citrifolia (Noni): a literature review and recent advances in Noni research. Acta Pharmacol Sin 2002;23(12):1127–41.
15. Hiramatsu T, Imoto M, Koyano T, Umezawa K. Induction of normal phenotypes in ras-transformed cells by damnacanthal from Morinda citrifolia. Cancer Lett 1993;73:161-6.
16. Hirazumi A, Furusawa E. An immunomodulatory polysaccharide-rich substance from the fruit juice of Morinda citrifolia (noni) with antitumor activity. Phytother Res 1999;13:380-7.
17. Millonig G, Stadlmann S, Vogel W. Herbal hepatotoxicity: acute hepatitis caused by a Noni preparation (Morinda citrifolia). Eur J Gastroenterol Hepatol 2005;17:445-7.
18. Mueller BA, Scott MK, Sowinski KM, Prag KA. Noni juice (Morinda citrifolia): hidden potential for hyperkalemia? Am J Kidney Dis 2000;35:310-2.
19. Yüce B, Gülberg V, Diebold J, Gerbes AL. Hepatitis induced by Noni juice from Morinda citrifolia: a rare cause of hepatotoxicity or the tip of the iceberg? Digestion 2006;73:167-70.