Research Article

Antibacterial efficacy of silver nanoparticles synthesized using herbal formulation against clinical pathogens

Ankita Komal Labh, S. Rajeshkumar*, T. Lakshmi, Anitha Roy

ABSTRACT

Introduction: Silver nanoparticles (AgNPs) have a wide range of application in a variety of fields such as medicine, food, cosmetics, and agriculture. They are used as antimicrobial and antifungal agents in agriculture and can potentially be used as targeted drugs for the human body. This antibacterial activity of AgNPs is being taken advantage of, in producing antibacterial textiles, coatings on medical devices, cosmetics, toothpastes, laundry detergents, soaps, home appliances, etc. The aim of this research is to study the antibacterial efficacy of AgNPs synthesized using herbal formulation against clinical pathogens. **Materials and Methods:** Herbal formulation was created using the leaves of *Andrographis paniculata* and *Moringa oleifera*. To this, silver nitrate solution was added and the solution was transferred to an orbital shaker for AgNP preparation. The powder of AgNPs was created. A fresh bacterial suspension of clinical pathogens such as Serratia, Aeromonas hydrophila, and Vibrio parahaemolyticus was cultured in Mueller-Hinton agar plates. Different concentrations of NPs (20, 40, and 60 µl) were incorporated into the wells and the plates were incubated at 37°C for 24 h. The antibiotics were used as positive control. Zone of inhibition was recorded in each plate. Proper documentation methods were used. The bioreduction of pure $AgNO_3$ was characterized by ultraviolet (UV)-visible spectroscopy. **Results:** Color change was seen after the preparation of AgNPs. The peak in UV-visible spectroscopy was seen to be at 390 nm. The zone of inhibition was seen to increase with an increase in the concentration of AgNPs in the agar wells. The zone was smallest in A. hydrophila sp. culture, whereas it was seen to be the largest in Serratia sp. culture. **Conclusion:** We can conclude by saying that herbal synthesis of AgNPs is highly eco-friendly and should be practiced more than chemical synthesis of the same. The antibacterial activity of AgNPs was comparably effective to the control used and in the future, they can be administered as antibiotics in the era of multidrug resistance.

KEY WORDS: Antibacterial, Clinical pathogens, Herbal synthesis, Silver nanoparticles

INTRODUCTION

Nanotechnology

Nanotechnology has applications in variety of fields. The rapidly rising interest of researchers in exploring the possibilities of using nanoparticles (NPs) for various applications.[1] This is mainly credited to the unique physical, chemical, and biological properties of NPs.[2] Out of these unique characteristics, the ones which are of most importance are – small particle size and large surface area.[3] Biological activity of NPs increases with an increase in surface area.[4] Researchers have also been striving for non-toxic synthesis of NPs. Physical and chemical methods of NP synthesis have major disadvantages due to the usage of toxic chemicals and radiation.[5] Possible eco-friendly alternatives have been suggested involving the usage of microorganisms,[6] enzymes,[7] plants, and plant extracts.[5]

Access this article online	
Website: jprsolutions.info	**ISSN:** 0975-7619

NPs are being used in a plethora of industries for drug delivery,[8] gene transfer,[9] management of insect pests in agriculture,[10] fabrication of bioelectronics devices,[11] antimicrobial action,[12] etc.

Silver NPs (AgNPs)

These are silver particles in the size range of 1–100 nm. They have a wide range of application in a variety of fields such as medicine, food, cosmetics, and agriculture.[13] Silver has been in long use in the medical industry where it is added to ointments to prevent infection of burns and open wounds.[14] Apart

Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, Chennai, Tamil Nadu, India

***Corresponding author:** Dr. S. Rajeshkumar, Department of Pharmacology, Saveetha Dental College, Saveetha Institute of Medical and Technical Sciences, Saveetha University, 162, Poonamallee High Road, Chennai - 600 077, Tamil Nadu, India. Phone: +91-9629739263. E-mail: ssrajeshkumar@hotmail.com

Received on: 21-03-2019; Revised on: 24-05-2019; Accepted on: 21-06-2019

from these AgNPs have very good antibacterial and antifungal activity. These properties have been taken advantage of, in employing the AgNPs in agriculture, against plant pathogens.[15]

Antibacterial Activity of AgNPs

AgNPs have been described as a new generation of antimicrobial agents.[16] They have gained a lot of attention due to their ability to act as broad-spectrum antimicrobial.[17] Their specialty is that they can act on both aerobic and anaerobic bacteria.[18] AgNPs attach themselves to the surface of bacterial cell membranes. Due to this, there is a loss in cell membrane potential. Further, they penetrate the bacterial cell membrane, release silver ions, generate reactive oxygen species (ROS), and deactivate cellular enzymes.[19] They have other actions as well such as inhibition of DNA replication and depletion in ATP levels which make it highly difficult for the bacteria to produce resistant strains.[20] This antibacterial activity of AgNPs has been wisely taken advantage of, in producing antibacterial textiles, coatings on medical devices, cosmetics, toothpastes, laundry detergents, soaps, home appliances, etc.[21]

MATERIALS AND METHODS

Preparation of Plant Extract

Leaves of *Andrographis paniculata* and *Moringa oleifera* were collected from Chennai. The collected leaves were washed 3–4 times using distilled water and dried in shade for 7–14 days. The well-dried leaves were then crushed into powder using mortar and pestle. The collected powder of the leaves was stored in airtight containers. About 1 g of *A. paniculata* leaf powder was dissolved in distilled water and boiled for 5–10 min at 60–70°C. The same was done for the powder of *M. oleifera*. The solutions were then filtered using Whatman No. 1 filter paper. The filtered extract was collected and stored in 4°C for further use.

Synthesis of NPs

One millimolar of silver nitrate was dissolved in 90 ml of double distilled water. The plant extracts of *A. paniculata* and *M. oleifera* were added with the metal solution and this solution was made into a 100 ml solution. The color change was observed visually and photographs were taken for the record. The solution was then kept in a magnetic stirrer/orbital shaker for NP synthesis.

Characterization of AgNPs

The synthesis of NPs is primarily characterized using ultraviolet (UV)-visible spectroscopy. About 3 ml of the solution is taken in a cuvette and scanned in UV-visible spectrometer under 350 nm–550 nm wavelength. The results were recorded for graphical analysis.

Preparation of AgNP Powder

The AgNP solution was centrifuged using Lark refrigerated centrifuge. The centrifugation was done at 8000 rpm for 10 min, and the pellet was collected and washed with distilled water twice. The final purified pellet is collected and dried at 60°C. Finally, the NPs powder was collected and stored in an airtight Eppendorf tube.

Study of Antibacterial Activity of NPs against Clinical Pathogens

The agar well diffusion method was used to determine the antibacterial activity of AgNPs. Different concentrations of AgNPs were tested against the clinical pathogens such as *Serratia*, *Aeromonas hydrophila,* and *Vibrio parahaemolyticus*. The fresh bacterial suspension was dispersed on the surface of Mueller-Hinton agar plates. Different concentrations of NPs (20, 40, and 60 µl) were incorporated into the wells and the plates were incubated at 37°C for 24 h. The antibiotics were used as positive control. The zone of inhibition was recorded in each plate.

RESULTS AND DISCUSSION

The color change was seen after the production of AgNPs [Figure 1]. Initially, the extract solution was green in color and after the synthesis, it turned dark brown. The color change from green to brown preliminarily confirms the synthesis of AgNPs. This change takes place due to the excitation of the surface plasmon resonance.

This was done to monitor the formation and stability of AgNPs. The peak was seen at 390 nm [Figure 2].

The zone of inhibition was seen to increase with an increase in the concentration of the AgNPs in the agar well [Figure 3]. It was also registered that the zone of inhibition was the largest for the bacteria *Serratia* and smallest for *A. hydrophila*.

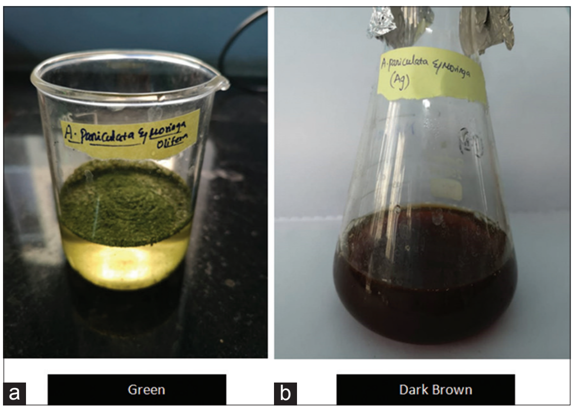

Figure 1: (a-b) Visual observation

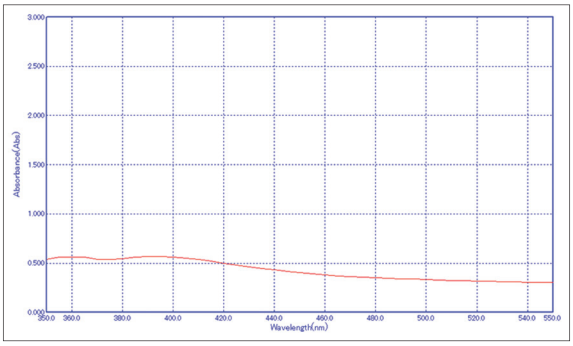

Figure 2: Ultraviolet-visible spectroscopy

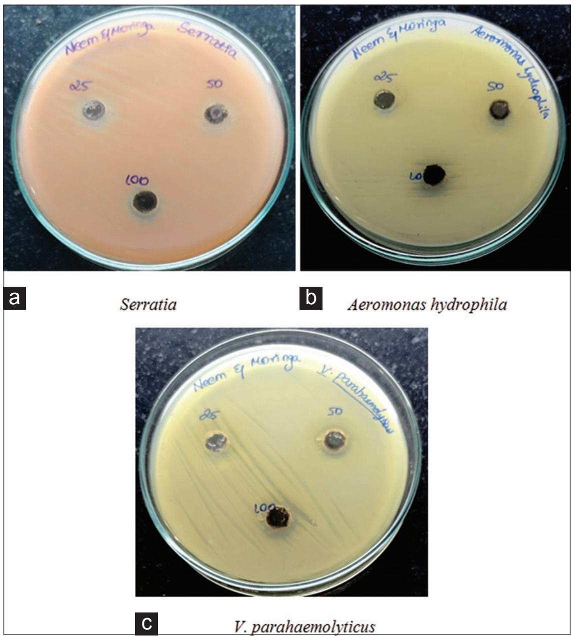

Figure 3: (a-c) Antibacterial activity

AgNPs exhibit strong antimicrobial activity against bacteria, viruses, and fungi.[22] This can be attributed to its membrane damage-causing potential which has a highly toxic effect on the bacteria.[23] Due to this property, it can be used in controlling infections in areas of mild to severe burns.[24] It can be used as an antibacterial agent in medical equipment, textiles, food packaging, and water treatment.[25] This can be done because AgNPs are applicable on glass, titanium, polymers, etc.[26]

Drug-resistant bacteria which are an issue of high medical concern can be dealt with using AgNPs.[27] NPs do not act the same way as standard antibiotics and thus have an extreme effect on multidrug-resistant bacteria. There are various mechanisms through which antibacterial activity can be exerted by NPs:
1. Direct interaction with bacterial cell wall
2. Inhibition of biofilm formation
3. Triggering of innate and adaptive host immune responses
4. Generation of ROS
5. Interactions with DNA and/or proteins.[28]

NPs can also be used as vectors for the delivery of antibiotics to the site of action. This enhances the biocidal activity of the antibiotic.[29]

CONCLUSION

Through the success of this study, we have come to a conclusion that AgNPs have potent antibacterial activity on clinically pathogenic species and in the future, they can be used as alternatives for conventional antibiotics.

REFERENCES

1. Sivakumar P, Kannaiyan P, Pandian K, Pandian S, Renganathgan S. Synthesis of silver nanorods from food industrial waste and their application in improving the keeping quality of milk. Ind Eng Chem Res 2013;52:17676-81.
2. Agarwal H, Kumar SV, Rajeshkumar S. A review on green synthesis of zinc oxide nanoparticles an eco-friendly approach. Res Efficient Technol 2017;3:406-13.
3. Santhoshkumar J, Kumar SV, Rajeshkumar S. Phyto-assisted synthesis, characterization and applications of gold nanoparticles a review. Biochem Biophys Rep 2017;11:46-57.
4. Menon S, Rajeshkumar S, Kumar SV. A review on biogenic synthesis of gold nanoparticles; characterization and its applications. Resour Efficient Technol 2017;3:516-27.
5. Rajeshkumar S, Naik P. Synthesis and biomedical applications of cerium oxide nanoparticles a review. Biotechnol Rep 2017;17:1-5.
6. Agarwal H, Menon S, Kumar SV, Rajeshkumar S. Mechanistic study on antibacterial action of zinc oxide nanoparticles synthesized using green route. Chem Biol Interact 2018;286:60-70.
7. Menon S, Devi KS, Santhiya R, Shanmugam R, Kumar SV. Selenium nanoparticles: A potent chemotherapeutic agent and an elucidation of its mechanism. Colloids Surf B Biointerfaces 2018;170:280-92.
8. Ghosh P, Han G, De M, Kim CK, Rotello VM. Gold nanoparticles in delivery applications. Adv Drug Deliv Rev 2008;60:1307-15.
9. Rai M, Deshmukh S, Gade A. Strategic nanoparticle mediated gene transfer in plants and animals a novel approach. Curr Nanosci 2012a;8:170-9.
10. Rai M, Ingle A. Role of nanotechnology in agriculture with special reference to management of insect pests. Appl Microbiol Biotechnol 2012;94:287-93.
11. Liu T, Zhong J, Gan X, Fan C, Li G, Matsuda N, et al. Wiring electrons of cytochrome c with silver nanoparticles in layered films. Chemphyschem 2003;4:1364-6.
12. Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: The powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol 2012;112:841-52.
13. Rajeshkumar S. Green synthesis of different sized antimicrobial silver nanoparticles using different parts of plants a review. Int J ChemTech Res 2016;9:197-208.
14. Rajeshkumar S, Bharath LV. Mechanism of plant-mediated synthesis of silver nanoparticles a review on biomolecules involved, characterisation and antibacterial activity. Chem Biol Interact 2017;273:219-27.
15. Rajeshkumar S. Antifungal impact of nanoparticles against different plant pathogenic fungi. Nanopart Plants Algae Microorganisms 2019;2:197-217.
16. Rai M, Yadav A, Gade A. Silver nanoparticles as a new generation of antimicrobials. Biotechnol Adv 2009;27:76-83.
17. Mlalila NG, Swai HS, Hilonga A, Kadam DM. Antimicrobial dependence of silver nanoparticles on surface plasmon resonance bands against Escherichia coli. Nanotechnol Sci Appl 2017;10:1-9.

18. Sondi I, Salopek-Sondi B. Silver nanoparticles as antimicrobial agent: A case study on E. coli as a model for gram-negative bacteria. J Colloid Interface Sci 2004;275:177-82.
19. Shrivastava S, Bera T, Roy A, Singh GV, Ramachandrarao P, Dash D. Characterisation of enhanced antibacterial effects of novel silver nanoparticles. Nanotechnology 2007;18;103-12.
20. Morones JR, Elechiguerra JL, Camacho A, Holt K, Kouri JB, Ramírez JT, et al. The bactericidal effect of silver nanoparticles. Nanotechnology 2005;16:2346-53.
21. Dror-Ehre A, Mamane H, Belenkova T, Markovich G, Adin A. Silver nanoparticle-E. coli colloidal interaction in water and effect on E. coli survival. J Colloid Interface Sci 2009;339:521-6.
22. Malarkodi C, Rajeshkumar S, Paulkumar K, Gnanajobitha G, Vanaja M, Annadurai G. Biosynthesis of semiconductor nanopar ticles by using sulfur reducing bacteria Serratia nematodiphila. Adv Nano Res 2013;1:83-91.
23. Manke A, Wang L, Rojanasakul Y. Mechanisms of nanoparticle-induced oxidative stress and toxicity. Bio Med Res Int 2013;2013:15.
24. Samuel U, Guggenbichler JP. Prevention of catheter-related infections: The potential of a new nano-silver impregnated catheter. Int J Antimicrob Agents 2004;23 Suppl 1:S75-8.
25. Bosetti M, Massè A, Tobin E, Cannas M. Silver coated materials for external fixation devices: In vitro biocompatibility and genotoxicity. Biomaterials 2002;23:887-92.
26. Rajeshkumar S, Malarkodi C. In vitro antibacterial activity and mechanism of silver nanoparticles against foodborne pathogens. Bioinorg Chem Appl 2014;2014:581890.
27. Boisselier E, Astruc D. Gold nanoparticles in nanomedicine: Preparations, imaging, diagnostics, therapies and toxicity. Chem Soc Rev 2009;38:1759-82.
28. Baptista PV, McCusker MP, Carvalho A, Ferreira DA, Mohan NM, Martins M, et al. Nano-strategies to fight multidrug resistant bacteria "A battle of the titans". Front Microbiol 2018;9:1441.
29. Wang L, Hu C, Shao L. The antimicrobial activity of nanoparticles: Present situation and prospects for the future. Int J Nanomedicine 2017;12:1227-49.

Source of support: Nil; Conflict of interest: None Declared