International Journal of Advanced Research in Biological Sciences
ISSN: 2348-8069
www.ijarbs.com
DOI: 10.22192/ijarbs Coden: IJARQG (USA) Volume 7, Issue 12 -2020

Research Article

DOI: http://dx.doi.org/10.22192/ijarbs.2020.07.12.021

Biogenic Silver Nanoparticles from *Ocimum sanctum* Leaves: Exploring Antibacterial Potential

Madhuchanda Banerjee
Department of Zoology, Midnapore College (Autonomous)
E-mail: *madhuchanda.banerjee@gmail.com*

Abstract

Silver nanoparticles (AgNPs) have garnered significant attention due to their unique physicochemical properties and broad-spectrum antimicrobial activity, with applications spanning medicine, textiles, electronics, and water treatment. Biological (green) synthesis of AgNPs, leveraging plant extracts is particularly advantageous due to its eco-friendliness, cost-effectiveness, and non-toxic nature. In this study, we synthesized AgNPs using aqueous leaf extract of *Ocimum sanctum* L. (Tulsi). The synthesized nanoparticles were characterized by UV–visible spectroscopy and transmission electron microscopy (TEM). Their antimicrobial efficacy was evaluated against representative Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*) and Gram-positive (*Staphylococcus aureus*, *Enterococcus faecalis*) bacterial strains. Results demonstrated that Tulsi-derived AgNPs exhibited potent antibacterial activity, with greater efficacy against Gram-negative bacteria. These findings underscore the potential of green-synthesized AgNPs as viable antibacterial agents, opening avenues for their incorporation into healthcare and food industry applications.

Keywords: silver nanoparticles, *Ocimum sanctum*, green synthesis, TEM, antibacterial activity, Gram-positive, Gram-negative

Introduction

Nanotechnology is an area of intense scientific research that deals with design, synthesis and application of particle structures ranging approximately between1-100nm[1,2]. Metallic nanoparticles such as silver, gold, and iron are well recognised to have significant applications in biomedical, optical and electric field.[3-8]One such important member of the noble metal NPs are silver nanoparticle (AgNPs). Silver is well known since ancient times because of its medical value and preservative properties. It is one of the basic elements that make up the Earth.

Silver nanoparticles (AgNPs) are particularly prominent due to their strong antimicrobial properties, which originate from their high surface area-to-volume ratio and unique surface chemistry. Their extremely large surface area which provide better contact with microorganisms. AgNPs have long been integrated into consumer products such as textiles, coatings, cosmetics, and medical devices.[8–12] These nanoparticles exhibit multifaceted biological activities, including antibacterial, antifungal, antiviral, and anticancer effects.[13–16] Although chemical and physical routes can synthesize AgNPs, green synthesis using plant-derived biomolecules offers a sustainable, low-cost, and biocompatible approach.[17–20]

Ocimum sanctum L., commonly known as Tulsi, is a revered medicinal plant renowned in traditional medicine for its antioxidant, antimicrobial, and anti-inflammatory properties.[21] Previously, Tulsi extract has been used to biofabricate metal nanoparticles, serving as both reducing and capping agents.[22–24]

However, comprehensive studies involving detailed nanoparticle characterization and comparative antibacterial assessment against diverse bacterial taxa remain limited.

In the present study AgNPs has been successfully synthesized via biological reduction using Tulsi leaf extract. These nanoparticles were characterized using UV–visible spectroscopy and TEM and their antibacterial activity against Gram-positive and Gram-negative bacteria was evaluated.

Materials and Methods

Preparation of Tulsi Leaf Extract

Fresh leaves of *Ocimum sanctum* were collected from a pesticide-free garden, thoroughly washed with tap water followed by distilled water to remove surface contaminants. Clean leaves were air-dried under shade for 5 days and then finely powdered using a sterile mortar and pestle. For extraction, 5 g of this powder was mixed with 100 mL of double-distilled water and boiled for 45 minutes in a borosilicate flask. After cooling to room temperature, the mixture was filtered through Whatman No. 1 filter paper, and the filtrate (pale brown in color) was stored in a sterile, amber-colored glass bottle at 4 °C until use (Fig 1).

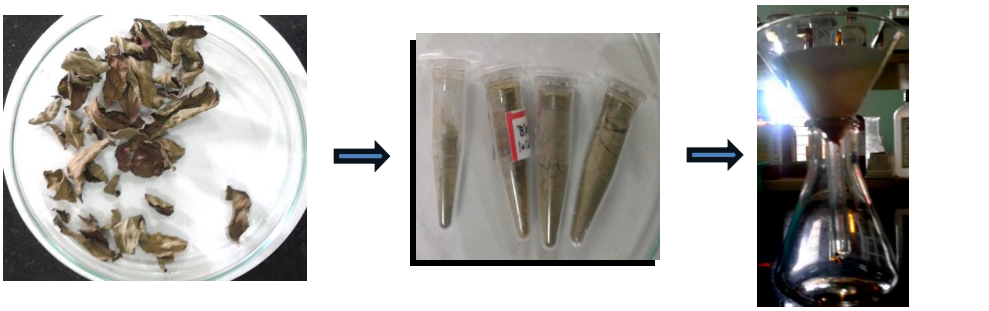

Figure 1. Preparation of Tulsi leaf extract

Green Synthesis of Silver Nanoparticles

Silver nitrate ($AgNO_3$, analytical grade, Merck) was used as the metal precursor. Aqueous $AgNO_3$ solution (0.5 M) was freshly prepared. To optimize nanoparticle formation, different volumes of the Tulsi extract were mixed with $AgNO_3$ solution to achieve final extract concentrations ranging from 1% to 10% (v/v). Each reaction mixture was made up to 5 mL total volume with distilled water. All mixtures were incubated in a water bath at 50 °C for 35 minutes. A color change from pale yellow to reddish-brown signified nanoparticle formation, attributed to surface plasmon resonance (SPR) of AgNPs.

UV–Visible Spectroscopy

The reduction of Ag^+ ions and stability of AgNPs were monitored using an Agilent Cary 60 UV–Vis spectrophotometer in the 200–900 nm range. Samples were scanned at regular intervals: immediately (0 h), and after 12 h, 24 h, 36 h, and 48 h of synthesis. Absorbance at ~415 nm indicated characteristic SPR of AgNPs. The optimal concentration of Tulsi extract yielding the most intense and stable peak was selected for all downstream experiments.

Purification and Characterization by TEM

AgNP suspensions were centrifuged at 10,000 rpm for 20 minutes at 25 °C. The size and shape of the biosynthesised AgNPs was determined by Transmission Electron Microscopy (JEOLJEM 2100, operating at 200KV). The sample for TEM was prepared by centrifuging the AgNP solution at 10,000 rpm at room temperature for 20minutes at 25°C, following which the concentrated NP was obtained as pellet.

Pellets were washed thrice with sterile deionized water and resuspended to remove unbound plant biomolecules and residual $AgNO_3$. For TEM analysis, a drop of this suspension was placed on carbon-coated copper grids, air-dried, and imaged using a JEOL JEM-2100 TEM operating at 200 kV. Particle size distribution was analyzed using **SIS Soft Imaging GmbH** image analysis software, measuring over 200 individual nanoparticles. Energy dispersive spectroscopy (EDS) was employed for elemental composition confirmation.

Antibacterial Activity Assay: MIC and MBC

Test organisms used for studying the antibacterial activity included both Gram-positive and Gram-negative bacteria such as *Escherichia coli* (MTCC 1687), *Pseudomonas aeruginosa* (MTCC 424), *Staphylococcus aureus* (MTCC 3160) and *Enterococcus faecalis* (MTCC 439).

Cultures were grown overnight in nutrient broth at 37 °C, adjusted to ~10^6 CFU/mL using McFarland standards. MICs were determined by broth microdilution. AgNPs were two-fold serially diluted (ranging from 1 µg/mL to 128 µg/mL) in Luria Bertani Broth (LBB), and 100 µL of each dilution was inoculated with 10 µL of bacterial suspension. Controls included wells with LBB + bacteria (growth control), LBB + AgNPs (sterility control), and LBB + Tulsi extract or $AgNO_3$ alone.

The culture tubes were incubated at 37 °C for 24 h. Optical density (OD) at 595 nm was measured using a UV-Vis Spectrophotometer. The lowest concentration with no visible turbidity (inhibiting visible growth) was recorded as MIC. For minimum bactericidal concentrations MBC determination, 10 µL from each MIC-negative tube were transferred on fresh media. Additionally, 10 µL from each MIC-negative tubewas streaked onto LB agar plates and incubated.MBC was defined as the lowest concentration yielding no colony formation after 24 h.

Results and Discussion

UV–Vis Spectroscopy of AgNPs

AgNPs were synthesized by using silver nitrate in the presence of tulsi leaf extract. No other chemical was used for this process. The production of silver nanoparticles using extract of tulsi leaf was indicated by the colour change from transparent to yellowish colour after the addition of tulsi leaf extract to the $AgNO_3$ solution.

A distinct surface plasmon resonance (SPR) peak emerged around 415 nm, confirming AgNP synthesis (Figure 2). Optimization experiments indicated that 6 % Tulsi extract yielded the most intense and stable absorbance peak. Lower extract concentrations yielded weaker SPR signals, while higher concentrations did not enhance nanoparticle formation. The SPR peak remained stable over 48 h, suggesting high colloidal stability, unlike chemically synthesized AgNPs that tend to aggregate within days.[25] This indicates that Tulsi-derived biomolecules function as both reducing and capping agents.

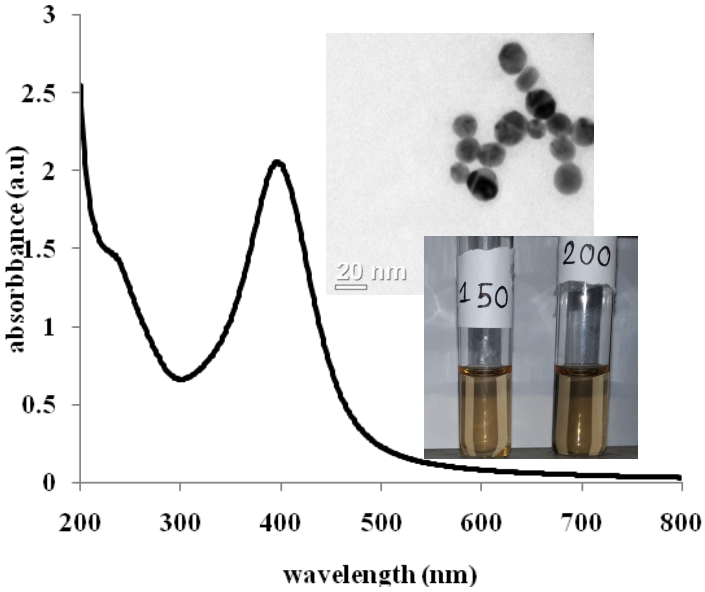

Figure 2. UV-visible spectra of AgNP synthesized using tulsi leaf extract Insets show the TEM images of corresponding particles and the AGNP solution.

TEM analysis

TEM micrographs (Figure 3) showed uniform, spherical nanoparticles, with size distribution between 8–20 nm (mean diameter ~14.7 nm). No significant aggregation was observed, and high-resolution images confirmed lattice fringes corresponding to silver's crystalline planes. Energy-dispersive X-ray spectroscopy (EDS) was employed to establish the chemical identity of the observed nanoparticles. EDS spectra demonstrated a strong Ag signal along with weak peaks for C and O, likely originating from capping phytochemicals in the extract.

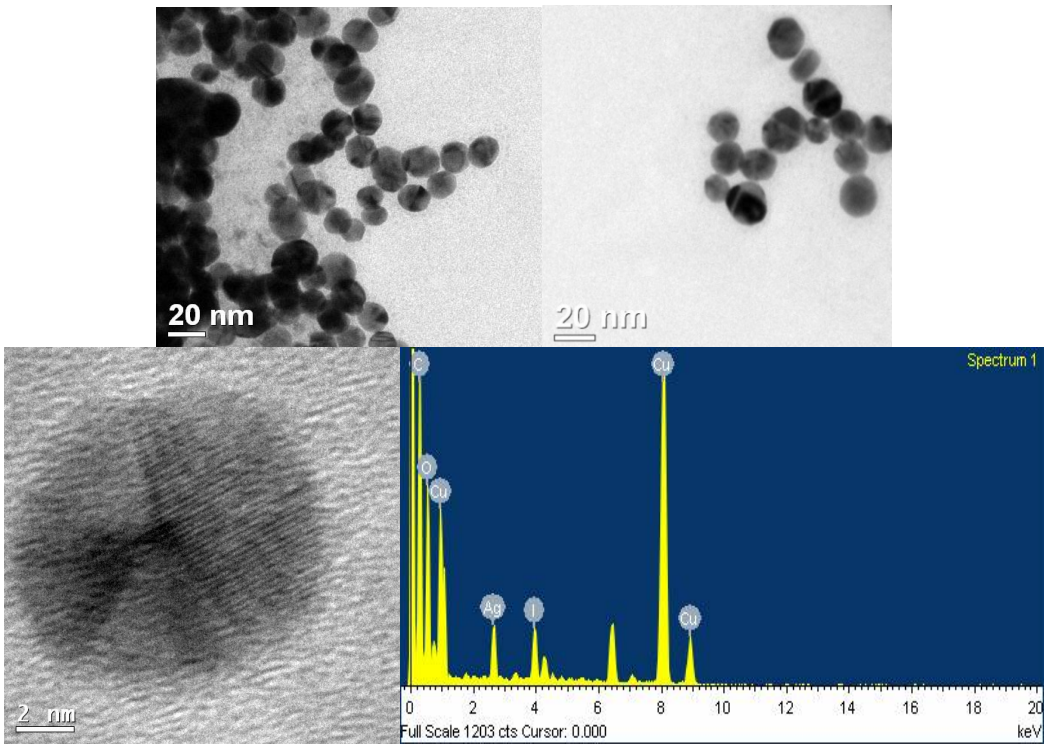

Figure 3. TEM micrograph Ag NPs. High resolution TEM micrograph showed the presence of lattice fringes corresponding to (111) planes of pure silver and EDX spectrum of Ag NPs. The copper peaks are due to the carbon-coated copper TEM grid used as a support for the sample.

Antimicrobial analysis

The biosynthesized AgNPs displayed broad-spectrum antibacterial activity as they are effective against both group of the test microorganisms(Table 1). Gram-negative strains (*E. coli*, *P. aeruginosa*) showed higher sensitivity, possibly due to their thinner peptidoglycan layer and outer membrane permeability. In contrast, Gram-positive strains required higher nanoparticle concentrations for inhibition, attributed to their thick peptidoglycan walls acting as a diffusion barrier.

The measurement of the OD (optical density) was performed at 595 nm and the bacterial growth was monitored at different time points. The OD value at 595 nm is due to the scattering of light by the bacterial cells and is a function of bacterial cell density and thus correlates with the growth of the colony. Control samples, which were either given only leaf extract or double distilled water along with LB media showed no significant growth inhibition (figure 4). The experiments were performed three times to ensure reproducibility.Negligible antibacterial activity of leaf extract and $AgNO_3$ controls exhibited at these concentrations indicate that silver ions or phytochemicals alone were insufficient at tested doses. This strongly supports the synergistic antibacterial mechanism of AgNPs synthesized via green routes.

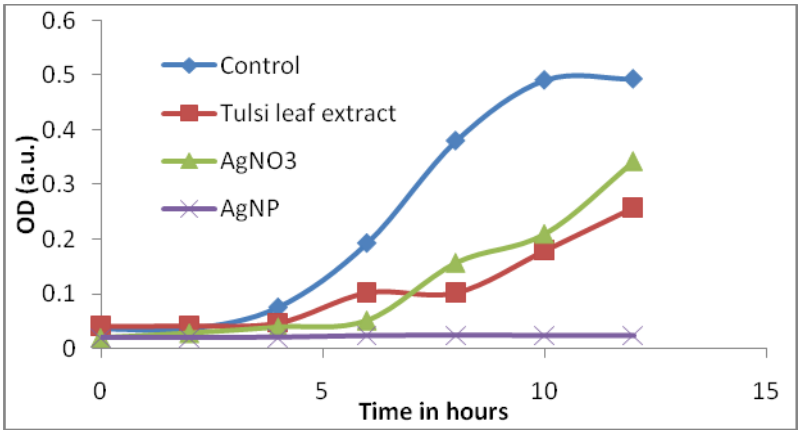

Figure 4. Effect of $AgNO_3$, aqueous extract of tulsi leaf and AgNPs on growth of *E.coli*

Table 1. MIC and MBC values of biosynthesized AgNPs synthesized on *Enterococcus faecalis*, *Staphylococcus aureus* (Gram-positive), and *Escherichia coli*, *Pseudomonas aeruginosa* (Gram-negative) bacteria

Bacteria	MIC (µg/mL)	MBC (µg/mL)
E. coli	25	50
P. aeruginosa	16.5	30
S. aureus	325	450
E. faecalis	3500	4000

It has also been found that the tulsi plant extract have shown more antibacterial activity than silver nitrate solution alone, which indicates that, the antimicrobial potency of tulsi extract is higher compared to $AgNO_3$ solutions at their respective concentration used in this study. However, when antibacterial activity of AgNPs synthesized using plant extract and $AgNO_3$solution was compared with the antibacterial activity of plant extract and $AgNO_3$ solution separately, it clearly shows that synthesised AgNPs were much more effective than that of both treatments ($AgNO_3$ solution and plant extract separately).This further proves that the inherent antibacterial activity of tulsi extract when coupled with antibacterial efficiency of AgNP synthesized from silver salt, show more beneficial to minimise bacterial growth than alone.

Possible Mechanism of Action

Silver nanoparticles exert their antimicrobial effect through multiple pathways. The small size facilitates close contact with microbial cells, leading to membrane disruption, increased permeability, and release of intracellular contents. [26–29]

AgNPs likely interact with bacterial membranes via electrostatic attraction, leading to membrane destabilization, leakage of cytoplasmic contents, and subsequent cell death. Additionally, smaller-sized nanoparticles may penetrate the membrane and interact with intracellular structures, generating ROS and interrupting DNA replication and protein synthesis.

Particularly, Ag^+ ions can collapse proton motive force in Gram-negative bacteria and inhibit key metabolic enzymes.[30–33]AgNPs also bind to thiol (-SH) groups in enzymes, impairing respiratory function.

Gram-negative bacteria typically have thinner peptidoglycan layers and negatively charged lipopolysaccharide-rich outer membranes, allowing efficient nanoparticle attachment and disruption. Gram-positive bacteria, with thicker peptidoglycan layers and teichoic acid-rich walls, exhibit more resistance to nanoparticle penetration.[34] Therefore, the structural differences in cell walls likely explain the observed efficacy differential.

This correlates with earlier findings[4,19] that smaller, bio-stabilized AgNPs exhibit enhanced bacterial penetration and target interaction compared to larger or chemically synthesized counterparts.

Conclusions

Tulsi-mediated green synthesis of silver nanoparticles provides a sustainable, affordable, and effective method for producing stable AgNPs with potent antibacterial properties. In the present work, nanoparticles were successfully synthesized, as confirmed by SPR (~415 nm peak). Further TEM confirmed shape (spherical, <20 nm), and EDS confirmed composition.

The antibacterial potency of silver is known for many years[14]. However, the exact mechanism by which AgNPs cause bacterial death is not clearly understood and several theories has been put forward to explain the microbicidal effect of AgNPs. Silver ions have been found to uncouple the respiratory chain from oxidative phosphorylation and lead to collapse of the proton motive force across the bacterial cytoplasmic membrane.AgNPs on the other hand may attach to the surface of cell membrane of microorganisms, leading to the disturbance of its functions like permeability and respiration. It is obvious, therefore, that the binding of particles to the microorganism depends on the surface area available for interaction. Small NPs have a larger surface area for interaction with bacteria, as compared to that of bigger particles, thus causing greater antibacterial activity[16-19]

Tulsi has been known to have antibacterial, antifungal, antiviral and anti-inflammatory activity from ancient periods for which they are extensively used as medicinal plant.[15]These biosynthesized AgNPs exhibit excellent stability and high antimicrobial activity against both Gram-positive and Gram-negative bacterial cells, however, there is a difference in the activity as Gram negative bacteria are more affected when compared to Gram positive cells. This could be due to their different cellular structure.

The dual properties of Tulsi extract as reducing and stabilizing agent, underpin nanoparticle stability and bioactivity. Future research should identify specific phytochemicals responsible, elucidate precise molecular mechanisms of antimicrobial action, assess cytotoxicity in mammalian systems, and evaluate efficacy in vivo. These advancements could pave the way for commercial applications in healthcare, food preservation, and water disinfection.

References

1. Lee, H. Y.; Park, H. K.; Lee, Y. M.; Kim, K.; Park, S. B. *Chem. Commun.*, 2007, *28*, 2885–2887.
2. Klaus, T.; Joerger, R.; Olsson, E.; Granqvist, C. G. *Proc. Natl. Acad. Sci. USA*, 1999, *96*, 13611–13614.
3. Dai, J.; Bruening, M. L. *Nano Lett.*, 2002, *2*, 497–501.
4. Chou, W.-L.; Yu, D.-G.; Yang, M.-C. *Polym. Adv. Technol.*, 2005, *16*, 600–607.
5. Navaladian, S.; Viswanathan, B.; Viswanath, R. P.; Varadarajan, T. K. *Nanoscale Res. Lett.*, 2007, *2*, 44.
6. Shameli, K.; Ahmad, M. B.; Yunus, W. M. Z. W.; Ibrahim, N. A.; Darroudi, M. *Int. J. Nanomed.*, 2010, *5*, 743–751.
7. Naik, R. R.; et al. *Nat. Mater.*, 2002, *1*, 169–172.
8. Rout, Y.; Behera, S.; Ojha, A. K.; Nayak, P. L. *J. Microbiol. Antimicrob.*, 2012, *4*, 103–109.
9. Singh, S.; Taneja, M.; Majumdar, D. K. *Indian J. Exp. Biol.*, 2007, *45*, 403–412.
10. Kaler, A.; Mittal, A. K.; Katariya, M.; Harde, H.; Agrawal, A. K.; Jain, S.; Banerjee, U. C. *J. Nanopart. Res.*, 2014, *16*, 2605.
11. Lalitha, A.; Subbaiya, R.; Ponmurugan, P. *Int. J. Curr. Microbiol. Appl. Sci.*, 2013, *2*, 228–235.
12. Hakkim, F. L.; Shankar, C. G.; Girija, S. *J. Agric. Food Chem.*, 2007, *55*, 9109–9117.
13. Mulvaney, P. *Langmuir*, 1996, *12*, 788–800.
14. Bauer, A. W.; Kirby, W. M.; Sherris, J. C.; Turck, M. *Am. J. Clin. Pathol.*, 1996, *45*, 493–496.
15. Sood, S.; Narang, D.; Dinda, A. K.; Maulik, S. K. *J. Pharm. Pharmacol.*, 2005, *57*, 127–133.
16. Naraginti, S.; Sivakumar, A. *Spectrochim. Acta A Mol. Biomol. Spectrosc.*, 2014, *128*, 357–362.
17. Tamayo, L. A.; et al. *Mater. Sci. Eng. C Mater. Biol. Appl.*, 2014, *40*, 24–31.
18. Choi, O.; Hu, Z. *Environ. Sci. Technol.*, 2008, *42*, 4583–4588.
19. Lu, Z.; et al. *Nanomedicine*, 2010, *5*, 1525–1533.
20. Franci, G.; Falanga, A.; Galdiero, S.; Palomba, L.; Rai, M.; Morelli, G.; Galdiero, M. *Molecules***2015**, *20*, 8856–8874.
21. Gautam, D. et al. *PeerJ***2023**, *11*, e15590.
22. Pirtarighat, S.; Ghannadnia, M.; Baghshahi, S. *Mater. Sci. Eng. C***2019**, *98*, 250–255.
23. Pal, S.; Tak, Y. K.; Song, J. M. *Appl. Environ. Microbiol.***2007**, *73*, 1712–1720.

24. Panacek, A. et al. *J. Phys. Chem. B***2006**, *110*, 16248–16253.
25. Ramteke, C.; Chakrabarti, T.; Sarangi, B. K.; *J. Chem.***2013**, *2013*, 1–7.
26. Rao, Y. S.; Kotakadi, V. S.; Prasad, T. N.; Reddy, A. V.; Gopal, D. S.*Acta A Mol. Biomol. Spectrosc.***2013**, *103*, 156–159.
27. Fayaz, A. M.; Balaji, K.; Girilal, M.; Yadav, R.; Kalaichelvan, P. T.; Venketesan, R. *Nanomedicine***2010**, *6*, 103–109.
28. Pattanayak, P.; Behera, P.; Das, D.; Panda, S. K. *Pharmacogn. Rev.***2010**, *4*, 95–105.
29. Sharma, V. K.; Yngard, R. A.; Lin, Y. *Adv. Colloid Interface Sci.***2009**, *145*, 83–96.
30. Gardea-Torresdey, J. L. et al. *Langmuir***2003**, *19*, 1357–1361.
31. Choi, S. H.; Oh, Y. K. *Int. J. Nanomedicine***2014**, *9*, 379–386.
32. Mittal, A. K.; Chisti, Y.; Banerjee, U. C. *Biotechnol. Adv.***2013**, *31*, 346–356.
33. Kharissova, O. V.; Dias, H. R.; Kharisov, B. I.; Pérez, B. O.; Pérez, V. M. J. *Trends Biotechnol.***2013**, *31*, 240–248.
34. Hajipour, M. J. et al. *Trends Biotechnol.***2012**, *30*, 499–511

Access this Article in Online	
Quick Response Code	Website: www.ijarbs.com
	Subject: Nanotechnology
DOI:10.22192/ijarbs.2020.07.12.021	

How to cite this article:
Madhuchanda Banerjee. (2020). Biogenic Silver Nanoparticles from *Ocimum sanctum* Leaves: Exploring Antibacterial Potential. Int. J. Adv. Res. Biol. Sci. 7(12): 190-196.
DOI: http://dx.doi.org/10.22192/ijarbs.2020.07.12.021