ijlpr 2020; doi 10.22376/ijpbs/lpr.2021.11.1.L148-154

International Journal of Life science and Pharma Research

Research Article

Microbiology for Health Care

# Green Synthesis of Silver Nanoparticles Using *Ocimum tenuiflorum* Leaf Extract and its Antimicrobial Activity against Certain Pathogens

**Pal Nidhi[1] and Shweta Agrawal [*2]**

[1]*Department of Microbiology, Shri Umiya Girls' College, Rau-453331, Indore, Madhya Pradesh, India*

[2]*Department of Life Science, Shri Vaishnav Institute of Science, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore-453111, Madhya Pradesh, India*

**Abstract:** Nanoparticles (NPs) are particles less than 100 nm in size. Silver nanoparticles have many important chemical and biological applications. These applications drag the attention of researchers for the production of silver nanoparticles. The objective of the study is to synthesize silver nanoparticles from the green method without using any hazardous chemicals. In the present study, *Ocimum tenuiflorum* (Tulsi) leaf extract was used to synthesize silver nanoparticles (Ag-NPs) from silver nitrate. Moreover, Tulsi leaf extract was also exploited, both traditionally and commercially, for their antibacterial potential which is essential oil components. The synthesis of Ag-NPs was visually deduced by a change in colour from light yellow to reddish-brown. UV-Visible Spectrometry, X-ray diffraction (XRD), and Transmission Electron Microscopic (TEM) analysis were then used to characterize synthesized nanoparticles. This study also determined the effect of temperature and effect of silver nitrate concentration on nanoparticle synthesis. Synthesized silver nanoparticles showed absorbance 'peaks' at 420 nm with UV-visible spectrometry. X-ray diffraction studies showed that the synthesized silver nanoparticles were crystalline. The mean crystallite size was estimated using the Debye-Scherrer equation. However, TEM showed that silver nanoparticles have spherical (40 nm) shape with a narrow size distribution. These synthesized nanoparticles were used to evaluate antimicrobial activity against the most common pathogens of nosocomial infections, MRSA, *E. coli, Salmonella typhi,* and *Klebsiella pneumoniae* (ESBL). *O. tenuiflorum* extract showed strong potential for the synthesis of silver nanoparticles from silver nitrate and the synthesized silver nanoparticles had efficient antimicrobial properties against different pathogens including drug-resistant pathogens *in vitro*. These synthesized nanoparticles showed maximum 'zones' of inhibition against MRSA among these four organisms.

**Keywords:** *Ocimum tenuiflorum,* MRSA, ESBL, Silver nanoparticles, XRD, TEM

***Corresponding Author**

**Dr. Shweta Agrawal , Department of Life Science, Shri Vaishnav Institute of Science, Shri Vaishnav Vidyapeeth Vishwavidyalaya, Indore-453111, Madhya Pradesh, India**

**Recieved On 04 July 2020**
**Revised On 23 September 2020**
**Accepted On 26 September 2020**
**Published On 04 January 2021**

**Funding** This research did not receive any specific grant from any funding agencies in the public, commercial or not for profit sectors.

**Citation** Pal Nidhi and Shweta Agrawal , Green Synthesis of Silver Nanoparticles Using Ocimum tenuiflorum Leaf Extract and its Antimicrobial Activity against Certain Pathogens.(2021).Int. J. Life Sci. Pharma Res.11(1), L148-154 http://dx.doi.org/10.22376/ijpbs/lpr.2021.11.1.L148-154

## 1. INTRODUCTION

Nanoparticles (NPs) are particles less than 100 nm in size. Nanoparticles show unique physical, chemical, and biological properties at the nanoscale as compared to their properties at higher scales. They also differ in shape and size from the bulk material.[1] Nanoparticles have significant antimicrobial property and are also considered as nano-antibiotics.[2] Silver nanoparticles have important medical application because they can work as bactericidal and fungicidal agents.[3] Silver nanoparticles can damage bacterial genetic material as well as the cell wall and their respiratory enzymatic pathway of bacteria.[4] It has been also reported that the antimicrobial property of silver is due to its ability to form free radicals which induce bacterial cell damage.[5-6] There are several chemical and physical methods available for the synthesis of silver nanoparticles.[7] The synthesis of silver nanoparticles from the green method is a bottom-up approach of synthesis of silver nanoparticles.[8-9] Biological systems such as the use of plant materials give an eco-friendly and alternative source for the production of nanoparticles.[10] There are several advantages of biosynthesis of nanoparticles using plant sources such as cost-effectiveness, eco-friendliness, fewer energy requirements than traditional synthesis methods.[11-12] Parameters like high temperature, energy, pressure, and harmful chemicals are avoided in the green synthesis method.[13] Various organic, inorganic, and capping agents are present in plant and are used for the reduction of silver ions ($Ag^+$) and stabilization of silver nanoparticles in plant-based synthesis.[14-15] Silver nanoparticles have various applications like in skin ointments to prevent burn and wound infections, in the field of agriculture, and also as an efficient inorganic catalyst.[16-18] In the present study, *Ocimum tenuiflorum* (commonly known as 'Tulsi') leaves were used to prepare silver nanoparticles. *O. tenuiflorum* is a well-known traditional medicinal plant in India. *O. tenuiflorum* contains bio reductants and stabilizers. This property is very important for the synthesis of silver nanoparticles. In the present study MRSA, *E. coli, S. typhi*, *Klebsiella pneumoniae* (ESBL) were used to evaluate the antimicrobial activity of synthesized silver nanoparticles. MRSA *(Methicillin-resistant Staphylococcus aureus*) is responsible for major hospital-acquired infection. MRSA does not respond to penicillin therefore other drugs like vancomycin must be used to treat the infection of MRSA.[19] *E. coli* is responsible for many common infections such as urinary tract infection, neonatal septicemia, etc.[20] *Salmonella typhi* is responsible for typhoid fever which infects the intestinal tract and blood of animals.[21] *Klebsiella pneumoniae* can cause pneumonia and bloodstream infections. It is an opportunistic pathogen that commonly infects hospitalized patients.[22]

## 2. MATERIALS AND METHODS

All the materials used in the present study were of analytical grade. Silver nitrate was purchased from Qualigens fine chemicals, Mumbai, India.

### *2.1. Synthesis of Silver Nanoparticles*

Fresh leaves of *O. tenuiflorum* were collected from the botanical garden of Umiya Girls' College, Indore, M.P., India, and washed several times with distilled water. 20 grams of leaves were taken and were ground in a pestle mortar. The crude extract obtained was then heated at 60 ± 2 °C in 100 ml sterilized distilled water. After cooling, the above plant extract was filtered by using Whatman filter paper No. 1. This extract can be stored in the refrigerator, below 5 °C, for future use. 10 ml of leaf extract was mixed with 45 ml of 1mM silver nitrate solution followed by stirring at 1000 rpm on a magnetic stirrer at 70 °C until the colour has changed.[23]

### *2.2. Effect of Temperature and Silver Nitrate Concentration on Silver Nanoparticles Synthesis*

To optimize the temperature of incubation, the reaction flasks containing both leaf extract and $AgNO_3$ were incubated at different temperatures (30, 40, 50, 60, and 70 °C) under shaking conditions (150 rpm). The Ag-NPs synthesized at different temperature was analysed using UV-Vis Spectrophotometer. To optimize the initial concentration of silver ion, the flasks were incubated at 35 ± 2 °C (150 rpm) containing different concentrations of $AgNO_3$ (0.2, 0.4, 0.8, and 1.0 mM) with a fixed concentration of leaf extract.[24]

### *2.3. Antimicrobial Activity of Ag-NPs*

Ag-NPs were tested for antimicrobial activity by agar well diffusion method against four clinical isolates (*E.coli, Salmonella typhi, Klebsiella* (ESBL) and MRSA). All the bacterial strains were subcultured in nutrient broth at 35 ± 2 ° C for 16-18 h on a rotary shaker at 150 rpm. Log phase bacterial inoculums ($10^6$ cells/mL) were standardized against McFarland's standard were swabbed uniformly on to the Nutrient agar plates using a sterile cotton swab. Three wells were punctured in each plate and were filled with 0.08 ml 1mM synthesized nanoparticles, 1 mM silver nitrate solution, and plant extract, respectively. All the plates were prepared in triplicates and incubated at 35 ± 2 ° C for 24 h. The different inhibition zones were measured.[25]

### *2.4. Assessment of increase in the fold area*

The increase in fold area was determined by calculating the mean surface area of the inhibition zone of each silver nitrate and Ag-NPs. The fold increase area of different test bacteria for silver nitrate and Ag-NPs was calculated by the equation.

$$\frac{(B^2 - A^2)}{A^2}$$

Where A and B are inhibition 'zones' of $AgNO_3$ and Ag-NPs respectively.[26]

### *2.5. Characterization of Silver Nanoparticles*

#### *2.5.1. UV-Vis Spectroscopy*

The preliminary detection of Ag-NPs was carried out by visual observation of colour change of the leaf extract and silver nitrate mixture. These samples were later subjected to optical measurements, carried out by using a UV-Vis spectrophotometer (Labtronics) and scanning the spectra between 300 and 700 nm at a resolution of 1 nm. Blanks were prepared with distilled and deionized (DI) water.[27]

#### *2.5.2. X-ray Diffraction Study*

X-ray powder diffraction (XRD) patterns were recorded using a 0.154 nm Cu Kα radiation between the range 30 ° to

90 °. The diffractometer was calibrated using a standard Si sample. The mean crystallite size was estimated using the Debye-Scherrer equation.

$$D = \frac{K\lambda}{\beta \cos \theta}$$

The above is the Debye-Scherrer equation. In this equation K = crystal shape factor usually 0.9, λ= wavelength of the x-ray radiation, β=FWHM i.e. peak width in half the maximum height and theta is the diffraction angle in degree.[25]

#### *2.5.3. TEM Analysis of Silver Nanoparticles*

Transmission electron microscopy was used to determine the size and shape of these biosynthesized nanoparticles. A drop of solution was loaded on the copper grid and a thin film of Ag-NPs sample was prepared. This Ag-NPs film was placed under the sample chamber of TEM and the results were recorded after focusing. TEM Model TALOS (Company FEI) was used for the measurement of silver nanoparticles at the accelerating voltage of 200 kV.[28]

### 2.6. STATISTICAL ANALYSIS

Data were analyzed by one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparisons test. Readings were considered significant when P was ≤0.05 using SPSS 16.0.

## 3. RESULTS AND DISCUSSIONS

### *3.1. Ag-NPs synthesis*

Before reaction, for all the experiments, the culture medium added with silver nitrate presents a weak transparent-yellow coloured solution. All experiments were carried out until observation of colour modification, light yellow to reddish-brown, of the culture medium (Figure 1). These colour changes arise because of the excitation of surface plasmon vibrations.[29]

### *3.2. Effect of operational parameters*

UV-Vis Spectroscopy analysis of Ag-NPs was used to investigate various parameters such as temperature and reactants concentration. Iravani *et.al* study, revealed that the parameters of the reaction such as temperature, silver nitrate concentration, reaction time play important role in the synthesis of Ag-NPs.[14] An increase in absorbance value of the reaction mixture with the increase in incubation temperature indicated higher production of Ag-NPs at an elevated temperature (Graph 2). This could be a characteristic fact that kinetic energy increases due to high temperature, which increases the rate of synthesis.[30] Absorption peak was found to improve upon increasing the incubation temperature from 30 to 70 °C and thereafter decreased. The maximum absorbance was observed at 70 °C indicating maximum yield. Maximum absorbance indicating the conversion of maximum silver ions in silver nanoparticles Various concentrations of silver nitrate solution (0.2–1 mM) were reacted with plant extract. After optimizing the reaction condition the sample obtained at 70 °C reaction temperature was the best sample. It was also observed that as the temperature increases to 70 °C the rate of reduction of silver ions increases. The yield of Ag-NPs increased with the increase in silver nitrate concentration (0.2–1 mM) and the maximum yield was obtained with 1 mM (Graph 3), this concentration was selected for further studies.

### *3.3. UV-Vis analysis*

This analysis showed an absorbance peak at 420 nm (Graph 1), specifically for the silver nanoparticles. Metal nanoparticles in the visible range exhibited strong absorption of electromagnetic waves due to surface plasmon resonance (SPR). It is observed that the silver surface plasmon resonance occurs at 420 nm and there is no change in peak position, suggesting that nucleation of silver nanoparticles starts with the initiation of reaction time only, and the size remains unchanged throughout the reaction. According to Mie theory, only a single SPR band is expected in the absorption spectra of spherical nanoparticles whereas, the number of 'peaks' increases as anisotropy increases.[27] In the present study, the SPR band reveals the spherical shape of silver nanoparticles, which was further confirmed by TEM. The reduction of silver ions was quite rapid. More than 90% of the reaction is complete within 35 minutes of the reaction time. Generally, biosynthetic methods are considered as time-consuming when compared with chemical methods. To the best of our knowledge, the reaction time of at least 12 h is required in plant-mediated nanomaterials synthesis. However, the time consumed in the present study for the reaction to complete is several-fold lesser than reported. Such alacrity in reaction time can be the outcome of the potent antioxidant activity of the Tulsi extract, which makes the reaction much more efficient than others.[26]

### *3.4. XRD spectra*

XRD spectra of silver nanoparticle solution showed 'peaks' at 38 $^0$, 41 $^0$, 53 $^0$, and 59 $^0$ of 2 theta. The characteristic sharp peaks indicate the cubic crystalline nature of the Ag-NPs synthesized from Tulsi leaf extract. The calculation with the Debye-Scherrer equation also revealed that the particle is in nanoscale. XRD studies concluded that the particles were crystalline and the estimated size was calculated 40.2 nm. (Graph 4).

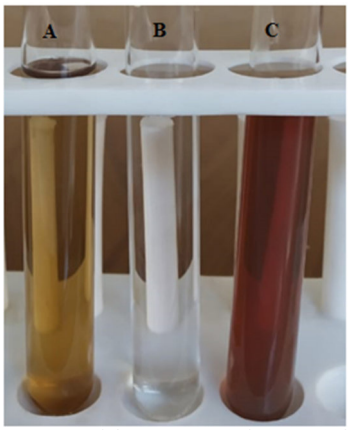
### *3.5. TEM image*

TEM studies of the synthesized nanoparticle sample showed the size of particle size ~ 40 nm and shape was spherical**.** These variations are common in biologically synthesized nanoparticles. The edges of nanoparticles were mainly lighter than the centres that were visible due to proteins of biomolecules capped with Ag-NPs (Figure 2). The Magnification image reveals the nanoparticles are embedded in a dense matrix which may be the organic stabilizing components of Tulsi leaf extract.

### *3.6. Antimicrobial activity of biosynthesized silver nanoparticles*

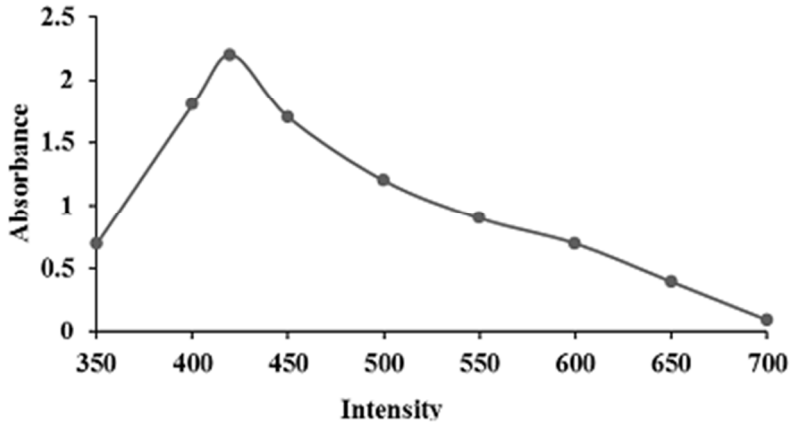
These synthesized silver nanoparticles show the zone of inhibition against *E.coli, Salmonella typhi,* MRSA*, Klebsiella* (ESBL*)*. The 'zones' diameter of synthesized silver nanoparticles, $AgNO_3$, and plant extract measured against these organisms and shown in table 1. In the present study, the Ag-NPs synthesized using *Ocimum tenuiflorum* extract and it exhibits a fairly significant antibacterial action against tested

bacteria. The diameter of the zone of inhibition values showed the antimicrobial property of Ag-NPs. Tulsi extract and distilled water were used as control. For all four bacterial strains, no zone of inhibition was observed for control. Bio reduced silver nanoparticles showed considerable growth inhibition of four well-known pathogenic bacterial species. Zones of 38 mm,17 mm,32 mm, and 16 mm were observed for *E.coli, Salmonella typhi,* MRSA, *and Klebsiella* (ESBL*)* respectively. Coupling of the inherent property of Tulsi extract with that of silver nanoparticles has proved to be beneficial to minimize the dose that needs to be administered for total microbial reduction. The antibacterial potential of silver is known for many years. Tulsi extract has also been much exploited, both traditionally and commercially, for their antibacterial potential which is an upshot of essential oil components.[28] There are the various suggested mechanism of Ag-NPs onto bacteria and the exact mode of action of silver nanoparticles are not known but according to one theory that silver nanoparticles form free radicals when these free radicles come in contact with bacterial cell they make pore in the cell membrane that leads to the death of the bacterial cell.[31] Ag-NPs do not only attach to the bacterial cell but also enter inside the cell alter ATP production and DNA replication and damage directly the bacterial structures.[32] Various antioxidants are present in *Ocimum tenuiflorum*, they uniquely trap free radicals which reduces the Ag metal ions.[33] The reduction of $Ag^{+}$ confirmed by colour changes after the addition of leaves extract in silver nitrate solution.

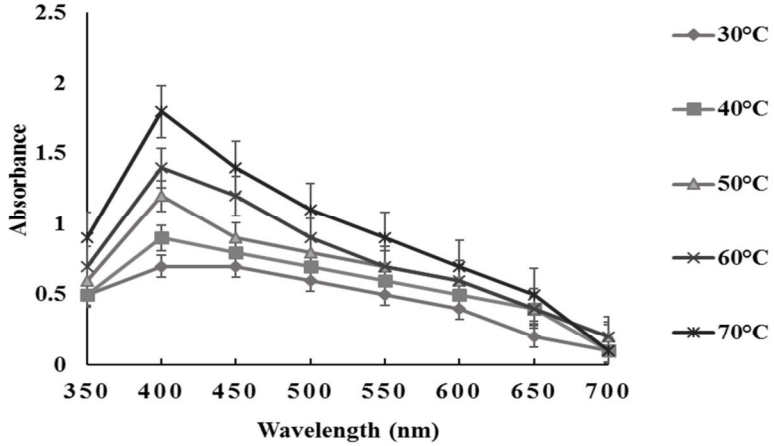



*(A), 1 mM $AgNO_3$ solution (B), and a mixture of Ocimum tenuiflorum leaf extract in 1 mM $AgNO_3$ reaction (C).*

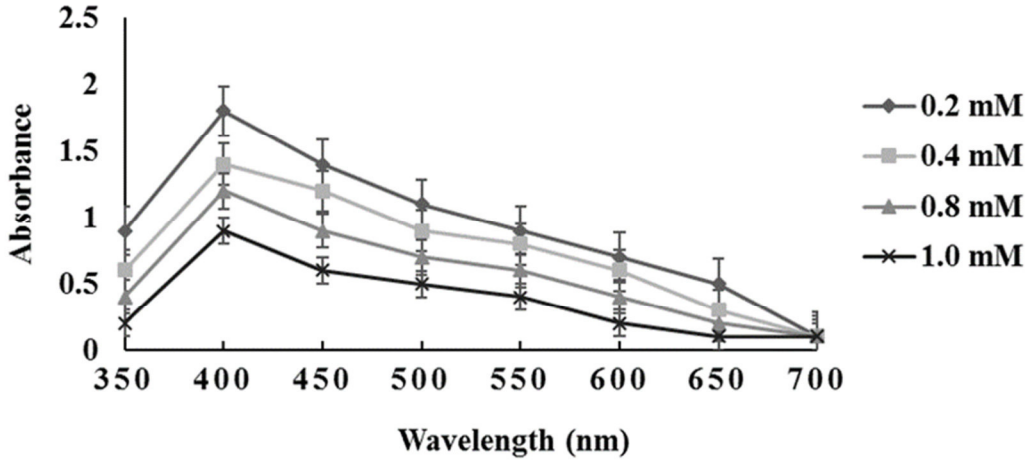
**Fig 1. *The Ocimum tenuiflorum leaf extract***



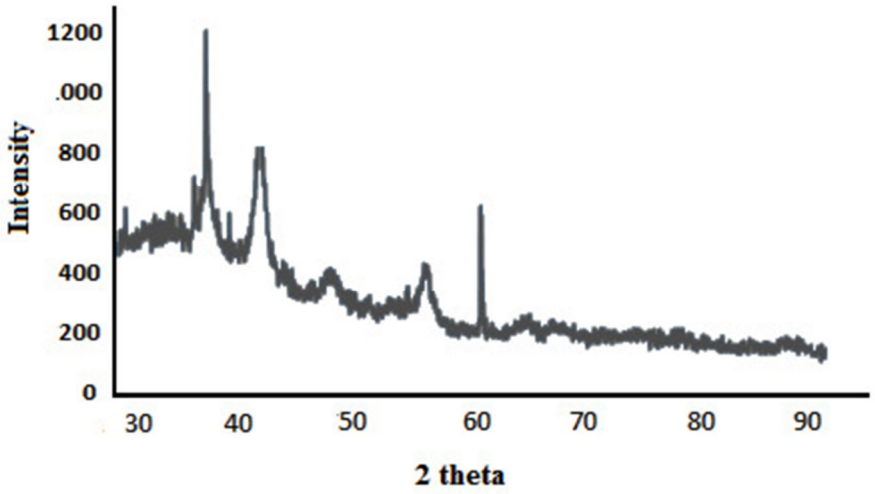

**Graph 1. UV- Vis spectra of silver nanoparticles synthesized from *Ocimum tenuiflorum* leaf extract.**




**Graph 2. UV –Vis spectra of silver nanoparticles synthesized from *Ocimum tenuiflorum* leaf extract at different reaction temperatures.**




**Graph 3. UV–Vis spectra of synthesized Ag-NPs showing the effect of variation of $AgNO_3$ concentration.**




**Graph 4. XRD pattern of Silver nanoparticles synthesized from *Ocimum tenuiflorum leaf extract*.**

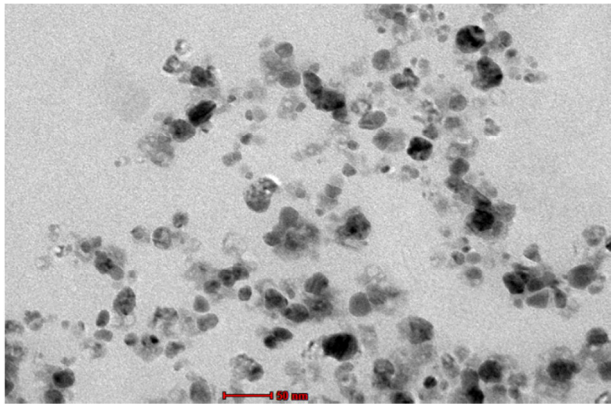
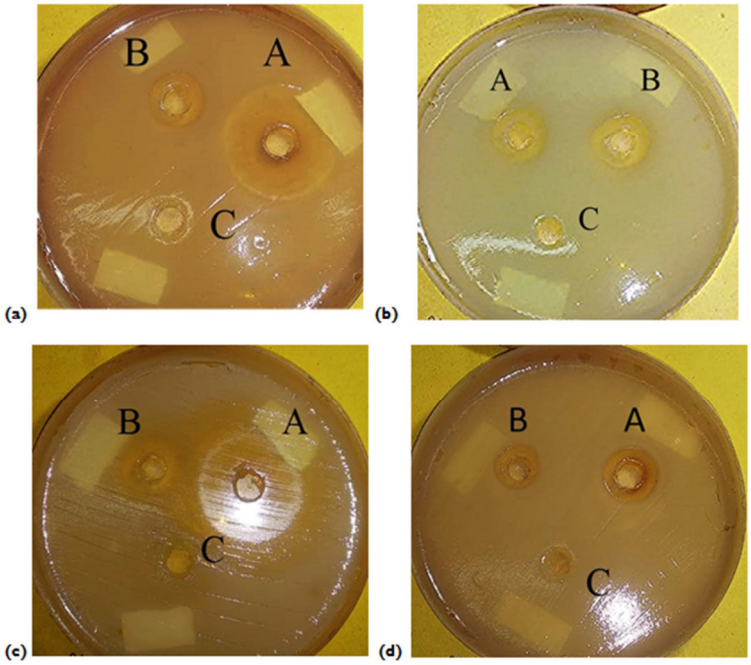


**Fig 2. TEM image of Silver nanoparticles synthesized from *Ocimum tenuiflorum leaf extract*.**

**Table 1. Zone diameter against MRSA, *E. coli, Salmonella typhi, Klebsiella* (ESBL)**

| Test Organisms | Zone of Inhibition (mm, mean±SD)* | | | Increase in the fold area |
|---|---|---|---|---|
| | Ag-NPs | 1 mM of $AgNO_3$ | Plant Extract | |
| MRSA | 37.66±0.57 | 18±1.0 | 14±1.0 | 3.37 |
| *E.coli* | 17.0±1.0 | 15±1.0 | 0.0 | 0.28 |
| *Salmonella typhi* | 32.33±0.57 | 16±0.0 | 0.0 | 3.08 |
| *Klebsiella* (ESBL) | 16.0±1.0 | 14.33±0.57 | 0.0 | 0.24 |

*± Mean surface area of the inhibition zone was calculated for each from the man diameter.*




*(a) MRSA, (b) E.coli, (c)-Salmonella typhi, (d) Klebsiella (ESBL). A=Ag-NPs, B= $AgNO_3$, C= Plant Extract of Ocimum tenuiflorum*

**Fig 3. Zone of inhibition of synthesized silver nanoparticles against selected pathogens**

## 4. CONCLUSION

The present method eludes the use of toxic chemicals for the synthesis of silver nanoparticles so it can be used for biological applications. The study showed that *Ocimum tenuiflorum* has strong potential to synthesize silver nanoparticles from silver nitrate and these silver nanoparticles have efficient antimicrobial properties against *E.coli, Salmonella typhi,* MRSA, *Klebsiella (*ESBL*) in vitro*. The method of synthesis of nanoparticles is rapid and eco-friendly. The synthesized nanoparticles were spherical and had an estimated size of about 40 nm. The zone of inhibition of silver nanoparticles is bigger than silver nitrate which reveals the high potential of Tulsi extract stabilized Ag-NPs to be used as an antimicrobial agent in the medical field as well as food and cosmetic industries.

## 5. AUTHORS CONTRIBUTION STATEMENT

Pal N., carried out the experiment and wrote the manuscript with support from Agrawal S. Agrawal S., supervised the findings of this work. All authors discussed the results and contributed to the final manuscript.

## 6. ACKNOWLEDGMENTS

The authors wish to express their sincere gratitude to UGC-DAE Consortium for Scientific Research Indore, M.P, India for providing XRD facility and Indian Institute of Science Education and Research (IISER) Bhopal, Madhya Pradesh, India for the TEM analysis.

## 7. CONFLICT OF INTEREST

Conflict of interest declared none.

## 8. REFERENCES

1. Pal N, Agrawal S. Green expertise methods for the synthesis of silver nanoparticles and their applications: a review. Alochana Chakra J. 2020;9(6):77-85.
2. Agarwal H, Venkat Kumar S, Rajeshkumar S. A review on green synthesis of zinc oxide nanoparticles – an eco-friendly approach. Resour Effic Technol. 2017;3(4):406-13. doi: 10.1016/j.reffit.2017.03.002.
3. Salem W, Leitner DR, Zingl FG, Schratter G, Prassl R, Goessler W, Reidl J, Schild S. Antibacterial activity of silver and zinc nanoparticles against Vibrio cholerae and enterotoxic Escherichia coli. Int J Med Microbiol. 2015;305(1):85-95. doi: 10.1016/j.ijmm.2014.11.005, PMID 25466205.
4. Lara HH, Ayala-Núñez NV, Ixtepan Turrent LdC, Rodríguez Padilla CR. Bactericidal effect of silver nanoparticles against multidrug-resistant bacteria. World J Microbiol Biotechnol. 2010;26(4):615-21. doi: 10.1007/s11274-009-0211-3.
5. Rai MK, Deshmukh SD, Ingle AP, Gade AK. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. J Appl Microbiol. 2012;112(5):841-52. doi: 10.1111/j.1365-2672.2012.05253.x, PMID 22324439.
6. Danilczuk M, Lund A, Sadlo J, Yamada H, Michalik J. Conduction electron spin resonance of small silver particles. Spectrochim Acta A Mol Biomol Spectrosc. 2006;63(1):189-91. doi: 10.1016/j.saa.2005.05.002, PMID 15978868.
7. Gudikandula K, Charya Maringanti S. Synthesis of silver nanoparticles by chemical and biological methods and their antimicrobial properties. J Exp Nanosci. 2016;11(9):714-21. doi: 10.1080/17458080.2016.1139196.
8. Elghanian R, Storhoff JJ, Mucic RC, Letsinger RL, Mirkin CA. Selective colorimetric detection of polynucleotides based on the distance-dependent

optical properties of gold nanoparticles. Science. 1997;277(5329):1078-81.
doi: 10.1126/science.277.5329.1078, PMID 9262471.
9. Pal N, Agrawal S. Classification and synthesis of nanoparticles. Innov Inventation & Ind Emerg Issues. 1997; ISBN-978-93-88237:58-1.
10. Antony E, Gunasekaran S, Sathiavelu M, Arunachalam S. A review on use of plant extracts for gold and silver nanoparticle synthesis and their potential activities against food pathogens. Asian J Pharm Clin Res. 2016;9:18-23.
11. Sun Y, Xia Y. Shape-controlled synthesis of gold and silver nanoparticles. Science. 2002;298(5601):2176-79. doi: 10.1126/science.1077229, PMID 12481134.
12. Agrawal S et al. Bacterial synthesis of metallic nanoparticles current trends and potential applications as soil fertilizer and plant protectants. In: Raliya, R. (ed.) Nanoscale Engineering in Agricultural Management. Vol. 1. London: CRC Press Press; 2019. p. 30-63. doi: 10.1201/9781315123950.
13. Ahmed S, Ahmad M, Swami BL, Ikram S. A review on plants extract mediated synthesis of silver nanoparticles for antimicrobial applications: a green expertise. J Adv Res. 2016;7(1):17-28.
doi: 10.1016/j.jare.2015.02.007, PMID 26843966.
14. Tran QH, Nguyen VQ, Le AT. Silver nanoparticles: synthesis, properties, toxicology, applications and perspectives. Adv Nat Sci: Nanosci Nanotechnol. 2013;4(3):1-20. doi: 10.1088/2043-6262/4/3/033001.
15. Iravani S, Korbekandi H, Mirmohammadi SV, Zolfaghari B. Synthesis of silver nanoparticles: chemical, physical and biological methods. Res Pharm Sci. 2014;9(6):385-406. PMID 26339255.
16. Bhusnure OG, Kuthar VS, Gholve SB, Giram PS, Shembekar VS, Zingade SG, Jadhav P. Green synthesis of silver nanoparticle using Catharanthus roseus extract for pharmacological activity. Int J Pharmacogn Phytochem Res. 2017;10:77-88.
17. Agrawal S, Rathore P. Nanotechnology pros and cons to agriculture: a review. Int J Curr Microbiol Appl Sci. 2014;3(3):43-55. doi: 10.13140/2.1.1648.1926.
18. Firdhouse MJ, Lalitha P. Biosynthesis of Silver Nanoparticles and Its Applications. J Nanotechnol. 2015;2015:1-18. doi: 10.1155/2015/829526.
19. Green BN, Johnson CD, Egan JT, Rosenthal M, Griffith EA, Evans MW. Methicillin-resistant Staphylococcus aureus: an overview for manual therapists(). J Chiropr Med. 2012;11(1):64-76.
doi: 10.1016/j.jcm.2011.12.001, PMID 22942844.
20. Lee DS, Lee SJ, Choe HS. Community-acquired urinary tract infection by Escherichia coli in the era of antibiotic resistance. BioMed Res Int. 2018;2018:7656752. doi: 10.1155/2018/7656752.
21. Veeraraghavan B, Pragasam AK, Bakthavatchalam YD, Ralph R. Typhoid fever: issues in laboratory detection, treatment options & concerns in management in developing countries. Future Sci OA. 2018;4(6):FSO312. doi: 10.4155/fsoa-2018-0003. PMID 30057789.
22. Parveen RM, Khan MA, Menezes GA, Harish BN, Parija SC, Hays JP. Extended-spectrum β-lactamase producing Klebsiella pneumoniae from blood cultures in Puducherry, India. Indian J Med Res. 2011;134:392-5. PMID 21985825.
23. K G. NEEM (AZADIRACHTA INDICA A. JUSS) AS A SOURCE FOR GREEN SYNTHESIS OF NANOPARTICLES. Asian J Pharm Clin Res;11(3).
doi: 10.22159/ajpcr.2018.v11i3.21939.
24. Ndikau M, Noah NM, Andala DM, Masika E. Green Synthesis and Characterization of Silver Nanoparticles Using Citrullus lanatus Fruit Rind Extract. Int J Anal Chem. 2017;2017:8108504.
doi: 10.1155/2017/8108504. PMID 28316627.
25. Bindhani BK, Panigrahi AK. Biosynthesis and Characterization of Silver Nanoparticles (Snps) by using Leaf Extracts of Ocimum Sanctum L (Tulsi) and Study of its Antibacterial Activities. J Nanomed Nanotechnol;s6. doi: 10.4172/2157-7439.S6-008.
26. Gajbhiye M, Kesharwani J, Ingle A, Gade A, Rai M. Fungus-mediated synthesis of silver nanoparticles and their activity against pathogenic fungi in combination with fluconazole. Nanomedicine. 2009;5(4):382-6.
doi: 10.1016/j.nano.2009.06.005, PMID 19616127.
27. Philip D, Unni C. Extracellular biosynthesis of gold and silver nanoparticles using Krishna tulsi (Ocimum sanctum) leaf. Phys E. 2011;43(7):1318-22.
doi: 10.1016/j.physe.2010.10.006.
28. Ramteke C, Chakrabarti T, Sarangi BK, Pandey RA. Synthesis of silver nanoparticles from the aqueous extract of leaves of Ocimum sanctum for enhanced antibacterial activity. J Chem. 2013;2013:1-7.
doi: 10.1155/2013/278925.
29. Singhal G, Bhavesh R, Kasariya K, Sharma AR, Singh RP. Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity. J Nanopart Res. 2011;13(7):2981-8. doi: 10.1007/s11051-010-0193-y.
30. Tomer AK, Rahi T, Neelam DK, Dadheech PK. Cyanobacterial extract-mediated synthesis of silver nanoparticles and their application in ammonia sensing. Int Microbiol. 2019;22(1):49-58.
doi: 10.1007/s10123-018-0026-x, PMID 30810931.
31. Kim JS, Kuk E, Yu KN, Kim JH, Park SJ, Lee HJ, Kim SH, Park YK, Park YH, Hwang CY, Kim YK, Lee YS, Jeong DH, Cho MH. Antimicrobial effects of silver nanoparticles. Nanomedicine. 2007;3(1):95-101.
doi: 10.1016/j.nano.2006.12.001, PMID 17379174.
32. Al-Shmgani HSA, Mohammed WH, Sulaiman GM, Saadoon AH. Biosynthesis of silver nanoparticles from Catharanthus roseus leaf extract and assessing their antioxidant, antimicrobial, and wound-healing activities. Artif Cells Nanomed Biotechnol. 2017;45(6):1-7. doi: 10.1080/21691401.2016.1220950. PMID 27534756.
33. Sukumaran P, Poulose E. Silver nanoparticles: mechanism of antimicrobial action, synthesis, medical applications, and toxicity effects. Int Nano Lett. 2012;2;5326:2228.