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SYNTHESIS OF SILVER NANOPARTICLES USING A MIXTURE OF *AZADIRACHTA INDICA*, *TINOSPORA CORDIFOLIA* AND *OCIMUM SANCTUM* AND THEIR ANTIMICROBIAL ACTIVITY

GUPTA T[*1], PANDEY G[2], CHATURVEDI P[3] AND DOTANIYA S[4]

1: Associate Professor, Department of Chemistry, IIS (Deemed to be) University, Jaipur

2: Assistant Professor, Department of Zoology, IIS (Deemed to be) University, Jaipur

3: Associate Professor, Department of Biotechnology, IIS (Deemed to be) University, Jaipur

4: PG Scholar, Department of Chemistry, IIS (Deemed to be) University, Jaipur

***Corresponding Author: Dr. Trapti Gupta: E Mail: trapti.gupta@iisuniv.ac.in**

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ABSTRACT

Silver nanoparticles (AgNPs) have already been reported as a viable remedy for microbial activities. There are a number of traditional procedures that use chemical processes and are linked to environmental harm. Green AgNP synthesis has been reported as safe, low-cost, and ecologically sustainable. Based on certain qualities such as size, distribution, and shape, nanoparticles demonstrate completely new or better properties. With decreasing nanoparticle size, the surface-to-volume ratio of nanoparticles increases. This approach reduces silver ions from $AgNO_3$ into nano-sized particles by using extracts from several plants. In this study, a mixture of several leaf extracts such as Tulsi (*Ocimum sanctum*), Neem (*Azadirachta indica*), and Giloy (*Tinospora cordifolia)* was successfully used for the biosynthesis of stable silver nanoparticles. Numerous techniques were employed to characterize and investigate the antimicrobial activity of synthesized AgNPs. Fourier-transform infrared spectroscopy was used to confirm the presence of plant-origin capping agents surrounding AgNPs. UV-Visible spectrophotometer showed absorbance peak in the range of 436-446 nm, SEM images showed that the silver nanoparticles are well dispersed and FTIR revealed that phytochemicals like flavonoids, terpenoids and reducing agents present in leaf extract were the main entities to stabilize the synthesized nanoparticles.

The zones of inhibition showed that the AgNPs had significant antibacterial action against strains of Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and Gram-negative (*Pseudomonas aeruginosa* and *Proteus vulgaris*) bacteria. Results revealed that AgNPs having potent antimicrobial activity could be prepared using different pure plant extracts and their mixtures.

Keywords: Silver Nanoparticles, Green synthesis, *Ocimum sanctum, Azadirachta indica, Tinospora cordifolia*

INTRODUCTION

The most introductory element in the construction of a nanostructure is a nanoparticle. Nanoparticles have a lesser face area to volume rate, which is their most essential and identifying point **[1], [2]**. The nature of nanoparticles can enhance whenever their specific face area expands due to an increase in face energy.

A nanoparticle's size generally ranges from 1 to 100 nanometres **[3], [4]**. Metallic nanoparticles differ from bulk essence in terms of their physical and chemical characteristics. For illustration, they may have lower melting points, advanced specific face area, specific optic parcels, mechanical strengths, and specific magnetizations **[5]**.

Because of its low cytotoxicity, silver was utilized as an antiseptic and antibacterial agent in ancient times to treat a variety of infectious disorders **[6]**. Further research revealed that silver nanoparticles also have antifungal properties **[7]**.

Chemical, physical, and green approaches are the three methods that can be used to make AgNPs. A number of research teams have formally introduced green plant-based reduction approaches, which are also thought to be safe, easy, and cost effective **[8]**. Plant extracts have been used in the green synthesis of AgNPs in several research **[8]–[13]**.

The existence of naturally occurring biomolecules such as proteins, enzymes, tannins, phenols, sugars, and flavonoids, which may be employed safely as reducing and stabilizing agents to generate stable nanometals, was the main advantage of the green methodology **[14]**.

Since ancient times, *Azadirachta indica* (commonly known as neem), a member of the Meliaceae family, has been used to treat bacterial, fungal, viral, and many forms of skin disorders. The aqueous neem extract is utilized to make a variety of nanoparticles, including gold, zinc oxide, and silver. Terpenoids and flavanones are two significant phytochemicals found in neem that play an important role in nanoparticle stabilization as well as capping and reducing agents **[15], [16]**.

Tinospora cordifolia, sometimes known as Giloy, is a big, deciduous climbing shrub that belongs to the Menispermaceae family. Alkaloids, steroids, diterpenoid lactones, aliphatics, and glycosides, among other active ingredients in giloy, play a vital role in nanoparticle stabilization as well as capping and reducing agents. The plant has recently piqued the interest of scientists all over the world due to its reported medicinal properties, which include anti-diabetic, anti-periodic, anti-spasmodic, anti-inflammatory, anti-arthritic, anti-oxidant, anti-allergic, anti-stress, anti-leprotic, anti-malarial, hepatoprotective, immunomodulatory, and anti-neoplastic properties **[17]– [19]**.

Ocimum sanctum, sometimes known as holy basil or tulsi, is a member of the Labiateae family. Flavonoids, alkaloids, saponins, phenols, anthocyanins, triterpenoids, and tannins are all found in the whole-plant extract. Flavonoids, alkaloids, saponins, tannins, phenols, anthocyanins and terpenoids, are all found in leaf extract **[20]–[22]**.

In this research work, the nanoparticles are synthesized by using an aqueous extract of a mixture of Neem leaf broth, Giloy and Tulsi and metal ions (such as silver). Silver was of particular interest due to its distinctive physical and chemical properties. A mixture of Neem leaf broth, Giloy, and Tulsi was selected as it is of high medicinal value and its applications in antidiarrheal, hypolipidemic, antiparasitic, antimicrobial and antiviral activities **[13], [18], [23]**.

MATERIAL AND METHODS

Collection of plant material

Leaves of Tulsi (*Ocimum sanctum*), Neem (*Azadirachta indica*), and Giloy (*Tinospora cordifolia*) were collected and dried under shade. Fine powder was prepared and aqueous extract was prepared by standard protocol.

Green synthesis of silver nanoparticles

Aqueous extract of plants was used as reducing agents in the green synthesis of AgNPs. The effect of extract, AgNO3 concentration, time and temperature of reaction were standardized to obtain the optimum values for the synthesis of AgNPs. For the synthesis of silver nanoparticles, 1.5 mL of plant extract was mixed with 30 mL of 1 mM aqueous silver nitrate solution and incubated at room temperature in dark conditions for 2 hours. The bio-reduction of the silver ions in the solution was monitored periodically by measuring the UV–Vis spectroscopy (200–800 nm) of the solutions. The formation of a yellowish brown-colored solution indicated the formation of the silver nanoparticles. The silver nanoparticles obtained from the solution were purified by repeated centrifugation at 12,000 rpm for 20 min followed by dispersion of the pellet in sterile deionized water three times to remove the water-soluble biomolecules such as proteins and secondary metabolites. The water–suspended nanoparticles were lyophilized. After freezing, dried silver nanoparticles were used to characterize the structure and composition.

Characterization of synthesized AgNPs

Morphological examination and particle-size measurement

AgNPs samples were studied by scanning electron microscopy. A drop of AgNPs dispersion was placed with distilled water onto a carbon-coated copper grid and then left to dry under ambient conditions.

UV-Vis Spectrophotometry

Ultraviolet-visible (UV-Vis) spectrophotometry is the most important and simple technique to confirm the formation of nanoparticles. The absorbance spectrum of the mixture was recorded using a UV-Vis spectrophotometer using the wavelength range from 190 to 800 nm.

Fourier-Transform Infrared Spectroscopy

AgNPs from all the plant extracts were freeze-dried and then analysed using a Fourier transform infrared (FTIR) spectrometer.

In Vitro Antimicrobial Activity of AgNPs:

The antibacterial potency of nanoparticles prepared by each plant extract and the mixture was evaluated against two strains of Gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and two strains of Gram-negative (*Pseudomonas aeruginosa* and *Proteus Vulgaris*) bacteria. The disk diffusion method was used to evaluate the antimicrobial activity of each plant extract and nanoparticles of mixture **[24]–[26]**. The plant extract and prepared nanoparticles were dissolved and loaded over sterile filter paper discs (8 mm in diameter). Nutrient agar medium was poured into sterile Petri dishes followed with 15 mL of seeded medium previously inoculated with bacterial suspension. Sterile filter paper discs loaded with plant extract and nanoparticles were placed on the top of agar plates. The plates were incubated at 37 °C for 24 hours. The presence of inhibition zones was measured and recorded.

RESULTS AND DISCUSSION

SEM analysis:

FESEM was used to analyze the surface morphology of Ag-NPs. The results of a SEM analysis of mixture of extracts containing Ag-NPs are shown in the figure below. Ag-NPs demonstrated the presence of a wide range of aggregated spherical-shaped particles with an average size of 25.83 to 46.29 nm. The creation of a clustered silver nanoparticle could be the result of particle agglomeration prior to examination. The coexistence of smaller and bigger Ag-NPs in the SEM image was due to Ag-NPs generated in the early and later stages of the reaction, indicating that both nucleation to form new NPs and aggregation to form larger particles occurred simultaneously **(Figure 1)**.

UV-visible spectrum analysis:

The samples were observed under a UV-vis spectrophotometer for their maximum absorbance and wavelength to confirm the reduction of silver nitrate. As a result, UV-visible absorbance measurements can be used to determine the shape and size of nanoparticles in aqueous suspension. The creation of silver nanoparticles is indicated by a change in color. Silver ions in an aqueous solution have been transformed to nanosized elemental silver, as evidenced by this creation. It is known very well that silver nanoparticles have a distinguished brown color in aqueous solution because of the surface plasmon resonance in silver nanoparticles. Colour change was observed till 18 hours This was the point where almost all the metal ions were converted into nanoforms. The reaction mixture was diluted 10 times and used for UV-vis spectrophotometry. To alter the baseline, deionized water was utilized. The reduction of $AgNO_3$ into NPs showed an absorbance peak at around 435 nm, which is a characteristic of AgNPs **(Figure 2)**.

IR analysis:

The IR measurements were carried out to identify the possible biomolecules responsible for the reduction of the Ag^+ ions. Identifying the biomolecules associated with the synthesis of nanoparticles by plant-mediated was performed by using IR. The IR spectra exposed the presence of different functional groups like alcohol (O-H stretching, H-bonded), alkene (C=C stretching), aromatic (C=C stretching) and alkane (-C-H bending), respectively. Infrared spectroscopy gives information on the vibrational and rotational modes of motion of a molecule and hence is an important technique for the identification and characterization of a substance. The band at 3431.87 cm^{-1} corresponds to O-H stretching H-bonded alcohols and phenols. The peak at 2925.1 cm^{-1} corresponds to O-H stretch carboxylic acids. The assignment at 1637.30 cm^{-1} corresponds to N-H bend primary amines. The peak at 1389.5 cm^{-1} corresponds to C-N stretching of the aromatic amine group **(Figure 3)**.

Antibacterial analysis

Nanoparticles prepared by the mixture were analyzed to evaluate their antibacterial activity against two strains of Gram-positive (*Bacillus subtilis* and

Staphylococcus aureus) and two strains of Gram-negative (*Pseudomonas aeruginosa* and *Proteus vulgaris*) bacteria using the disc diffusion method. The zone of inhibition obtained was shown in **Table 1** and illustrated in **Figure 4-8**.

The results revealed that nanoparticles of Neem, Tulsi and Giloy showed antibacterial activity. Nanoparticles of Tulsi were found to be most effective for retarding microbial growth against all four strains which might be due to the presence of Eugenol, Linalool, β-Caryophyllene, Carvacrol, Apigenin, Rosmarinic acid and Ursolic acid. Neem showed effective antimicrobial activity against *Pseudomonas aeruginosa*. Antimicrobial activity of Neem might be correlated with the presence of triterpenoids, phenolic compounds, carotenoids, steroids, flavonoids, ketones and tetra-triterpenoids azadirachtin. Giloy also showed antimicrobial activity against all four strains but the zone of inhibition was comparatively lesser than the other two i.e. Tulsi and Neem. Nanoparticles prepared by a mixture of all the three plant extracts (Neem, Tulsi and Giloy) revealed maximum antibacterial activity against *Proteus vulgaris* and *Bacillus subtilis* **[27]–[30]**.

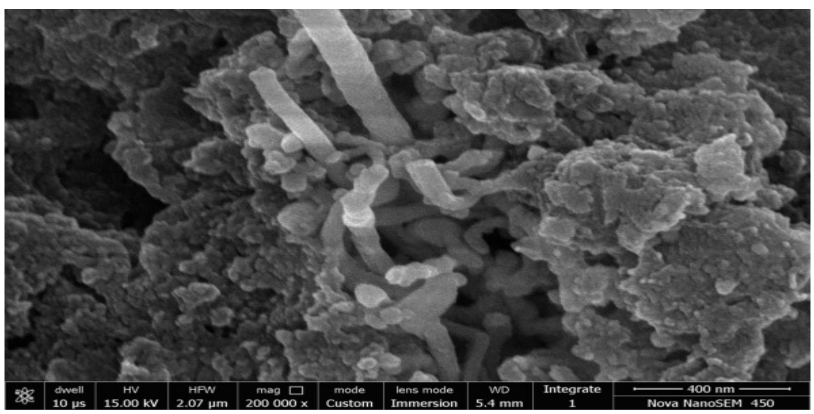

Figure 1: SEM Analysis of AgNP's

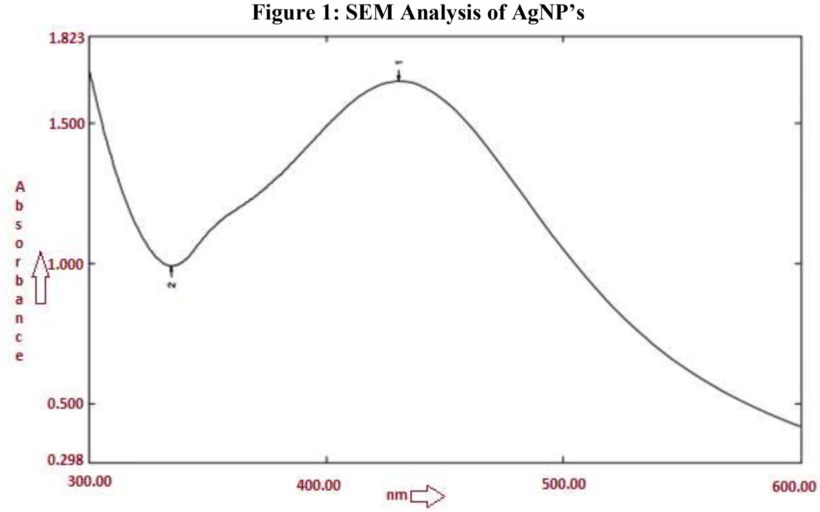

Figure 2: UV-vis absorption spectrum

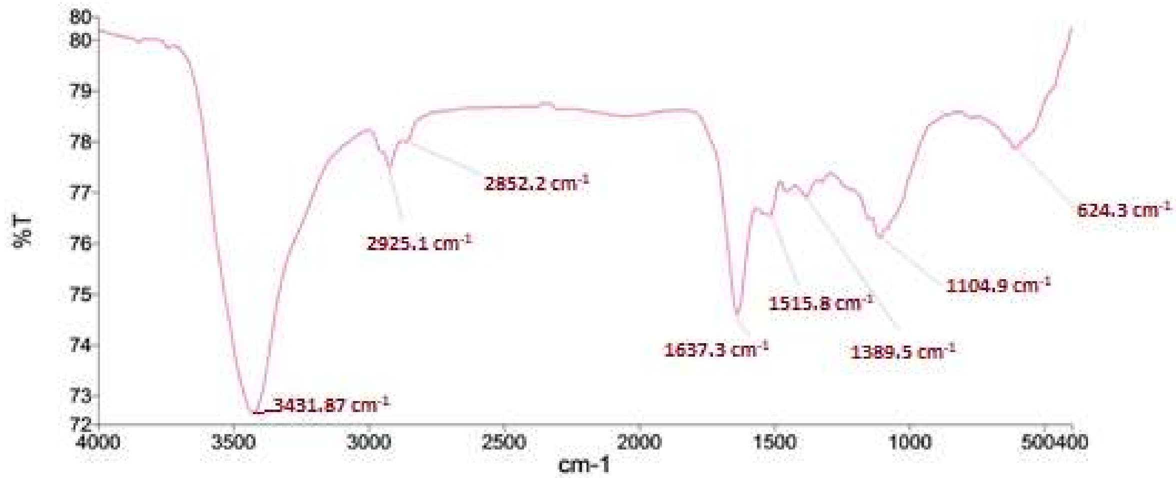

Figure 3: IR spectra of AgNPs of mixture of leaf extract

Table 1: Zone of inhibition for bacterial strains with AgNPs

S. No.	Bacterial strains		Zone of Inhibition (in cm.)			
			Mixture	Neem	Tulsi	Giloy
1	Gram negativ	*Pseudomonas aeruginosa*	1.47± 0.06	1.76 ± 0.04	1.76 ± 0.04	1.46 ± 0.16
2		*Proteus vulgaris*	1.7± 0.05	1.43 ± 0.09	1.66 ± 0.09	1.5 ± 0.02
3	Gram positive	*Bacillus subtilis*	1.7± 0.03	1.53± 0.04	1.61 ± 0.02	1.56 ± 0.20
4		*Staphylococcus aureus*	1.24 ± 0.06	1.6 ± 0.31	1.7 ± 0.02	1.5± 0.02

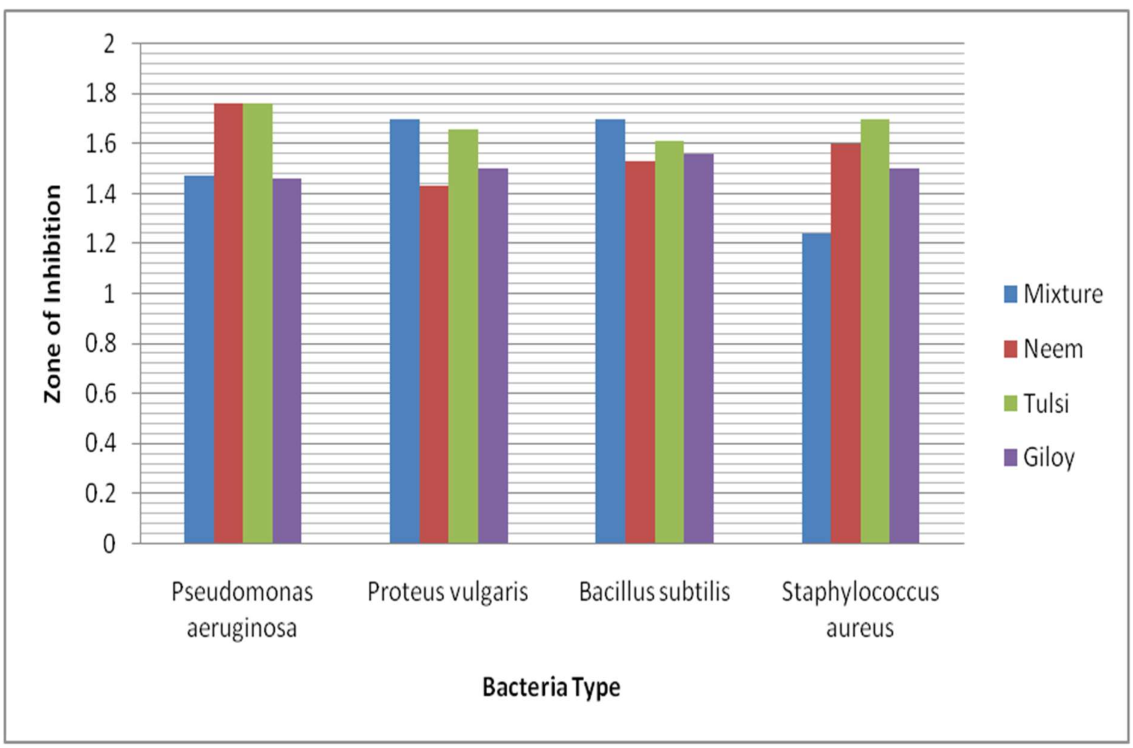

Figure 4: **IR spectra of AgNPs of the mixture of leaf extract**

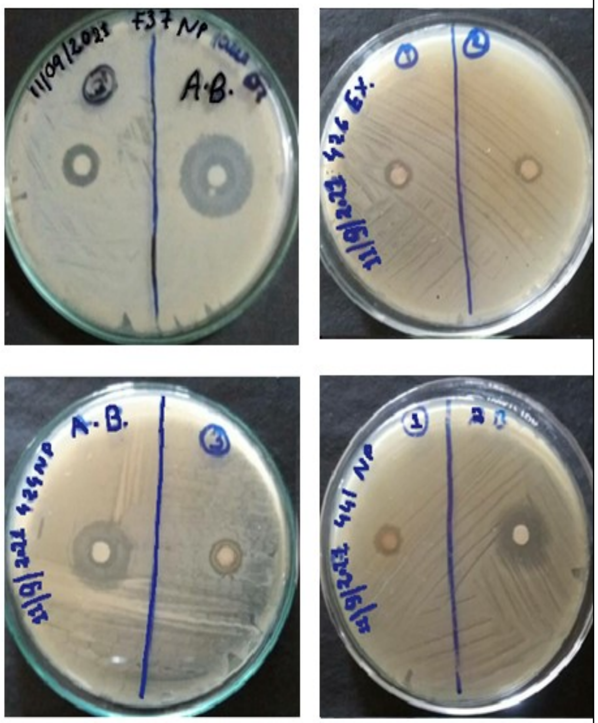

Figure 5: ZOI of silver nanoparticles of Giloy

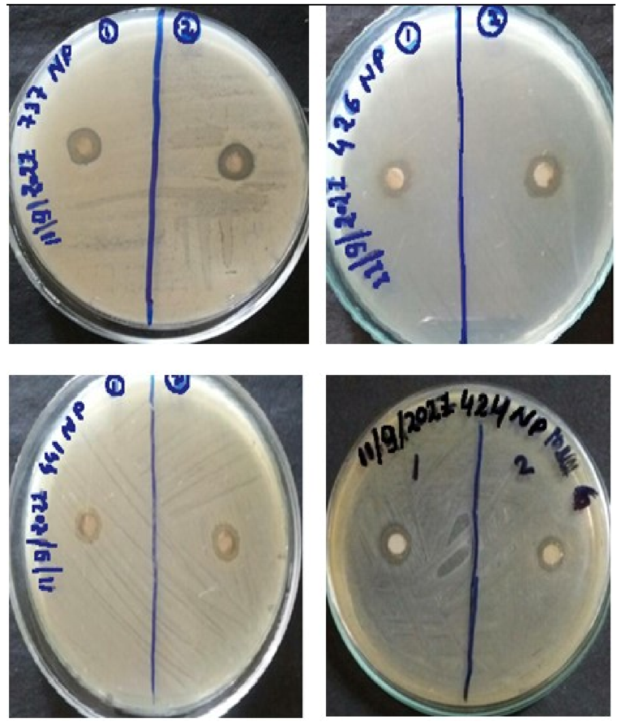

Figure 6: ZOI of silver nanoparticles of Tulsi

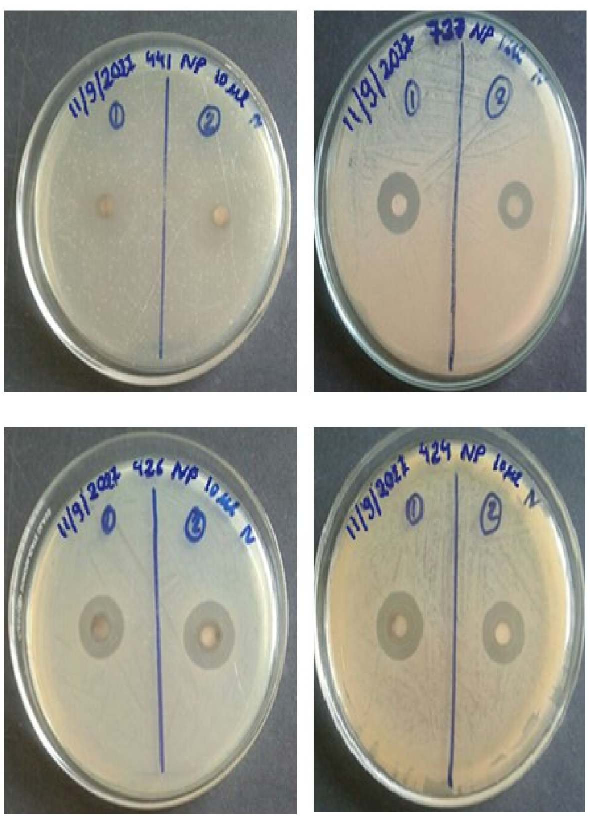

Figure 7: ZOI of silver nanoparticles of Neem

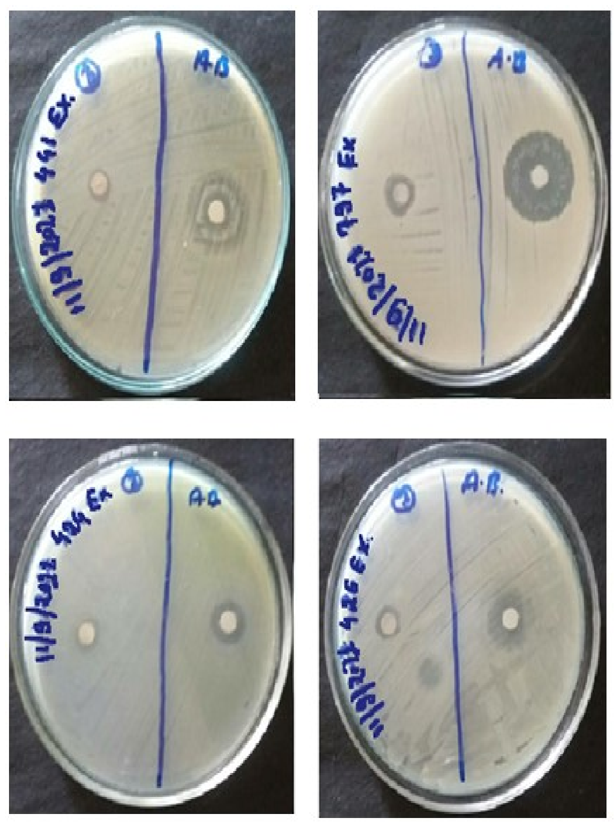

Figure 8: ZOI of silver nanoparticles of Mixture

CONCLUSIONS

The present work represents an economical, non-toxic and eco-friendly method for synthesizing silver nanoparticles. These silver nanoparticles showed a characteristic absorption peak at 435 nm in UV spectra. It is clearly seen that the mixture of leaf extract successfully reduces silver ions to silver nanoparticles. Further characterization of synthesized nanoparticles using SEM showed the nanoparticles are small-sized (1-100nm), spherical in shape and well distributed. Antibacterial activities of the synthesized nano silver particles of mixture show improved sensitivity against *Bacillus subtilis* and *Proteus vulgaris* bacteria.

REFERENCES

[1] J. R. Morones *et al.*, "The bactericidal effect of silver nanoparticles," *Nanotechnology*, vol. 16, no. 10, 2005, doi: 10.1088/0957-4484/16/10/059.

[2] A. A. Ashour, D. Raafat, H. M. El-Gowelli, and A. H. El-Kamel, "Green synthesis of silver nanoparticles using cranberry powder aqueous extract: Characterization and antimicrobial properties," *International Journal of Nanomedicine*, vol. 10, 2015, doi: 10.2147/IJN.S87268.

[3] C. G. Granqvist and R. A. Buhrman, "Ultrafine metal particles," *Journal of Applied Physics*, vol. 47, no. 5, 1976, doi: 10.1063/1.322870.

[4] K. Natarajan, S. Selvaraj, and V. R. Murty, "Microbial production of silver nanoparticles," *Digest Journal of Nanomaterials and Biostructures*, vol. 5, no. 1, 2010.

[5] S. Mourdikoudis, R. M. Pallares, and N. T. K. Thanh, "Characterization techniques for nanoparticles: Comparison and complementarity upon studying nanoparticle properties," *Nanoscale*, vol. 10, no. 27. 2018. doi: 10.1039/c8nr02278j.

[6] J. RubenMorones-Ramirez, J. A. Winkler, C. S. Spina, and J. J. Collins, "Silver enhances antibiotic activity against gram-negative bacteria," *Science Translational Medicine*, vol. 5, no. 190, 2013, doi: 10.1126/scitranslmed.3006276.

[7] A. S. Pozdnyakov *et al.*, "Nontoxic hydrophilic polymeric nanocomposites containing silver nanoparticles with strong antimicrobial activity," *International Journal of Nanomedicine*, vol. 11, 2016, doi: 10.2147/IJN.S98995.

[8] M. Rafique, I. Sadaf, M. S. Rafique, and M. B. Tahir, “A review on green synthesis of silver nanoparticles and their applications,” *Artificial Cells, Nanomedicine and Biotechnology*, vol. 45, no. 7. 2017. doi: 10.1080/21691401.2016.1241792.

[9] S. Ankanna, T. N. V. K. V. Prasad, E. K. Elumalai, and N. Savithramma, “Production of biogenic silver nanoparticles using Boswellia Ovalifoliolata stem bark,” *Digest Journal of Nanomaterials and Biostructures*, vol. 5, no. 2, 2010.

[10] S. P. Chandran, M. Chaudhary, R. Pasricha, A. Ahmad, and M. Sastry, “Synthesis of gold nanotriangles and silver nanoparticles using Aloe vera plant extract,” *Biotechnology Progress*, vol. 22, no. 2, 2006, doi: 10.1021/bp0501423.

[11] J. Huang *et al.*, “Biosynthesis of silver and gold nanoparticles by novel sundried Cinnamomum camphora leaf,” *Nanotechnology*, vol. 18, no. 10, 2007, doi: 10.1088/0957-4484/18/10/105104.

[12] V. Kumar and S. K. Yadav, “Plant-mediated synthesis of silver and gold nanoparticles and their applications,” *Journal of Chemical Technology and Biotechnology*, vol. 84, no. 2. 2009. doi: 10.1002/jctb.2023.

[13] A. Verma and M. S. Mehata, “Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity,” *Journal of Radiation Research and Applied Sciences*, vol. 9, no. 1, 2016, doi: 10.1016/j.jrras.2015.11.001.

[14] V. K. Sharma, R. A. Yngard, and Y. Lin, “Silver nanoparticles: Green synthesis and their antimicrobial activities,” *Advances in Colloid and Interface Science*, vol. 145, no. 1–2. 2009. doi: 10.1016/j.cis.2008.09.002.

[15] P. Banerjee, M. Satapathy, A. Mukhopahayay, and P. Das, “Leaf extract mediated green synthesis of silver nanoparticles from widely available Indian plants: Synthesis, characterization, antimicrobial property and toxicity analysis,” *Bioresources and Bioprocessing*, vol. 1, no. 1, 2014, doi: 10.1186/s40643-014-0003-y.

[16] P. Roy, B. Das, A. Mohanty, and S. Mohapatra, “Green synthesis of silver nanoparticles using azadirachta indica leaf extract and its antimicrobial study,” *Applied*

Nanoscience (Switzerland), vol. 7, no. 8, 2017, doi: 10.1007/s13204-017-0621-8.

[17] A. Upadhyay, K. Kumar, A. Kumar, and H. Mishra, "Tinospora cordifolia (Willd.) Hook. f. and Thoms. (Guduchi) - validation of the Ayurvedic pharmacology through experimental and clinical studies," *International Journal of Ayurveda Research*, vol. 1, no. 2, 2010, doi: 10.4103/0974-7788.64405.

[18] K. Singh, M. Panghal, S. Kadyan, U. Chaudhary, and J. P. Yadav, "Antibacterial activity of synthesized silver nanoparticles from Tinospora cordifolia against multi drug resistant strains of Pseudomonas aeruginosa isolated from burn patients," *Journal of Nanomedicine and Nanotechnology*, vol. 5, no. 2, 2014, doi: 10.4172/2157-7439.1000192.

[19] S. A. Anuj and K. B. Ishnava, "Plant mediated synthesis of silver nanoparticles by using dried stem powder of Tinospora Cordifolia, its antibacterial activity and comparison with antibiotics," *International Journal of Pharma and Bio Sciences*, vol. 4, no. 4, 2013.

[20] P. Pattanayak, P. Behera, D. Das, and S. Panda, "Ocimum sanctum Linn. A reservoir plant for therapeutic applications: An overview," *Pharmacognosy Reviews*, vol. 4, no. 7. 2010. doi: 10.4103/0973-7847.65323.

[21] R. K. Jaggi, R. Madaan, and B. Singh, "Anticonvulsant potential of holy basil, Ocimum sanctum Linn., and its cultures," *Indian Journal of Experimental Biology*, vol. 41, no. 11, 2003.

[22] M. A. Kelm, M. G. Nair, G. M. Strasburg, and D. L. DeWitt, "Antioxidant and cyclooxygenase inhibitory phenolic compounds from Ocimum sanctum Linn.," *Phytomedicine*, vol. 7, no. 1, 2000, doi: 10.1016/S0944-7113(00)80015-X.

[23] G. Singhal, R. Bhavesh, K. Kasariya, A. R. Sharma, and R. P. Singh, "Biosynthesis of silver nanoparticles using Ocimum sanctum (Tulsi) leaf extract and screening its antimicrobial activity," *Journal of Nanoparticle Research*, vol. 13, no. 7, 2011, doi: 10.1007/s11051-010-0193-y.

[24] Q. Wang *et al.*, "Evaluation of the Etest and disk diffusion method for detection of the activity of

ceftazidime-avibactam against Enterobacterales and Pseudomonas aeruginosa in China," *BMC Microbiology*, vol. 20, no. 1, 2020, doi: 10.1186/s12866-020-01870-z.

[25] L. Dubreuil *et al.*, "Improvement of a disk diffusion method for antibiotic susceptibility testing of anaerobic bacteria. French recommendations revisited for 2020," *Anaerobe*, vol. 64, 2020, doi: 10.1016/j.anaerobe.2020.102213.

[26] H. Yao, J. Liu, X. Jiang, F. Chen, X. Lu, and J. Zhang, "Analysis of the clinical effect of combined drug susceptibility to guide medication for carbapenem-resistant klebsiella pneumoniae patients based on the Kirby–Bauer disk diffusion method," *Infection and Drug Resistance*, vol. 14, 2021, doi: 10.2147/IDR.S282386.

[27] M. Reda, A. Ashames, Z. Edis, S. Bloukh, R. Bhandare, and H. A. Sara, "Green synthesis of potent antimicrobial silver nanoparticles using different plant extracts and their mixtures," *Processes*, vol. 7, no. 8, 2019, doi: 10.3390/pr7080510.

[28] P. R. Rathi Sre, M. Reka, R. Poovazhagi, M. Arul Kumar, and K. Murugesan, "Antibacterial and cytotoxic effect of biologically synthesized silver nanoparticles using aqueous root extract of Erythrina indica lam," *Spectrochimica Acta - Part A: Molecular and Biomolecular Spectroscopy*, vol. 135, 2015, doi: 10.1016/j.saa.2014.08.019.

[29] K. Anandalakshmi, J. Venugobal, and V. Ramasamy, "Characterization of silver nanoparticles by green synthesis method using Pedalium murex leaf extract and their antibacterial activity," *Applied Nanoscience (Switzerland)*, vol. 6, no. 3, 2016, doi: 10.1007/s13204-015-0449-z.

[30] A. A. El-Refai, G. A. Ghoniem, A. Y. El-Khateeb, and M. M. Hassaan, "Eco-friendly synthesis of metal nanoparticles using ginger and garlic extracts as biocompatible novel antioxidant and antimicrobial agents," *Journal of Nanostructure in Chemistry*, vol. 8, no. 1, 2018, doi: 10.1007/s40097-018-0255-8.