History of Medicine, 2024, 10(2): 138–152

DOI: 10.17720/2409-5834.v10.2.2024.17

Green Synthesis and Conjugation of Silver Nanoparticles for Antimicrobial Analysis

Maria Ashraf [1], Sana Ijaz [1], Aisha Hamid [2], Aina Rao [3], Sidra Iqbal [4], Saania iftikhar [4], salman haider [4], Farah Liaqat [5], Aimen Jamil [4], Syed Asif Nawaz [6], Hassan Raza [7], Tabish Arif[4], Zara Abid[4*], Farhat Batool [8], Muhammad Raza Malik* [9]

1. Institute of molecular biology and biotechnology, The University of Lahore
2. Department of Pharmacy, The University of Lahore, Pakistan
3. Department of Biochemistry, University of management & Technology
4. Department of Life Sciences, University of Management & Technology
5. Department of Zoology, University of Sialkot
6. Faculty of Biological and Health Sciences, HU Mansehra KP Pakistan
7. Institute of Biomedicine, University of Eastern Finland
8. Department of Botany, Minjah University, Lahore, Pakistan
9. School of Chemistry, University of Punjab, Lahore, Pakistan

*Correspondence

zahraabid3811@gmail.com, chemistofpu@gmail.com

Abstract:
Nanoparticles have become increasingly important as biomedicines within the field of health biotechnology, with transition metals playing a crucial role in various biological applications and the advancement of nanotechnology. Among the diverse range of metallic nanoparticles, silver nanoparticles (AgNPs) stand out due to their remarkable contributions to biomedical applications. Silver nanoparticles are particularly significant as they offer a non-toxic, inorganic alternative to traditional antibiotics, acting as potent antibacterial agents. This unique property makes them highly valuable in the fight against microbial infections, especially given the growing concern over antibiotic resistance. The primary objective of this study is to develop a sustainable and environmentally friendly method for synthesizing silver nanoparticles. This is achieved by conjugating silver nanoparticles with extracts from plants such as mint, neem, and ginger. This green synthesis approach not only leverages the natural antimicrobial properties of these plant extracts but also enhances the overall effectiveness of the silver nanoparticles. By combining these natural extracts with silver nanoparticles, we aim to create a powerful antimicrobial agent that can be used in various biomedical applications, offering a promising alternative to conventional antibiotics and advancing the field of nanomedicine.

Key words: Antimicrobial, Biomedical applications, Nanparticles, nanomedicine, AgNPs

Introduction:
The purposeless use of antibiotics promote greater threats to global community due to microbial community resistance. So, its need to explore effective and new antimicrobial agents. Metal nanoparticles are widely used from past 20 years due to their precise form, size, and melodic chemical-physical properties. Nanoparticles have ability to affect host pathogen simultaneously due to metal ions release (Feroze et al., 2020).

Nanoparticles are present in productive form of food-cooking, combustion, volcanic and photochemical activity in nature. Nanoparticles are of great interest due to their optical behavior and chemical reactivity. The green synthesis method is used to biosynthesize the silver nanoparticles. The eco-friendly, adaptable, and quick process takes place in nanobiofactories made of microorganisms and plants. The pathogen, plant, and nanoparticles communicate with one another. (paul et al., 2021). The nanoparticles can regulate the expression of proteins, transcription, and host plant metabolism as well as damage the membrane integrity of phytopathogens and cause oxidative stress.

The silver nanoparticles have a diameter of 100 nm, a three-dimensional structure, and are created by the reduction of silver ions into silver oxide groups that are then calmed by coating ligands. (Shahwany et al., 2016). Many studies focus on the biosynthesis of silver nanoparticles with antimicrobial properties that is aided by extracts of plant components, such as seeds, leaves, stems, and roots. By modifying biological activities, medicinal plants and their extracts play a part in the management of illness. The current generation of study scholars has focused their studies on natural bioactive substances.

Zingiber officinale, one of the most significant medicinal plants, is primarily utilized in food and pharmaceutical goods. The Zingiberaceae family includes ginger (Zingiber officinale), which has a number of medicinal properties include antioxidant, anti-diabetic, anti-bacterial, gastroprotective, and anti-inflammatory properties. (hazim et al., 2020). The active constituents of ginger include curcumin, 6-paradol, 6-shogaol and 6-gingerol (Zhang et al., 2020). It has very high antimicrobial activity against bacteria and fungi.

Nano particles also synthesized by the plant leaf mentha which available easily and has potential to ion reduction. Mint plant rich of flavonoids and phenol. Nanoparticles are very expensive although it can be minimized by using plants as a source. Basic material required for the synthesis of nanoparticles includes reducing agents, protective agents and metal ions and these material must be in chemical form. Medical value of mint is very well known as ginger. Additionally, peppermint has been discovered to have antiviral and fungicidal properties. In an egg and cell culture system, aqueous preparations of the leaves significantly inhibited Influenza A, Newcastle disease, Herpes simplex, Vaccinia, Semliki Forest, and West Nile viruses.

Azadirachta indica (Neem), a plant belonging to the Meliaceae family, is thought to have a health-encouraging impact due to the abundance of antioxidants it contains. Various plant parts, including seeds, leaves, bark, and flowers, are used to cure both acute and chronic human illnesses. They are also used as insecticides and have larvicidal, antimicrobial, antibacterial, spermicidal, antiviral, and antimalarial properties. (Alzohairy 2016). Additionally used to cure gangrene, diabetic food, and healing wounds. Numerous substances produced by neem extraction contain components such as flavonoids, alkaloids, phenolic compounds, triterpenoids, ketones, steroids, and carotenoids in their chemical makeup.

Material and Method:

Fresh mint and neem leaves, ginger rhizomes, and lemon peels were collected from the botanical park, while various chemicals, including polyethylene glycol, $AgNO3$, ethanol, TBS, distilled water, and others, were obtained from the facility. The ginger rhizomes were cleaned with deionized water, peeled, dried at room temperature, ground into a powder, and mixed with distilled water, resulting in a dark yellow extract that was filtered. Mint leaves were washed, chopped, and blended with distilled water to obtain a dark green extract, which was also filtered. Similarly, neem leaves were processed to produce a dark green extract.For the synthesis of silver nanoparticles (AgNPs), lemon juice was used as the base and silver nitrate as the reducing and stabilizing agent, with distilled water employed throughout the procedures. A 0.1 M silver nitrate solution and 1 mM lemon juice were combined in a 1:4 ratio using thermal reduction. Fresh lemon juice was heated on a magnetic stirrer, and silver nitrate was added dropwise until a color change indicated the formation of dark gray or black precipitation. The product was frozen, thawed, reconstituted in TBS, and stored at room temperature.To synthesize extract-encapsulated AgNPs, 10 cc of lemon juice was heated on a magnetic stirrer, followed by the addition of 5 ml of polyethylene glycol and 10 ml each of neem, ginger, and mint extracts. Finally, 10 ml of AgNO3 was incorporated into the mixture. The solution was stirred continuously for one hour, during which the color change indicated the formation of stable silver nanoparticles. These nanoparticles, encapsulated with the respective extracts, were stored in a refrigerator for future use.

Characterization of AgNps

The characterization of silver nanoparticles was conducted using UV-visible spectroscopy. This technique, which compares absorption in the ultraviolet and visible spectral regions, is generally referred to as UV-Vis spectroscopy and is used to analyze molecules in solution. Although the broad features of UV-Vis spectra are not suitable for precise sample identification, they are highly effective for quantitative analysis. UV-Vis spectroscopy is a widely employed method to assess the structural stability of silver nanoparticles (AgNPs). The UV-vis spectrophotometer was utilized to investigate absorption bands within the 300 to 650 nm range, with deionized water serving as the solvent. The highest absorbance was observed in formulations of dry AgNPs with the optimal production rate. This method was used to study the growth of AgNPs, focusing on how their morphology and particle size varied with wavelength. Absorption bands were recorded between 300 and 650 nm.

Analysis by FTIR

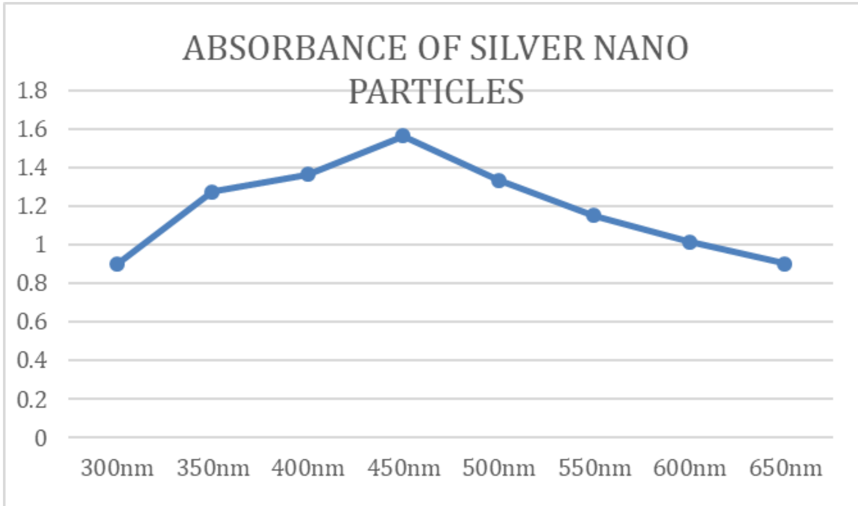
The FTIR spectra of AgNPs, along with their conjugations with ginger, neem, and mint extracts, were obtained using an FT-IR spectrometer. Fourier transform infrared spectroscopy (FTIR) offers several advantages and provides significant insights into the various functional groups present in a system by analyzing the vibrational frequencies of chemical bonds. In-situ FTIR spectroscopy of interfaces allows for the study of surface adherence of atoms from functional groups at nanoparticles. To obtain the Fourier transform infrared spectra of lyophilized silver nanoparticles, potassium bromide was combined with the nanoparticles, and the spectra were recorded using a Perkin Elmer Spectrum 100. This spectrometer operates with a resolution measuring frequencies between 400 and 225 Hz per centimeter.

Microscopy and Antibacterial Analysis of Plant-Extract-Conjugated Silver Nanoparticles

Silver nanoparticles (AgNPs) and their conjugates with mint, neem, and ginger extracts were examined under a compound microscope at 10X and 100X magnifications.The antimicrobial activities of these conjugated AgNPs were assessed using the well diffusion method against Bacillus subtilis and E. coli. Nutrient agar media was prepared by dissolving 5.6 g of nutrient agar in 200 ml of distilled water and autoclaved. The media was poured into Petri dishes and, once solidified, inoculated with bacterial cultures. Wells were created in each plate, and plant extracts (ginger, neem, and mint) were added at concentrations of 5, 10, 15, and 20 microliters, respectively. Plates were incubated for 24 hours to observe bacterial inhibition. For test tube analysis, LB broth was prepared with 1 g tryptone, 1 g NaCl, and 0.5 g yeast in 100 ml distilled water, distributed into test containers, and autoclaved. Each container was inoculated with 100 microliters of bacterial culture, followed by the addition of AgNPs conjugated with plant extracts. The test containers were incubated with shaking for three hours, and the bacterial growth was measured using spectrophotometry at 600 nm.

Results:

Spectrophotometric analysis of conjugated AgNps



:

Figure1: absorbance od AgNPS in spectrophotometer without conjugation

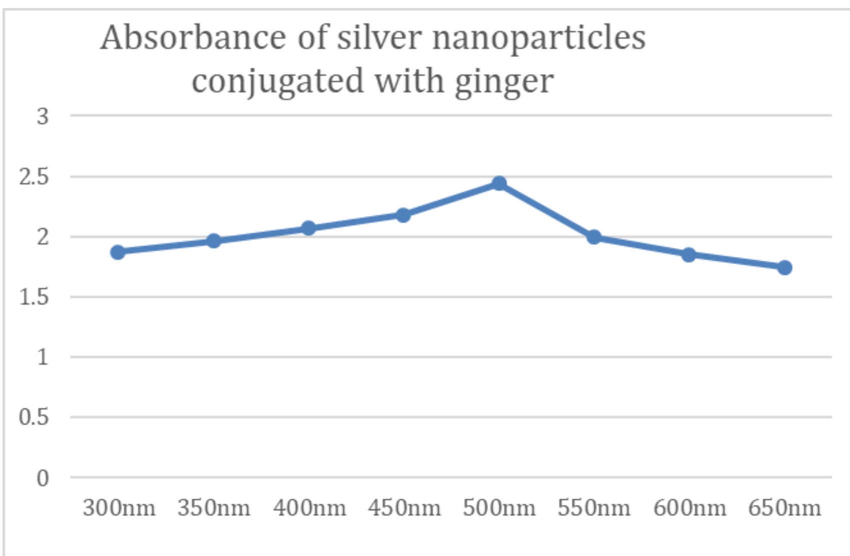


Figure 2: Comparison of conjugation of plant extract ginger with AgNPs

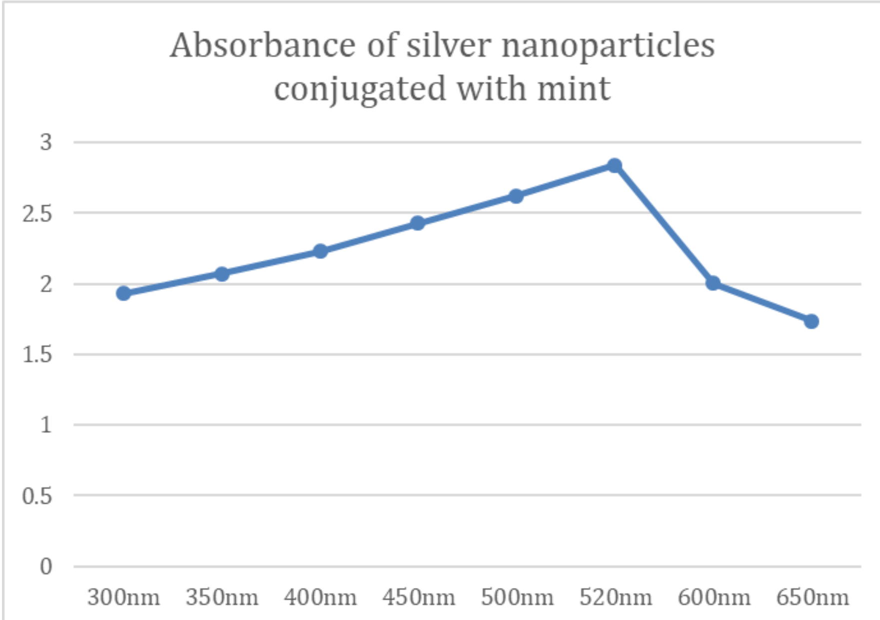


Figure 3: Comparison of conjugation of plant extract mint with AgNPs

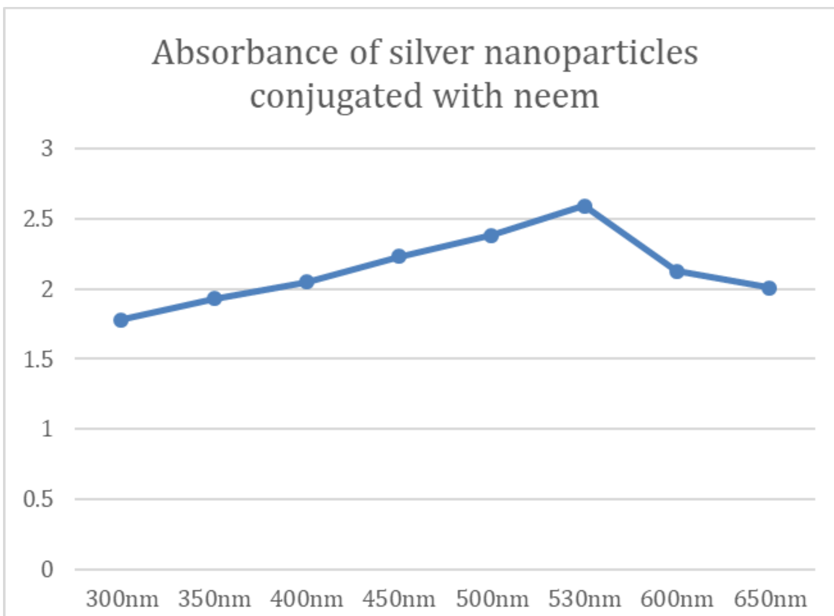


Figure 4: Comparison of conjugation of plant extract neem with AgNPs

The absorbance values of silver nanoparticles (AgNPs) and their conjugates with ginger, mint, and neem varied across different wavelengths. The peaks in the spectra indicate maximum absorbance, which increases with higher substance concentration. Consequently, the conjugated AgNPs exhibited different peaks compared to the simple AgNPs. The peak for simple AgNPs

appeared at 450 nm. However, when AgNPs were conjugated with mint, neem, and ginger, the peaks shifted towards the red end of the spectrum, indicating an increase in concentration.
4o

Antibacterial analysis via petri plate method with *Bacillus cereus*

Resukts shows the zone of inhibition around each well to assess the antibacterial activity, these zones are compared with inhibition to evaluate the effectiveness of each conjugated AgNP.

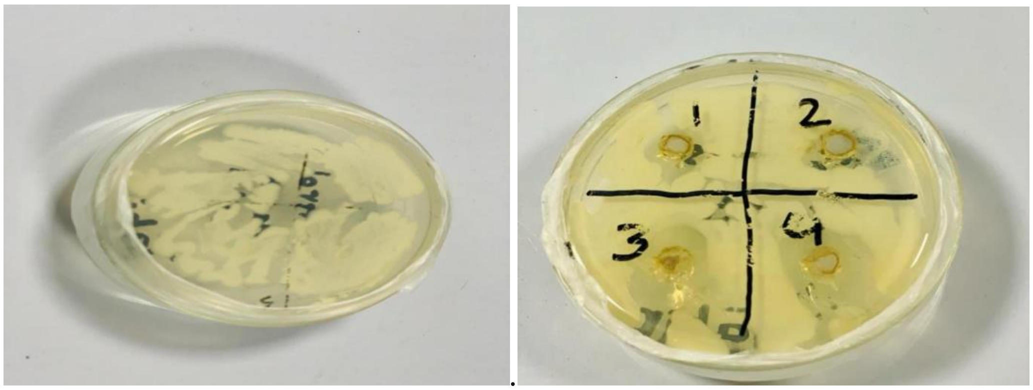


Figure 5: Shows the diffusion with *bacillus cereus*

This image shows the zone of inhibition on the culture of *Bacillus cereus*. Via the agar well diffusion methods, different concentrations of AgNPs encapsulated with ginger were loaded into the wells. Respective measurements have been recorded.

AgNPs encapsulated with ginger loaded into wells	**Zone of inhibitions of *Bacillus cereus***
5µl	0.51cm
10µl	0.82cm
15µl	1.01cm
20µl	1.41cm

Diffusion of AgNPs with silver nanoparticles encapsulated with mint

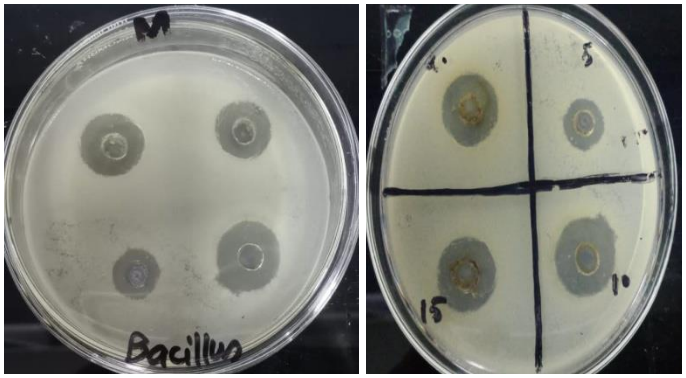


Figure 6: Shows the zone formation and inhibition pf AgNPs with *Bacillus Cereus*

Concentration of AgNPs conjugated with mint	**Zone of inhibition in *Bacillus cereus***
5µl	3mm
10µl	5mm
15µl	5mm
20µl	6mm

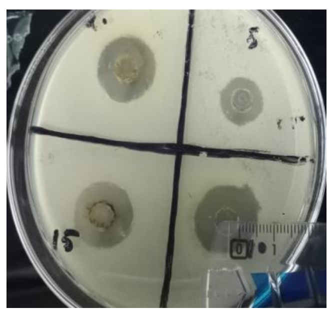


Figure 7: Shows the zone formation and inhibition pf AgNPs with *Bacillus Cereus* in mint

Diffusion of silver nanoparticles encapsulated with neem

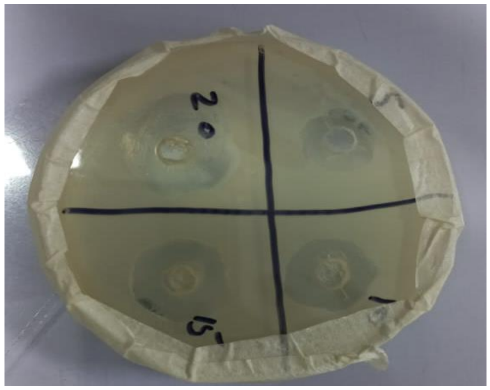


Figure 8: Shows the zone formation and inhibition pf AgNPs with *Bacillus Cereus* with neem

The images display the zones of inhibition on Bacillus cereus cultures, obtained through the agar well diffusion method. Different concentrations of AgNPs encapsulated with neem were loaded into the wells.

Concentration of AgNPs conjugated with neem	**Zone of inhibition in *Bacillus cereus***
5µl	2 mm
10µl	3.5mm
15µl	4 mm
20µl	5 mm

The zone of inhibitions in the *Bacillus cereus* are maximum by the silver nanoparticles conjugated with ginger when 20 µl of concentration was loaded into the wells. After ginger, neem is more effective as antibacterial agent. The least effective is mint.

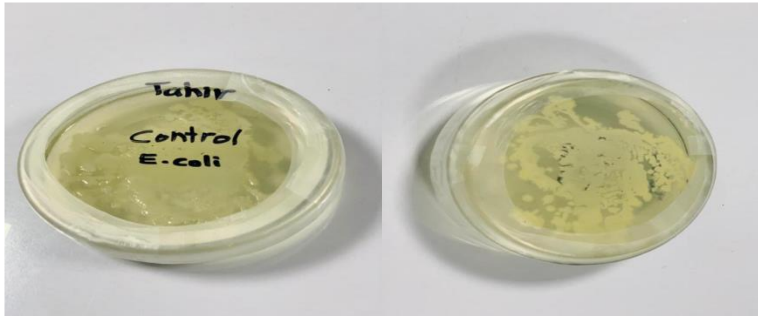


Figure 9: These images show the control of *E.coli*.

Well diffusion method via silver nanoparticles encapsulated with ginger

Figure 10: Shows the zone formation and inhibition pf AgNPs with *E.Coli* with ginger

This image shows the zone of inhibition on the culture of *E. coli*. Via the agar well diffusion methods, different concentrations of AgNPs encapsulated with ginger were loaded into the wells. Respective measurements have been recorded.

Concentrations of silver nanoparticles encapsulated with ginger loaded into wells	**Zone of inhibitions in *E. coli***
5µl	0.4cm
10µl	0.8cm
15µl	0.9cm
20µl	1.3cm

- **Well diffusion method via silver nanoparticles encapsulated with mint**

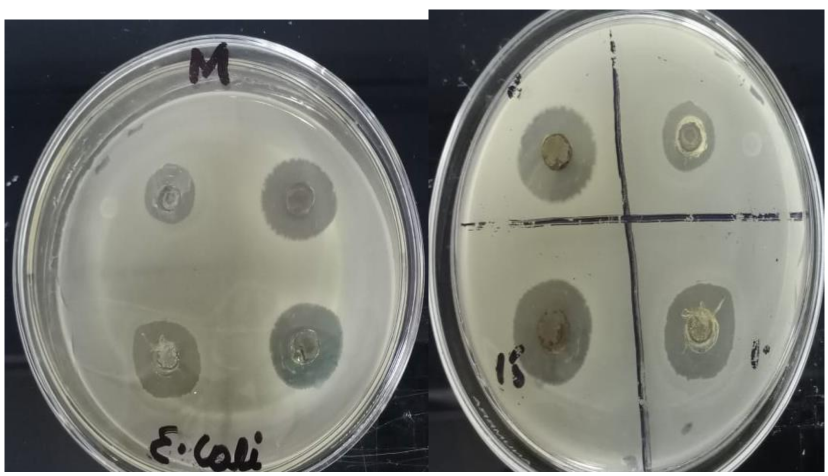


Figure 11: Shows the zone formation and inhibition pf AgNPs with *E.Coli* and Mint

This image shows the zone of inhibition on the culture of *E. coli*. Via the agar well diffusion methods, different concentrations of AgNPs encapsulated with mint were loaded into the wells. Respective measurements have been recorded.

Concentration of silver nanoparticles conjugated with mint loaded into wells	**Zone of inhibition in *E.coli***
5µl	3mm
10µl	5mm
15µl	6mm
20µl	7mm

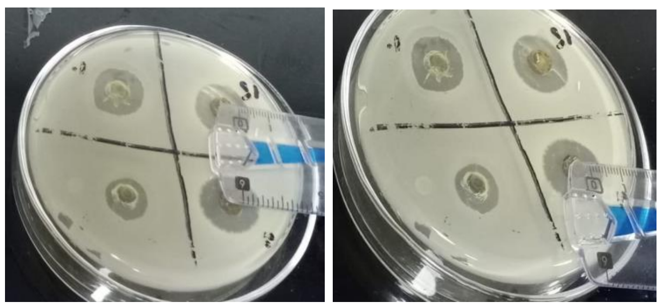


Figure 12: Shows the zone formation and inhibition pf AgNPs with *E.Coli* and Mint

Well diffusion method via silver nanoparticles encapsulated with neem

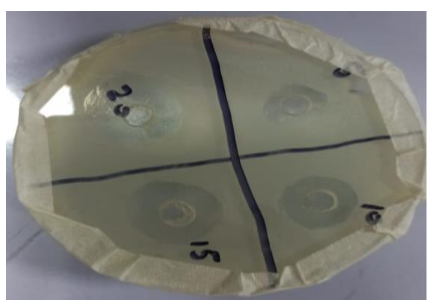


Figure 12: Shows the zone formation and inhibition pf AgNPs with *E.Coli* and Neem

This image shows the zone of inhibition on the culture of *E. coli*. Via the agar well diffusion method, different concentrations of AgNPs encapsulated with neem were loaded into the wells. Respective measurements have been recorded.

Concentration of silver nanoparticles conjugated with neem	Zone of inhibition in *E.coli*
5µl	2mm
10µl	4mm
15µl	5mm
20µl	7mm

The zone of inhibitions in the *E.coli* are maximum by the silver nanoparticles conjugated with ginger when 20 µl of concentration was loaded into the wells. After ginger, mint is effective. The least effective is neem. .

Discussion:

Nanoparticles, particularly silver nanoparticles (AgNPs), represent a significant advancement in addressing antibiotic resistance in biomedicine. This study aimed to develop an eco-friendly synthesis method by conjugating AgNPs with plant extracts like mint, neem, and ginger. Spectroscopic analyses confirmed successful synthesis and encapsulation of AgNPs with plant extracts, while microscopic examination verified their presence. Antibacterial testing against Bacillus cereus revealed ginger-encapsulated AgNPs as the most effective, followed by neem and mint. These findings underscore the potential of plant-extract-conjugated AgNPs as potent antibacterial agents, with ginger showing the highest efficacy. Further investigation into their mechanisms of action and broader biomedical applications is warranted to harness their full potential in combating antibiotic resistance. The antimicrobial efficacy of silver nanoparticles (AgNPs) combined with plant extracts significantly affects isolated strains of bacteria and fungus. Studies indicate that AgNPs can induce structural changes in microbial cells, rendering them more susceptible to penetration. This is attributed to the interaction between Ag-NP and cell membranes, leading to destabilization and increased permeability, ultimately resulting in bacterial cell death. AgNPs possess unique properties such as large surface area, small size, thermal and mechanical stability, electrical conductivity, and antimicrobial activity, positioning them as potential tools in disease management. The enhanced antibacterial efficacy of green synthesis AgNPs compared to plant extracts alone is attributed to synergistic interactions between Ag^+ ions and phytochemicals. Silver nanoparticles exhibit multiple modes of action, including inhibition of protein synthesis, cell wall synthesis, nucleic acid formation, and metabolic processes. Studies have shown that certain concentrations of AgNPs can inhibit bacterial growth by over 99%. Plant extracts contain a diverse array of metabolites, including carbohydrates, terpenoids, phenolic substances, alkaloids, and reducing agents, making them effective precursors for biosynthesizing AgNPs. The biosynthesis process involves nucleation, condensation, surface reduction, and stabilization stages. Fourier transform infrared (FTIR) spectroscopy analysis reveals that proteins present in the plant extracts act as capping materials, facilitating the bio-reduction of Ag^+ ions to AgNPs by providing free carboxyl and amino groups for attachment.

Conclusion:

Bio-synthesized silver nanoparticles, when combined with compounds from ginger, mint, and neem, have demonstrated significant antimicrobial activity, particularly evident during antibacterial assays. The well diffusion method clearly delineated the zones of inhibition. Spectrophotometric analysis was conducted on all materials, while Fourier transform infrared (FTIR) data supported the conjugation process. Examination under a compound microscope revealed the presence of silver nanoparticles both individually and in combination with mint, neem, and ginger. Notably, silver nanoparticles synthesized using ginger exhibited the most potent antibacterial properties. Overall, the silver nanoparticles showed exceptional efficacy in inhibiting microbial growth on a broad scale.

REFERENCES

● Ingle, A., Gade, A., Pierrat, S., Sonnichsen, C., & Rai, M. (2008). Mycosynthesis of silver nanoparticles using the fungus Fusarium acuminatum and its activity against some human pathogenic bacteria. Current Nanoscience, 4(2), 141-144.

● Naqvi, S. Z. H., Kiran, U., Ali, M. I., Jamal, A., Hameed, A., Ahmed, S., & Ali, N. (2013). Combined efficacy of biologically synthesized silver nanoparticles and different antibiotics against multidrug-resistant bacteria. International journal of nanomedicine, 8, 3187.

● Nasrollahi, A., Pourshamsian, K. H., & Mansourkiaee, P. (2011). Antifungal activity of silver nanoparticles on some of fungi.

● KarimiPourSaryazdi, A., Tavakoli, P., Barati, M., Ghaffarifar, F., Dalir Ghaffari, A., & KarimiPourSaryazdi, Y. (2019). Anti-Toxoplasma Effects of Silver Nanoparticles Based on Ginger Extract: An in Vitro Study. Journal of Archives in Military Medicine, 7(4).

● Feroze, N., Arshad, B., Younas, M., Afridi, M. I., Saqib, S., & Ayaz, A. (2020). Fungal mediated synthesis of silver nanoparticles and evaluation of antibacterial activity. Microscopy Research and Technique, 83(1), 72-80.

● Shahwany, A. W., Tawfeeq, H. K., & Hamed, S. E. (2016). Antibacterial and anti-biofilm activity of three phenolic plant extracts and silver nanoparticles on Staphylococcus aureus and Klebsiella pneumoniae. J. Biomed. Biotechnol, 4, 12-18.

● Hazim, I., Abd, K. Y., & Abachi, F. T. (2020). Newly formulated extract of Zingiber officinale as reducing agent for Silver nitrate Nanoparticals.

● Yang, X. X., Li, C. M., & Huang, C. Z. (2016). Curcumin modified silver nanoparticles for highly efficient inhibition of respiratory syncytial virus infection. Nanoscale, 8(5), 3040-3048.

● Adelere, I. A., Babayi, H., Aboyeji, D. O., Adams, H., Adabara, N. U., & Jagaba, A. (2021). Antibacterial activities of silver nanoparticles synthesized using aqueous extract of ginger (Zingiber officinale) rhizome.

● Hussein, E. A. M., Mohammad, A. A. H., Harraz, F. A., & Ahsan, M. F. (2019). Biologically synthesized silver nanoparticles for enhancing tetracycline activity against staphylococcus aureus and klebsiella pneumoniae. Brazilian Archives of Biology and Technology, 62.

● Roy, S., & Das, T. K. (2013). Activity of biosynthesized silver nanoparticles in combination with synthetic and natural fungicide against some pathogenic fungi. Asian Journal of Chemistry, 25(Supplementary Issue), S315.52

● Paul, A., & Roychoudhury, A. (2021). Go green to protect plants: repurposing the antimicrobial activity of biosynthesized silver nanoparticles to combat phytopathogens. Nanotechnology for Environmental Engineering, 6(1), 1-22.

● Matei, A., Matei, S., Matei, G. M., Cogălniceanu, G., & Cornea, C. P. (2020). Biosynthesis of silver nanoparticles mediated by culture filtrate of lactic acid bacteria, characterization and antifungal activity. The EuroBiotech Journal, 4(2), 97-103.

● Ma, R. H., Ni, Z. J., Zhu, Y. Y., Thakur, K., Zhang, F., Zhang, Y. Y., ... & Wei, Z. J. (2021). A recent update on the multifaceted health benefits associated with ginger and its bioactive components. Food & Function, 12(2), 519-542.

● Nouri, A., Yaraki, M. T., Lajevardi, A., Rezaei, Z., Ghorbanpour, M., & Tanzifi, M. (2020). Ultrasonic-assisted green synthesis of silver nanoparticles using Mentha aquatica leaf extract for enhanced antibacterial properties and catalytic activity. Colloid and Interface Science Communications, 35, 100252.

● Abd El-Mongy, M., & Mohamed, A. (2017). Synergistic effect of some medicinal plants oils incorporated with silver Nanoparticles against different Gram negative bacteria. THE EGYPTIAN JOURNAL OF EXPERIMENTAL BIOLOGY (Botany), 13(2), 333-340.

● Fathi, H., Ramedani, S., Heidari, D., YazdanNejat, H., Habibpour, M., & Ebrahimnejad, P. (2017). f silver nanoparticles using Mentha aquatic L extract as the reducing agent. Journal of Kerman University of Medical Sciences, 24(1), 28-37.

● Jayaramudu, T., Varaprasad, K., Raghavendra, G. M., Sadiku, E. R., Mohana Raju, K., & Amalraj, J. (2017). Green synthesis of tea Ag nanocomposite hydrogels via mint leaf extraction for effective antibacterial activity. Journal of Biomaterials science, Polymer edition, 28(14), 1588-1602.

● Aziz, W. J., & Jassim, H. A. (2018). Green chemistry for the preparation of silver nanoparticles using mint leaf leaves extracts and evaluation of their antimicrobial potential. World News of Natural Sciences, 18(2)

● Iravani, S. (2011). Green synthesis of metal nanoparticles using plants. Green Chemistry, 13(10), 2638-2650.53

● Naqvi, S. Z. H., Kiran, U., Ali, M. I., Jamal, A., Hameed, A., Ahmed, S., & Ali, N. (2013). Combined efficacy of biologically synthesized silver nanoparticles and different antibiotics against multidrug-resistant bacteria. International journal of nanomedicine, 8, 3187.

● Zhang, X. F., Liu, Z. G., Shen, W., & Gurunathan, S. (2016). Silver nanoparticles: synthesis, characterization, properties, applications, and therapeutic approaches. International journal of molecular sciences, 17(9), 1534.

● Alzohairy, M. A. (2016). Therapeutics role of Azadirachta indica (Neem) and their active constituents in diseases prevention and treatment. Evidence-Based Complementary and Alternative Medicine, 2016.

● Chinnasamy, G., Chandrasekharan, S., Koh, T. W., & Bhatnagar, S. (2021). Synthesis, Characterization, Antibacterial and Wound Healing Efficacy of Silver Nanoparticles From Azadirachta indica. Frontiers in Microbiology, 12, 204.

● Choukade, R., Jaiswal, A., & Kango, N. (2020). Characterization of biogenically synthesized silver nanoparticles for therapeutic applications and enzyme nanocomplex generation. 3 Biotech, 10(11), 1-13.

● Gupta, S. C., Prasad, S., Tyagi, A. K., Kunnumakkara, A. B., & Aggarwal, B. B. (2017). Neem (Azadirachta indica): An indian traditional panacea with modern molecular basis. Phytomedicine, 34, 14-20.

● Rather, M. A., Bhat, I. A., Sharma, N., Gora, A., Ganie, P. A., & Sharma, R. (2017). Synthesis and characterization of Azadirachta indica constructed silver nanoparticles and their immunomodulatory activity in fish. Aquaculture Research, 48(7), 3742-3754.

● Uthaya Kumar, U. S., Abdulmadjid, S. N., Olaiya, N. G., Amirul, A. A., Rizal, S., Rahman, A. A., ... & Abdul Khalil, H. P. S. (2020). Extracted compounds from neem leaves as antimicrobial agent on the physico-chemical properties of seaweed-based biopolymer films. Polymers, 12(5), 1119.

● Verma, A., & Mehata, M. S. (2016). Controllable synthesis of silver nanoparticles using Neem leaves and their antimicrobial activity. Journal of radiation Research and applied sciences, 9(1), 109-115.

● Vinay, S. P. (2021). Synthesis of Fullerene (C 60)-Silver Nanoparticles Using Neem Gum Extract Under Microwave Irradiation. BioNanoScience, 11(1), 1-7.

● Kumar, S., Taneja, S., Banyal, S., Singhal, M., Kumar, V., Sahare, S., ... & Choubey, R. K. (2021). Bio-synthesised Silver Nanoparticle-Conjugated l-Cysteine Ceiled Mn: ZnS Quantum 54 Dots for Eco-friendly Biosensor and Antimicrobial Applications. Journal of Electronic Materials, 50(7), 3986-3995.

● Haider, A., & Kang, I. K. (2015). Preparation of silver nanoparticles and their industrial and biomedical applications: a comprehensive review. Advances in materials science and engineering, 2015.

● Banach, M., Szczygłowska, R., Pulit, J., & Bryk, M. (2014). Building materials with antifungal efficacy enriched with silver nanoparticles.

● Wicki, A., Witzigmann, D., Balasubramanian, V., & Huwyler, J. (2015). Nanomedicine in cancer therapy: challenges, opportunities, and clinical applications. Journal of controlled release, 200, 138-157.