Journal of Commercializable Technologies
Volume 1(01), June 2026, pp.31-44

Mushroom-Mediated Green Synthesis of Silver and Iron Nanoparticles and Their Antimicrobial, Antioxidant, and Antifungal Potential

Megha, Monika Yadav, Nitika Dhaka, Nikita Kadian, Krishna Kant Sharma, Dr. Rajeev Kumar Kapoor*

*Department of Microbiology, MaharshiDayanand University, Rohtak 124001, Haryana,India

* Corresponding author.

E-mail address:rajeev.micro@mdurohtak.ac.in

Abstract

Technology Area

Nanotechnology; Green Nanobiotechnology; Microbial/Mushroom-based Bioprocessing; Biomedical Applications

Technology

The present technology involves the green synthesis of silver (AgNPs) and iron nanoparticles (FeNPs) using extracellular extracts of Pleurotuscitrinopileatus and Ganoderma spp. The process utilizes mushroom-derived biomolecules as natural reducing and stabilizing agents, eliminating the need for toxic chemicals.

The synthesis is simple, cost-effective, and environmentally sustainable, indicated by a characteristic color change due to reduction of metal ions. The nanoparticles are characterized using standard analytical techniques such as UV–Visible spectroscopy (peak at 425 nm for AgNPs), FTIR, SEM, and zeta potential analysis.

The synthesized nanoparticles exhibit significant antibacterial, antifungal, and antioxidant activities, making them suitable for biomedical and environmental applications.

Potential Market / Customers

Pharmaceutical and biomedical industries (antimicrobial formulations, wound dressings), Healthcare sector (infection control, coatings, therapeutics), Food industry (food preservation and packaging), Water treatment and environmental remediation industries, Cosmetic industry (antioxidant-based products), Research and nanotechnology companies

Technology Valuation (Commercial Potential & Value Proposition)

This technology offers strong commercial potential due to its eco-friendly, scalable, and low-cost production process compared to conventional nanoparticle synthesis methods. The use of readily available mushroom biomass reduces production costs and enhances sustainability.

Keywords

Green synthesis, Silver nanoparticles, Iron nanoparticles, Mushroom extract, Antimicrobial activity, Antioxidant activity

1. Introduction

Nanotechnology, derived from the Greek word "nano" meaning "dwarf," refers to the science and engineering of materials at the nanoscale level (1–100 nm). It is an interdisciplinary field integrating principles from physics, chemistry, biology, materials science, and engineering, enabling the development of novel materials with unique physicochemical properties. Nanoparticles, due to their high surface area-to-volume ratio and distinctive optical, chemical, and biological characteristics, have gained considerable attention for applications in medicine, environmental remediation, and food technology **(1–3)**.

In recent years, green synthesis of nanoparticles has emerged as a sustainable alternative to conventional physical and chemical methods. Traditional approaches often involve toxic chemicals, high energy input, and environmentally hazardous by-products. In contrast, biological or green synthesis utilizes natural sources such as plants, microorganisms, and fungi, offering advantages including eco-friendliness, cost-effectiveness, scalability, and biocompatibility **(4–7)**.

Among biological systems, mushrooms have gained prominence as efficient biofactories for nanoparticle synthesis. Mushrooms are rich in bioactive compounds such as polyphenols, terpenoids, proteins, and enzymes, which act as reducing and stabilizing agents in the conversion of metal ions into nanoparticles. This process, known as mushroom-mediated biosynthesis, is simple, rapid, and does not require harsh reaction conditions or toxic reagents **(8–10)**. Additionally, mushrooms have been used for centuries as both food and medicine, largely due to their rich phenolic content and therapeutic properties **(8)**.

Metal-based nanoparticles, particularly silver (AgNPs) and iron nanoparticles (FeNPs), have attracted significant attention owing to their potent antimicrobial properties. These nanoparticles exhibit broad-spectrum antibacterial activity and are widely explored for applications in medical therapeutics, food preservation, and water treatment **(11–13)**. The genus *Pleurotus* (oyster mushrooms), commonly found across tropical and subtropical regions, is especially suitable for nanoparticle synthesis due to its rapid growth, high biomass yield, and rich biochemical composition **(8, 14)**.

The biosynthesis of nanoparticles is influenced by several physicochemical parameters, including metal ion concentration, extract volume, pH, temperature, and incubation time. Optimization of these parameters is essential to achieve maximum yield and stability of nanoparticles **(15, 16)**. Typically, synthesis is confirmed by a visible color change, followed by detailed characterization using techniques such as UV–Visible spectroscopy, Fourier-transform infrared spectroscopy (FTIR), scanning electron microscopy (SEM), and zeta potential analysis to determine particle size, morphology, and stability **(9, 17)**.

Furthermore, biologically synthesized nanoparticles have demonstrated significant antimicrobial activity against pathogenic microorganisms such as *Escherichia coli*, *Bacillus subtilis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. Their effectiveness highlights their potential as alternative antimicrobial agents in the face of rising antibiotic resistance **(18–19)**.

2. Materials and Methods

2.1 Chemicals and Reagents

All chemicals and culture media used in this study were of analytical grade. Potato Dextrose Agar (PDA), Nutrient Agar (NA), Agar, Sodium

hydroxide (NaOH), Silver nitrate ($AgNO_3$), and Ferric chloride ($FeCl_3$) were procured from HiMedia Laboratories (India), while Mueller–Hinton Agar (MHA) was obtained from SRL Chemicals (India).

2.2 Instruments

The experimental work was carried out using standard laboratory instruments, including a weighing balance (Shimadzu Pvt. Ltd.), autoclave, pH meter (Sartorius), laminar airflow chamber (Hicon Pvt. Ltd., India), shaking incubator (Scigenics Biotech Pvt. Ltd.), FTIR spectrometer (Bruker, Germany), hot air oven (SANYO Electric Co., Ltd.), refrigerator (Godrej Pvt. Ltd.), centrifuge (Genetix Pvt. Ltd.), zeta sizer (Malvern Nano ZS), UV–Visible spectrophotometer (Spectro UV-Vis), and scanning electron microscope (Zeiss Smart EDX).

2.3 Preparation of Mushroom Extract

Mushroom extracts of *Pleurotuscitrinopileatus* and *Ganoderma* spp. were prepared using both culture filtrate and fruiting body extraction methods.

For culture-based extraction, 250 mL Erlenmeyer flasks containing 50 mL Potato Dextrose (PD) broth were inoculated with mycelial discs obtained from actively growing 7-day-old cultures on PDA plates. The flasks were incubated at 25°C under static conditions for 5 days. The culture was then filtered through muslin cloth followed by Whatman filter paper to obtain a clear extracellular extract.

For fruiting body extraction, dried *Ganoderma* fruiting bodies were powdered, and 10 g of powder was mixed with 100 mL distilled water. The mixture was heated in a water bath at 100°C for 2 h, followed by filtration using Whatman filter paper. The extract was stored at 4°C for further use.

2.4 Biosynthesis of Silver Nanoparticles (AgNPs)

Silver nanoparticles were synthesized using mushroom extract as a reducing and stabilizing agent. Briefly, 2 mL of mushroom extract was added dropwise to 10 mL of 1 mM$AgNO_3$ solution under dark conditions to prevent photoreduction. The reaction mixture was incubated at 60°C for 24 h in a shaking incubator. Formation of AgNPs was indicated by a visible color change from pale yellow to brown. The synthesis was further confirmed by UV–Visible spectroscopy in the range of 200–800 nm.

2.5 Optimization of Nanoparticle Synthesis (OFAT Approach)

Optimization of nanoparticle synthesis was carried out using a one-factor-at-a-time (OFAT) approach by varying individual parameters while keeping others constant. The parameters optimized included silver nitrate concentration, volume of mushroom extract, incubation time, temperature, and pH.

2.5.1 Effect of $AgNO_3$ Concentration

The concentration of $AgNO_3$ was varied from 0.25 mM to 2 mM to determine its effect on nanoparticle synthesis. The optimal concentration was identified based on maximum absorbance using UV–Visible spectroscopy.

2.5.2 Effect of Mushroom Extract Volume

Different volumes of mushroom extract (1–5 mL) were tested with 1 mM$AgNO_3$ solution. Nanoparticle synthesis was monitored spectrophotometrically.

2.5.3 Effect of Incubation Time

The reaction mixture was incubated for varying time intervals (0–92 h), and nanoparticle formation was monitored periodically using UV–Visible spectroscopy to determine the optimal incubation time.

2.5.4 Effect of Temperature

The reaction mixtures were incubated at different temperatures (4°C, 25°C, 37°C, and 60°C) to determine the optimal temperature for nanoparticle synthesis.

2.5.5 Effect of pH

The pH of the reaction mixture was adjusted between 6 and 11 to study its effect on nanoparticle synthesis. Absorbance readings were recorded to determine optimal pH conditions.

2.6 Characterization of Nanoparticles

2.6.1 UV–Visible Spectroscopy

Preliminary confirmation of nanoparticle synthesis was achieved by observing the color change and recording absorbance spectra in the range of 200–800 nm.

2.6.2 Zeta Size and Zeta Potential Analysis

Particle size distribution and stability of nanoparticles were analyzed using a zeta sizer. Zeta potential measurements were performed to determine surface charge and colloidal stability.

2.6.3 Fourier Transform Infrared Spectroscopy (FTIR)

Nanoparticles were centrifuged at 10,000 rpm for 15 min, and the pellet was dried at 40°C. The dried samples were analyzed using FTIR to identify functional groups involved in nanoparticle synthesis.

2.6.4 Scanning Electron Microscopy (SEM)

The morphology and surface topology of nanoparticles were examined using SEM. Dried samples were mounted and analyzed to obtain high-resolution images.

2.7 Biological Applications of Synthesized Nanoparticles

2.7.1 Antibacterial Activity

Antibacterial activity was evaluated using the agar well diffusion method. Mueller–Hinton Agar plates were inoculated with bacterial strains (*Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli,* and *Bacillus subtilis*). Wells (6 mm diameter) were loaded with 50 µL of mushroom extract, AgNPs, FeNPs, and standard antibiotic (amoxicillin, 50 µg/mL). Plates were incubated at 37°C for 24 h, and zones of inhibition were measured.

2.7.2 Antioxidant Activity (DPPH Assay)

DPPH radical scavenging activity was determined using 0.1 mM DPPH solution in methanol. Equal volumes (2 mL each) of DPPH solution and sample were mixed and incubated at 30°C for 30–40 min. Absorbance was recorded at 517 nm. Percentage scavenging activity was calculated using standard formula.

2.7.3 Antifungal Activity

Antifungal activity was evaluated using a 96-well microtiter plate assay. Potato Dextrose Broth (50 µL) was added to each well, followed by serial dilution of the nanoparticle sample. Fungal spores (20 µL) were added to each well and incubated for 24 h. Subsequently, 20 µL of resazurin dye was added and incubated for 30 min. Color change was observed to determine the minimum inhibitory concentration (MIC).

4. Results

4. Results

4.1 Biosynthesis of Silver nanoparticles

The synthesis of different biomolecules and enzymes holding industrial importance has been reported by *P. citrinopileatus*and *Ganodermaspp,* due to which extracellularly silver and Iron nanoparticles synthesizing ability of this fungus have been reported. Many

methods have been reported for the synthesis of silver nanoparticles but the biological method is beneficial in many aspects including inexpensive and ecological properties. Mushroom extract contains biomolecules like polysaccharides, amino acids, vitamins, enzymes and proteins, which enables the reduction of $AgNO_3$ into AgNPs and fecl3 into FeNPs assured by visual observation of colour change from light milkiness to dark brown. In a reaction mixture culture filtrate supernatant of *P.citrinopileatus*and *Ganodermaspp.*reacted with $AgNO_3$ (aqueous) in dark condition. The colour turned dark brown after increase in reaction time. The occurrence of a band at 425 nm in visible absorption spectroscopy confirmed nanoparticles formation

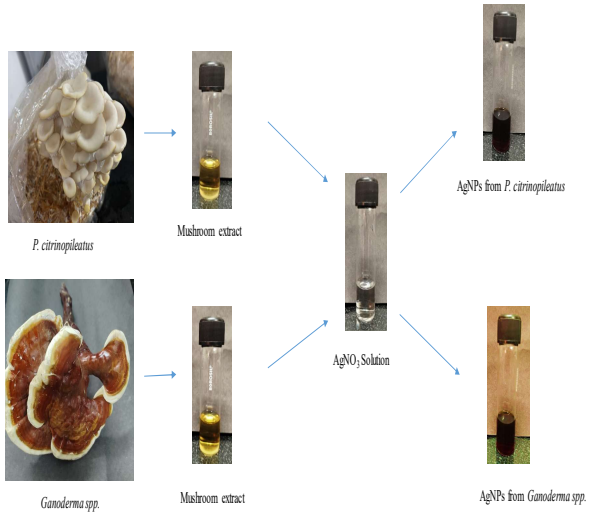


Figure 4.1 Steps of biosynthesis of AgNPs by *P. citrinopileatus*and *Ganoderma spp.*

4.1.1 Visual Observation of colour change during Green synthesis

Aqueous mushroom extract along with aqueous $AgNO_3$ (1mM) was subjected to change colour of reaction mixture changed from light yellow to yellowish brown colour (Figure 4.2 d) without any deposition indicating the formation of AgNPs after 24 hours.

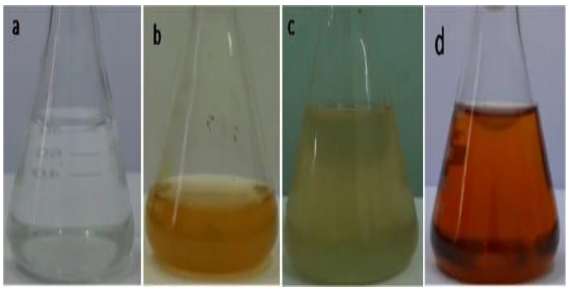


Figure 4.2 Visual observation of colour change. **a)** $AgNO_3$ Solution0.1mM. **b)** Mushroom Extract. **c)** Initial colour of AgNPs and **d)**Colour of AgNPs after 24 hours (brownish colour appear).

4.1.2 UV- visible Spectroscopy-

The UV visible spectra between 200 and 800 nm were examined for the synthesis of both FeNPs and AgNPs. Peak for AgNPs was observed at 425 nm (Figure 4.3). After 48 hours of incubation, the reaction mixture shows maximum synthesis of Nanoparticles.

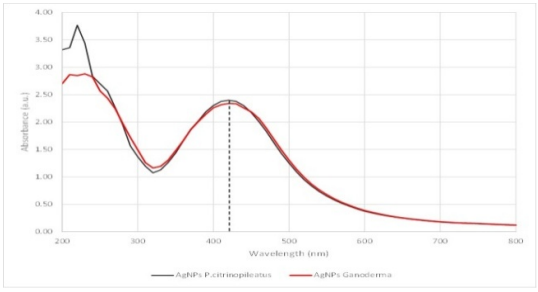


Figure 4.3 - The ultraviolet- visible spectra of AgNPs from *P. citrinopileatus*and *Ganoderma spp.* (showing peak at 425nm).

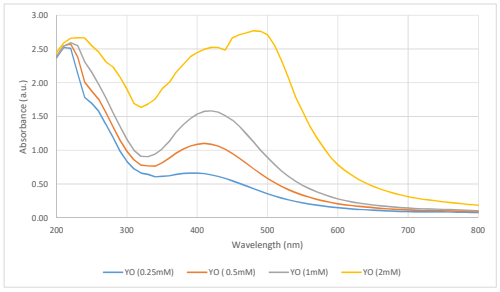
4.2 Process optimization of AgNPs biosynthesis through OFAT approach

The growth and metabolism of mushroom are greatly influenced by environmental factors. The rate of synthesis and stability of AgNPs were subject of numerous investigations. The crucial factors that directly affect productivity and economics of the process are cultural conditions. Optimizing physical and chemical parameters will enhance growth while also increasing product yield. The growing conditions directly track the rate of enzyme activity, which reflects the synthesis of AgNPs, AgNPs were studied and optimized the different parameters by using the OFAT approach, such parameters are volume

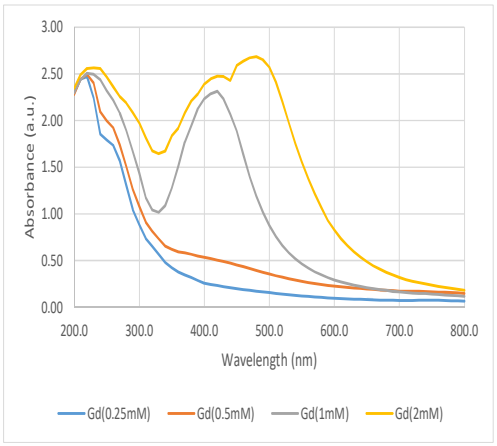
of mushroom extract, Effect of temperature, Concentration of $AgNO_3$, Reaction time, Effect of pH.

4.2.1 Effect of silver nitrate concentration

*P. citrinopileatus and Ganodermaspp.*boththeextract was used to examine the AgNPs biosynthesis at several $AgNO_3$ concentration solution, ranging from 0.25mM to 2mM. In both the case maximum synthesis of silver nanoparticles (Figure 4.4) occur when we use 1mM solution of Silver nitrate.



A.)



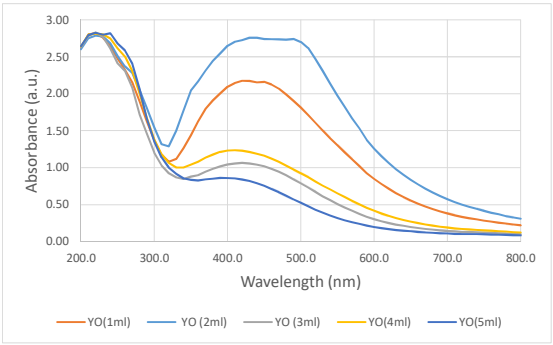
B.)

Figure 4.4 Effect of ($AgNO_3$) concentration on biosynthesis of Silver nanoparticles from **A.)***P. citrinopleatus*and**B.)** *Ganoderma spp.* (showing best result when 1mM $AgNO_3$ was used)**.**

4.2.2 Effect of Mushroom extract Volume

The result of Volume of Mushroom extract on the production of AgNPs was examined by using 1mL to 5mL extract. The result reveal a maximum peak at 425 nm (Figure 4.5) .When we use 2mL Mushroom extract maximum synthesis of Nanoparticles occur in both the cases.

A.)



B.)

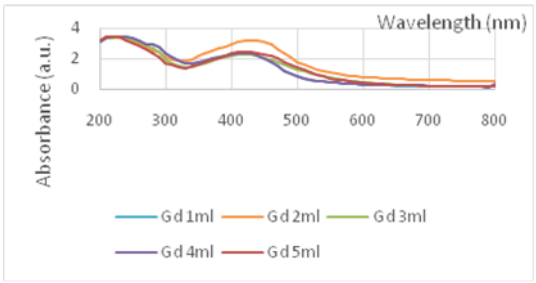


Figure 4.5 Effect of Volume mushroom extract on production of AgNPs from **A.)** *P. citrinopleatus*and **B.)***Ganoderma spp.* (showing best result when 2mL Mushroom extract was used for 10mL (1mM) $AgNO_3$ Solution).

4.2.3 Effect of Temperature

Temperature play important role for synthesis of Nanoparticles. Reaction mixture was incubated at different temperature (4℃, 25℃, 37℃, 60℃) in a BOD incubator. Compared to other temperatures, the highest synthesis of AgNPs was seen at 60℃ (Figure 4.6) through a colour change in 12 hours, as well as by UV- visible absorption spectra, 60℃ has the highest enzyme activity.

A.)

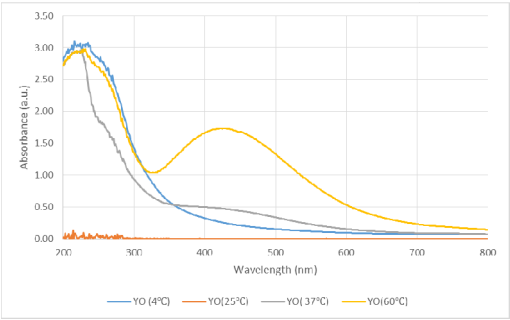


Figure 4.6.1 Effect of temperature on production of AgNPs from **A.)** *P. citrinopleatus*

B.)

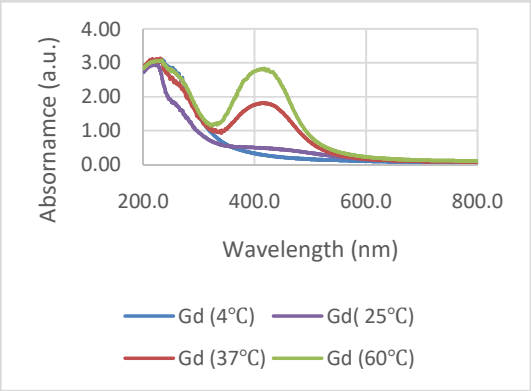
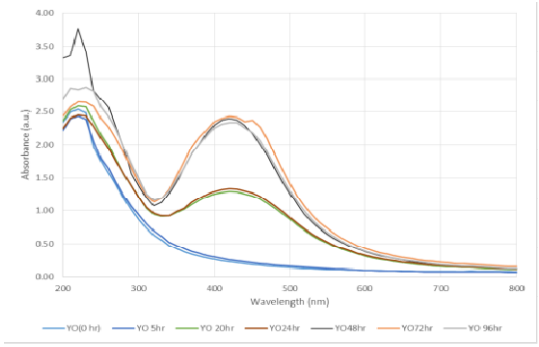


Figure 4.6.2 Effect of temperature on production of AgNPs from **B.)** *Ganoderma spp.*(Maximum synthesis at 60℃).

4.2.4 Effect of Reaction Time

For the biosynthesis of AgNPs silver nitrate solution along with mushroom extract, long incubation is required under particular chemical and physical condition. Reaction time largely affect the biosynthesis, the amount of AgNPs increase with increase in time but for a certain period of time. The experiment was performed reaction time was used (0 – 96 hours) out of which 48 hour was optimized (Figure 4.7). The optimal concentration for the synthesis of AgNPs was observed using UV- visible absorption spectroscopy.

A.)



B.)

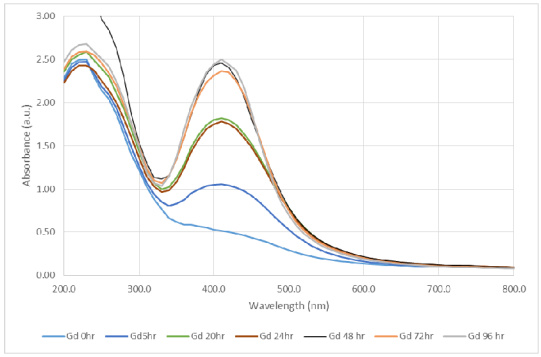
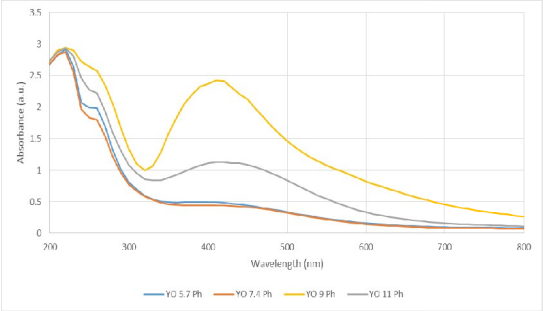


Figure 4.7 Effect of reaction time on production of AgNPs from**A)***P. citrinopleatus*and **B)** *Ganoderma spp.*(showing best results when we incubate our reaction mixture for 48 hours).

4.2.5 Effect of pH

pH play important role in the synthesis of Nanoparticles . Reaction occur at several pH ranging from 6 to 12 the optimized pH for the AgNPs synthesis is 9 pH at pH 9 maximum nanoparticles synthesis occur (Figure4.8).

A.)



B.)

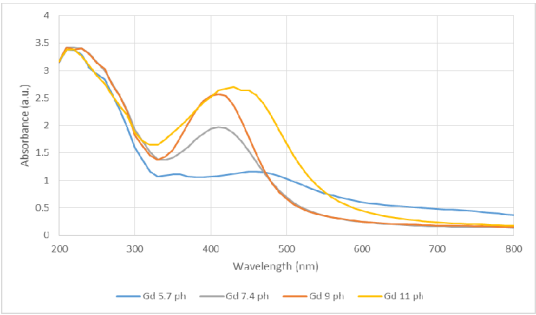


Figure 4.8 Effect of pH on production of AgNPs from **A.)** *P. citrinopleatus*and **B.)** *Ganoderma spp.* (Maximum synthesis at pH 9).

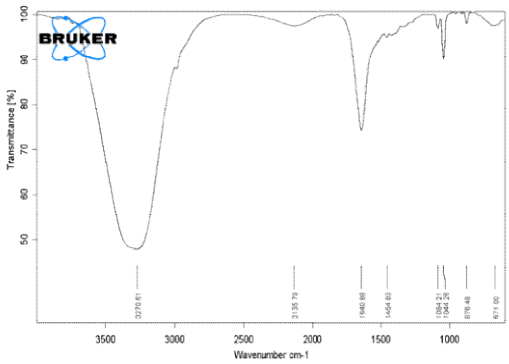
4.3 Studies on the characterization of biosynthesized silver and Iron Nanoparticles

4.3.1 FTIR Spectroscopy

To determine any potential interaction between silver and bioactive molecules that might be in charge of the certain and stabilization of AgNPs, FTIR measurements of the dried and powered sample were performed. Bands at 3278.36, 2127.27, 1637.78 and 678.27 cm-1(Figure 4.9) in the FT-IR spectra of AgNPs produced by *P. citrinopileatus* showed the participation of several amino acid functional groups in the creation.

The band at 3278.36 cm^{-1} is attributed to N-H Stretching a strong indicator of the presence of nitrogen-containing functional groups, especially those with N-H bonds like primary amines, secondary amines, and amides, while the peak at 2127.27 cm^{-1} suggests the presence ofcarbon-carbon triple bond (C≡C). The band at 1637.38 cm^{-1} is assigned to the C=O stretching of the carbonyl group, also from the capping agent.The absence of a distinct peak around 1454.83cm^{-1} indicates the C-C bond is not significantly contributing to the spectrum at this stage.The absence of peaks in the 1000–600 cm^{-1} region suggests the lack of aromatic compound vibrations, indicating that non-aromatic functional groups may be involved. This spectral pattern supports the successful synthesis of nanoparticles.

A.)



B.)

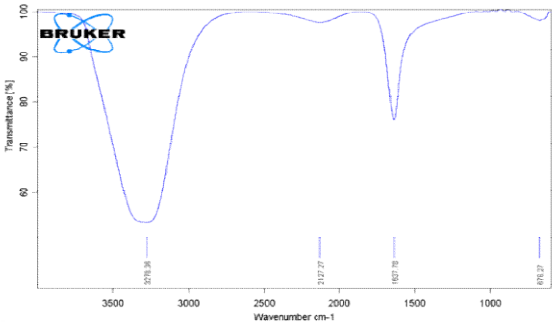
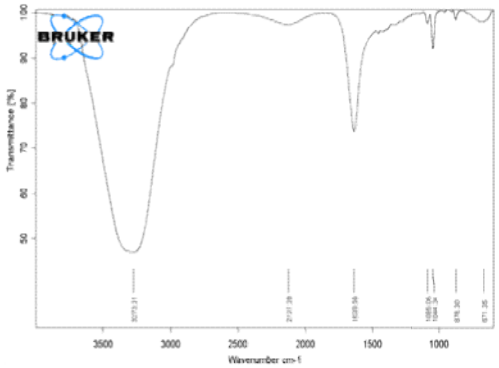


Figure 4.9a) FTIR spectrum of **A**.)*P. citrinopileatus.* extract**B**.) *P. citrinopileatus*AgNPs(Nanoparticles synthesis due to absence of peaks in the 1000–600 cm^{-1} region).

Similarly FT-IR spectra of AgNPs produced by *Ganoderma spp.* shows more bands than *P. citrinopileatus* at 3273.31, 2127.28, 1639.58, 1085.06, 1044.34, 876.30 and 671.35 cm-1 (Figure 4.10) which showed the participation of several amino acid functional groups in the creation. The band at 3273.31 cm^{-1} is attributed to N-H Stretching a strong indicator of the

presence of nitrogen-containing functional groups, especially those with N-H bonds like primary amines, secondary amines, and amides, while the peak at 2127.28 cm^{-1} suggests the presence ofcarbon-carbon triple bond (C≡C). The band at 1639.58 cm^{-1} is assigned to the C=O stretching of the carbonyl group, also from the capping agent.Peak in the 1000-600 cm^{-1} region indicates the ring vibrations in aromatic compounds. Due to band at 1639.58 cm^{-1} which is for C=O stretching nanoparticles synthesized.

A.)



B.)

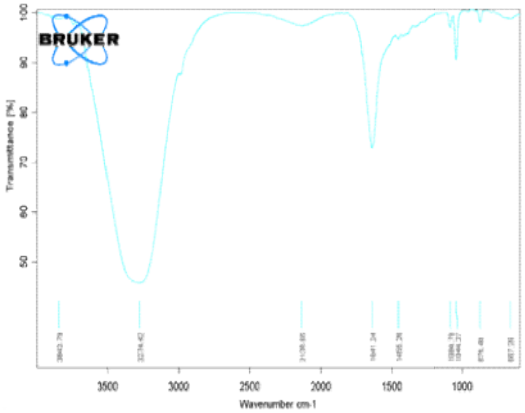


Figure 4.10 a) FTIR spectrum of *Ganodermaspp.*AgNPs**b)***Ganodermaspp.*extract (band at 1639.58 cm^{-1} which is for C=O stretching shows nanoparticles synthesis).

FT-IR spectrum obtained by $FeCl_3$ shows bands at 3852.01, 3742.25, 3673.21, 3614.52, 3214.16, 3116.93, 2359.11, 1916.95,1743.39,1696.31, 1649.94, 1520.94, 1463.95, 1045.84,687.44cm^{-1} (Figure 4.11) which showed the participation of several amino acid functional groups in the creation. The band at 3852.01, 3742.25, 3673.21, 3614.52, 3214.16 and 3116.93cm^{-1} is attributed to N-H Stretching a strong indicator of the presence of nitrogen-containing functional groups, especially those with N-H bonds like primary amines, secondary amines, and amides, while the peak at 2359.11 cm^{-1} Suggests the presence ofcarbon-carbon triple bond (C≡C). The band at 1916.95,1743.39,1696.31, 1649.94 and 1520.94cm^{-1} is assigned to the C=O stretching of the carbonyl group, also from the capping agent. The bands at 1463.95cm^{-1} indicates the C-C bond is not significantly contributing to the spectrum at this stage.Peak in the 1000-600 cm^{-1} region indicates the ring vibrations in aromatic compounds.

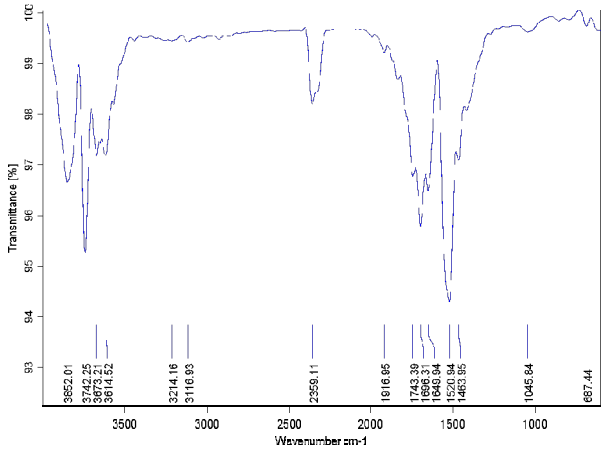


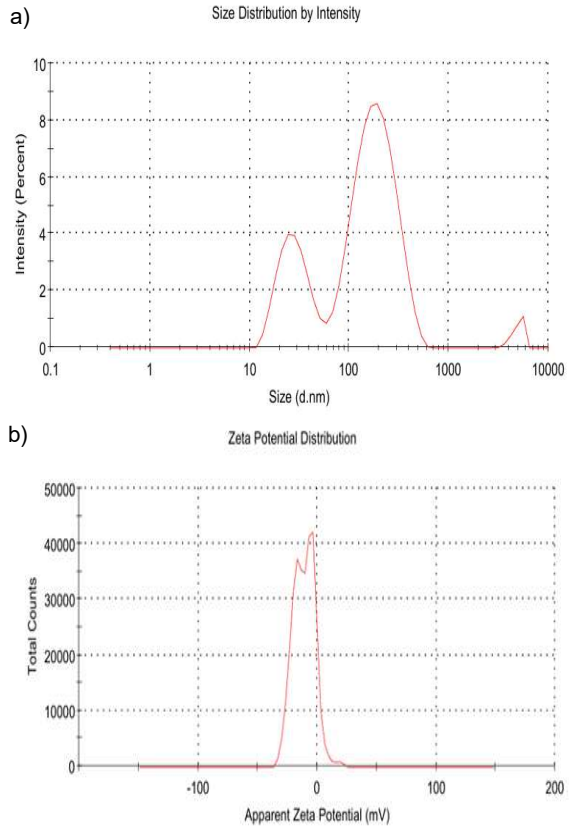
Figure 4.11 FTIR spectrum of FeNPs produced by *P. citrinopileatus* (Showing Nanoparticles synthesis due to N-H Stretching and C=O stretching).

4.3.2 Zeta potential

The appropriate size and charge plays a major role in the structural and functional properties of any particle used in biological applications in nanoscience, though the nano-size of each particle is appropriately small, several tiny nanoparticles are needed to improve activity, which they do by increasing the surface area. The AgNPs produced by *P. citrinopileatus*zeta potential and particle size were found to be

(82.51nm, -10.9 mV) and AgNPs produced by *Ganoderma*Potential and Particle size is (39.48nm, -25.1mV), which indicates that the AgNPs were relatively stable (Figure 4.12a, 4.12c). The homogeneity of the dispersions is indicated by the PDI of 0.546 and 0.447 within the allowed limit of <1.0. Zeta potential is one such method that verifies the applicability of nanoparticles based on those mentioned criteria. A Zeta potential analyzer was used to analyze bio- stabilized AgNPs in a colloidal solution. The last result that was seen was -10.9 mV and -25.1mV (4.12a, 4.12c). This proves that AgNPs are safe to use and are redistributed in colloidal form.

$FeCl_3$ produced by *P. citrinopileatus*zeta potential and particle size were found to be (80.62nm, -14.6 mV) (Figure 4.12e, 4.12f) which indicates that the $FeCl_3$ were relatively stable. The homogeneity of the dispersions is indicated by the PDI of 0.262 within the allowed limit of <1.0. Zeta potential is one such method that verifies the applicability of nanoparticles based on those mentioned criteria. This proves that $FeCl_3$ are safe to use and are redistributed in colloidal form.



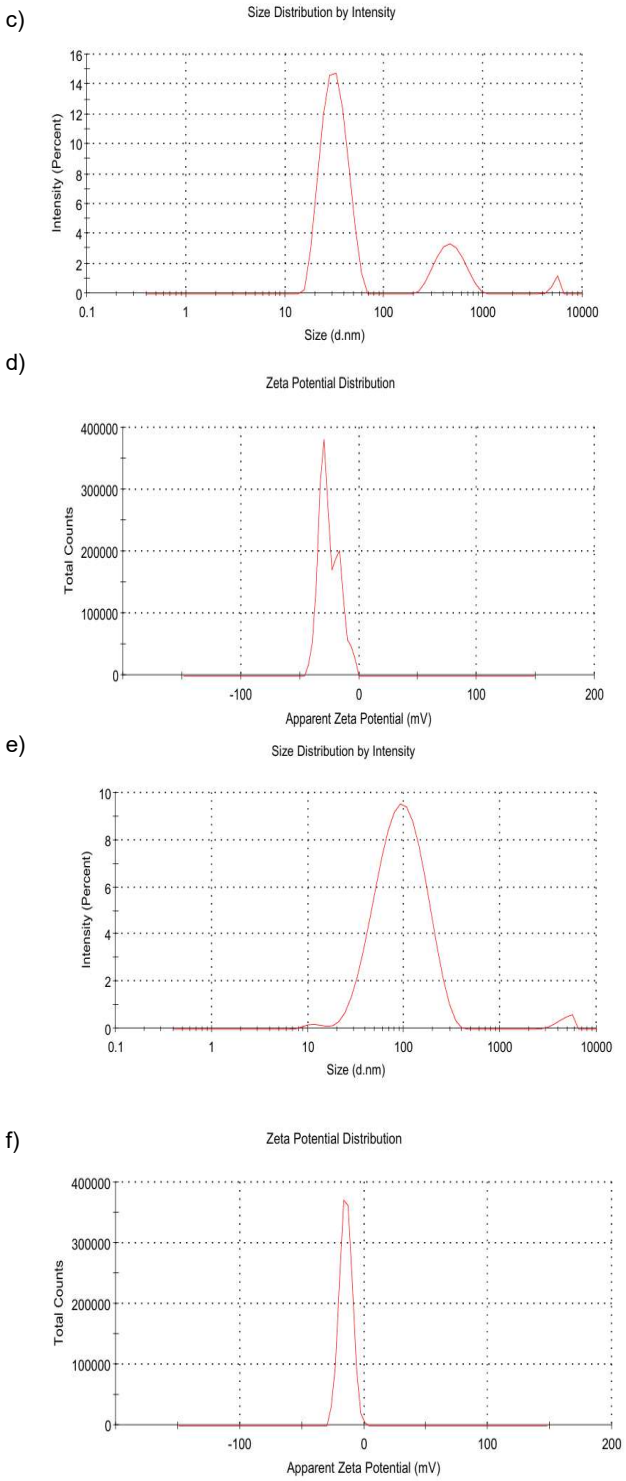


Figure 4.12 a) Zeta size and PDI of the AgNPs produced by *P. citrinopileatus.* ***b)*** Zeta potential of AgNPs produced by *P. citrinopileatus.* **c)** Zeta size and PDI of the AgNPs produced by *Ganoderma spp.* ***d)***Zeta potential of AgNPs produced by *Ganoderma spp.* **e)** Zeta size and PDI of the $FeCl_3$ produced by *P. citrinopileatus.* **f)** Zeta potential of FeNPs produced by *P. citrinopileatus*(Size indicates that AgNPs andFeNPs were relatively stable).

4.4.3 SEM (Scanning Electron Microscope)

This technique makes 3D image of the nanoparticles. The surface area of the nanoparticles are scaned by the SEM which confirms the Nanoparticles are present or not in an sample. The spherical tiny particles appears. Their size is in the range of 25-30 nm.

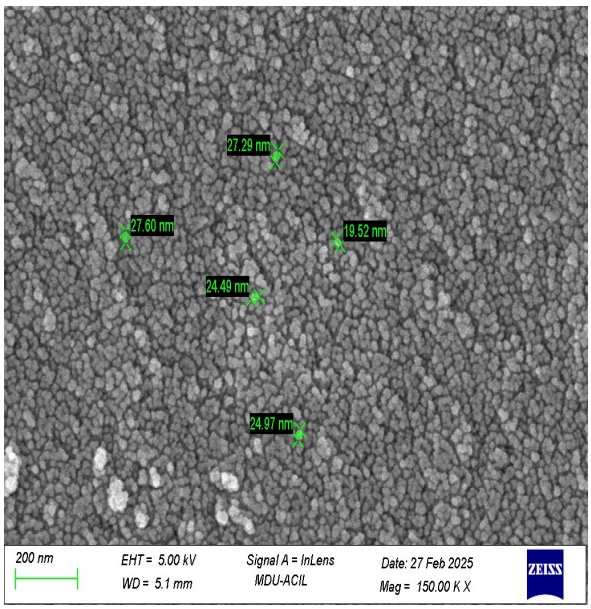
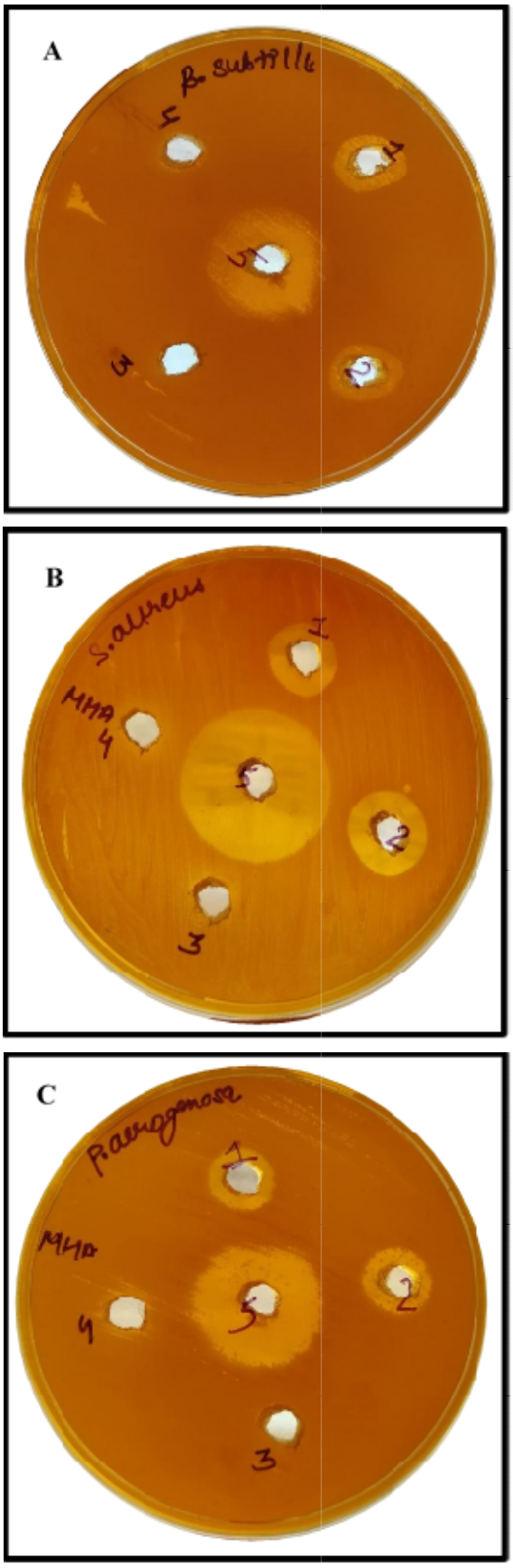


Figure 4.13 Scanning Electron Microscope Image of FeNPsSynthesized from *P. citrinopileatus*(FeNPsSize is in the range of 25-30 nm).

4.4 Applications of silver and iron nanoparticles

4.4.1 Antibacterial Activity of MycosynthesizedFeNPs and AgNPs

The antimicrobial Activity of AgNPs and FeNPS was tested against 4 bacterial strainsgram positive and gram negative bacteria (*Pseudomonas aeruginosa, Staphylococcus aureus , Escherichia coli , Bacillus subtilis)* The antibacterial activity was better observed in *Escherichia coli , Bacillus subtilis*(Figure 4.14).



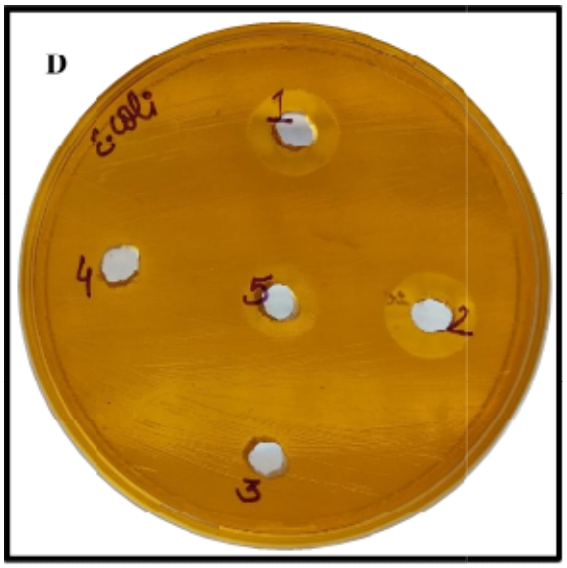


Figure 4.14- Antimicrobial activity of synthesized AgNPs against **A)***B. subtilis***B)** *S. aureus***C)** *P. aeruginosa,* and **D)***E. coli* (Showing maximum results against *Escherichia coli* and *Bacillus subtilis*with zone of Inhibition of 9mm).

Table 4.1 Assigned well positions of nanoparticles and mushroom extract.

Sr. No.	Well No.	Sample
1.	1	*Ganodermaspp.*AgNPs
2.	2	*P. citrinopileatus*AgNPs
3.	3	*Ganodermaspp.*extract
4.	4	*P. citrinopileatus*extract
5.	5	Antibiotic (Amoxicillin)

AgNPs produced by both the Mushrooms showed Maximum results against *Escherichia coli* and *Bacillus subtilis*with zone of Inhibition of 9mm (Table 4.2).This may be due to protein molecules, phospholipid bilayers and negatively charged phosphate present in membrane of bacterial cell which leads to the generation of Ag^{+} ions from AgNPs.

Consequently, Ag^{+} ions attract and associate together with bacteria cell membrane results in modification in bacterial structure with major cell membrane damage. Moreover, inactivation or even loss of bacterial enzymes due to significant involvement of Ag^{+} ions with sulfhydryl group (-SH) of bacterial enzyme (Brandt et al., 2012; Zhao et al., 2013)

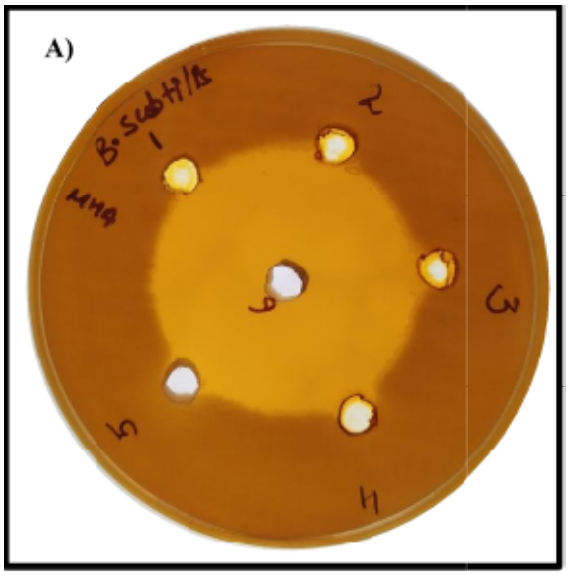
Development of zones of inhibition against different bacteria are listed below in table.

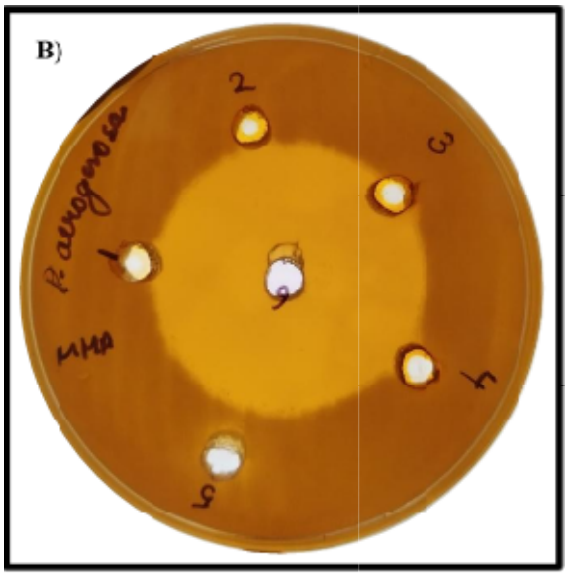
Table 4.2 Antibacterial activity of synthesized silver nanoparticles and Mushroom extract(*P. citrinopileatus*and *Ganoderma spp.)*

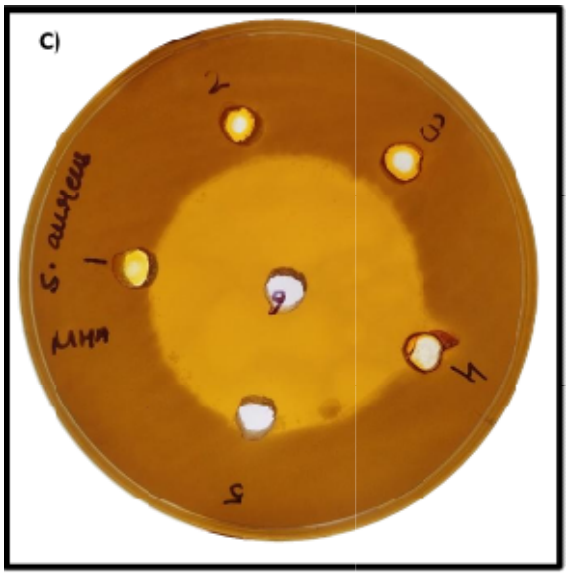
Sr No.	**Microbial Organisms**	**Zone of Inhibition (mm)**				
		Antibiotic (mm)	**Mushroom extract (*Ganoderma spp.*)**	***Pleurotuscitrinopileatus* extract**	**AgNPs (*Ganoderma spp.*)**	**AgNPs *Pleurotuscitrinopileatus***
1.	*Escherichia coli*	4	-	-	9	9
2.	*Pseudomonas aeruginosa*	14	-	-	7	4
3.	*Staphylococcus aureus*	19	-	-	4	4
4.	*Bacillus subtilis*	17	-	-	9	8

Iron nanoparticles synthesized from *P. citrinopileatus*and *Ganodermaspp.*did not shown any antibacterial activity against all the four bacterial strains. Four concentration of biosynthesized FeNPs

(25%, 50%, 75%, 100%) were investigated towards four bacterial cultures (*Pseudomonas aeruginosa, Staphylococcus aureus , Escherichia coli , Bacillus subtilis).* FeNPs does not show any antibacterial activity (Figure 4.15).







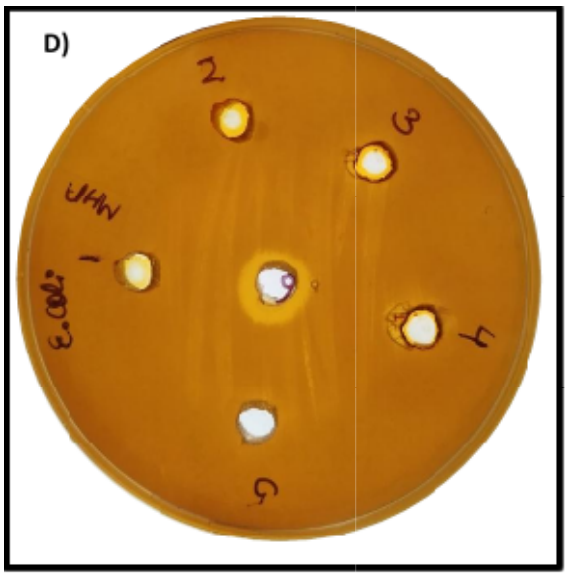


Figure 4.15 Antimicrobial activity of synthesized FeNPs**A)***B. subtilis.* **B)** *P. aeruginosa,* **C)** *S. aureus*and **D)***E. coli.*

Table 4.3 Antibacterial activity of synthesized Iron nanoparticles and Mushroom extract *P. citrinopileatus).*

Sr No.	**Microbial organism**	**Zone of Inhibition(mm)**					
		Antibiotic (mm)	Mushroom extract (P. citrinopileatus)	FeNPs			
				100	75	50	25
1.	Escherichia coli	6	-	-	-	-	-
2.	Pseudomonas aeruginosa	40	-	-	-	-	-
3.	Staphylococcus aureus	44	-	-	-	-	-
4.	Bacillus subtilis	49	-	-	-	-	-

4.4.2 Antifungal Activity

Antifungal activity of synthesized AgNPs was examined against three fungus by 96 well plate method. AgNPs of *Ganodermaspp.*and*P. citrinopileatus* both do not show antifungal activity against lab culture VS9 (*Aspergillusniger*) as shown in 1st and 2nd row for AgNPs of *Ganodermaspp.*and*P. citrinopileatus*. 3rd and 4th row is for lab cultureVS17 (*Aspergillusfumigatus)* which show MIC (Minimum Inhibitory concentration) in 2nd column upto 25µl concentration (Table 4.4). Similarly AgNPs synthesized from *Ganodermaspp.*and*P. citrinopileatus*both show good antifungal activity against lab culture VS23 (*Aspergillusoryzae*) culture shows MIC upto 25µl conc. So we can say *Ganodermaspp.*and*P. citrinopileatus*both show good antifungal activity against VS17 and VS23 lab culture (Figure4.16).

Table 4.4 Different dilutions of Samples for Antifungal activity

Sr No.	1	2	3	4	5	6	7	8	9	10	11	12
Conc.(µL)	50	25	12.5	6.25	3.12	1.56	0.78	0.39	0.19	0.09	-ve	+ve

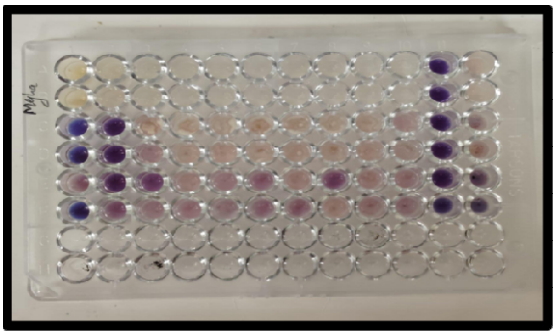


Figure 4.16 Antifungal Activity using microtiterPlate(*Ganoderma spp.* and *P. citrinopileatus*(AgNPS shows good antifungal activity against lab culture VS17 (*Aspergillusfumigatus*) and VS23 (*Aspergillusoryzae*) MIC upto 25µl concentration).

4.4.3 Antioxidant Property

The reducing ability of any component is related to its antioxidant capacity. The antioxidant compounds are based on free radicals reduction. DPPH is stable free radical and is often used to determine radical scavenging activity in the chemical analysis. DPPH radicles have a single electron and it also demonstrates the highest absorption at 517 nm. The colour changed of solution after cooled down indicated the presence of antioxidants in Mushroom extract (Figure4.17).

$$\%\text{DPPH scavenging activity} = \frac{Absorbance\ of\ control - Absorbance\ of\ sample}{Absorbance\ of\ control} \times 100$$

*P. citrinopileatus*AgNPs show maximum % DPPH scavenging activity 50.31%, *Ganoderma spp.* AgNPs shows 46.91% and Iron nanoparticles shows 32.06% of DPPH scavenging activity.

4.	*Ganoderma spp. extract*	0.819	15.57
5.	$FeCl_3$	0.659	32.06
Control (DPPH) = 0.97			

Figure 4.17 Antioxidant activity of AgNPs and *Ganodermaspp.*and *P. citrinopileatus*extract.

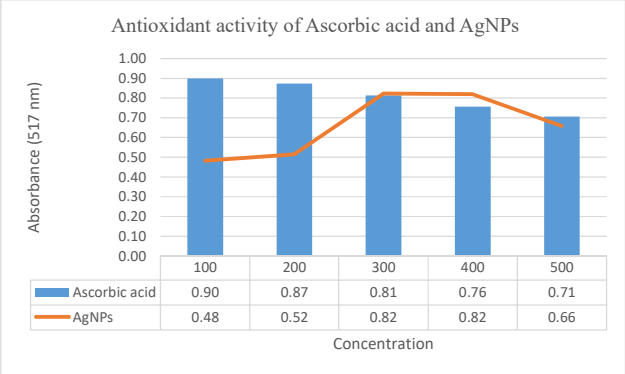


Table 4.5 DPPH radical scavenging activity of Silver and Iron nanoparticle.

Figure 4.18 Antioxidant activity of Ascorbic acid and AgNPs*(P.citrinopileatus*and *Ganoderma spp.)(P. citrinopileatus*AgNPs (50.31%) and *Ganoderma spp.* AgNPs (46.91%) showing good % DPPH scavenging activity than Ascorbicacid).

Sr. No.	Conc. (µl)	Absorbance (517nm)			Average	DPPH Scavenging Activity (%)
1.	100	0.9	0.9	0.9	0.9	7.22
2.	200	0.88	0.87	0.87	0.87	10.31
3.	300	0.81	0.81	0.82	0.81	16.49
4.	400	0.76	0.75	0.76	0.76	15.56
5.	500	0.71	0.7	0.71	0.71	26.80

Table 4.6 Antioxidant activity of ascorbic acidat different concentrations.

5. Discussion

It has been noted that fungi grow more readily on potato dextrose agar (PDA) media. On the basis of maximum synthesis of silver nanoparticle, we select 2 Mushrooms *P.citrinopileatus*and *Ganoderma spp.* For the synthesis of Silver (Ag) and Iron (Fe) nanoparticles, metals such as Silver nitrate ($AgNO_3$) and Ferric chloride ($FeCl_3$) were applied. When Mushroom extract was added in silver nitrate ($AgNO_3$), it changed colour after 12 hours to brown(Ahmad et al., 2010; Kalimuthu et al., 2008; V. Kumar &Yadav, 2009), found similar findings. In these studies isolates show less brown colour, indicating low synthesis while, the Mushroom extract of *P. citrinopileatus* and *Ganodermaspp.*detected dark brown colour, indicating highest production of silver and Iron Nanoparticles. Also determining the quantity, UV visible spectroscopy was used which indicated that *Ganodermaspp.*synthesized less AgNPs than *P.citrinopileatus*.According to (Devi et al., 2012), the highest peak was observed at 390nm and indicated the formation of silver nanoparticles. In contrast, the enzyme activity decreases at low temperature 4°C which slows down synthesis of AgNPs in the reaction.

Effect of different factor such as silver nitrate concentration, Mushroom extract Volume, temperature, pH and incubation time were analysed for maximum biosynthesis of nanoparticles. The results showed that whereas absorbance was measured at a wavelength in the region of 200-800nm, UV spectral readings of Silver and Iron Nanoparticles of Mushroom were taken between 200- 800 nm. $AgNO_3$ concentration 0.25mM-2mM, mushroom extract 1mL-5mL, temperature 4°C-60°C, pH 6-11, incubation time 0-92 hours were checked from which 1mM silver nitrate concentration, 2mL volume of mushroom extract, 60°C temperature, 9 pH, and 48 hour incubation time were optimized for the maximum biosynthesis of Silver nanoparticles. In (Vahabi&Dorcheh, 2014) Similar results were found.

Characterization of AgNPs and FeNPs were examination of UV-visible absorption spectroscopy, Zeta size, Zeta Potential, FT-IR and SEM. The first visible indication of the synthesis of silver and iron nanoparticles was found in the mushroom extract colour, which turned brownish in colour after adding in $AgNO_3$. After 48 hours of incubation, AgNPs from Synthesized *P. citrinopileatus* and *Ganodermaspp.*displayed a maximum absorbance peak at 425 nm, showing that bio-reduction of$AgNO_3$ had occurred with the mushroom extract. In FTIR spectroscopy various bands showed the participation of several amino acid functional groups in synthesis of nanoparticles. The AgNPs and FeNPs Zeta potential and particle size were found to be (82.51, 39.48 and 80.62nm), which indicated that the AgNPs and FeNPs were relatively stable. . The zeta potential analyzer was used to analyse bio- stabilized AgNPs and FeNPs in a colloidal solution.

References

1. Bonaccorsi, A., &Thoma, G. (2005). Scientific and technological regimes in nanotechnology. *Research Policy*, 34(9), 144–165.
2. Patil, N., Deshmukh, R., &Bhamare, S. (2021). Overview on methods of synthesis of nanoparticles. *Materials Today: Proceedings*, 43, 2007–2012.
3. Sajanlal, P. R., Sreeprasad, T. S., Samal, A. K., & Pradeep, T. (2011). Anisotropic nanomaterials: Structure, growth, assembly, and functions. *Nano Reviews*, 2(1), 5883.
4. Hurtado, R. B., Cortez-Valadez, M., & Flores-López, N. S. (2017). Green synthesis of metallic nanostructures and their applications. *Journal of Nanomaterials*, 2017, Article ID 123456.
5. Kumar, V., &Yadav, S. K. (2009). Plant-mediated synthesis of silver and gold nanoparticles and their applications. *Journal of Chemical Technology & Biotechnology*, 84(2), 151–157.
6. MohammadiDargah, M., Hosseini, S. M., &Ahmadi, M. (2024). Biomimetic synthesis of nanoparticles: A sustainable approach. *Green Chemistry Letters and Reviews*, 17(1), 45–60.
7. V., P., Kumar, R., & Singh, S. (2017). Green strategy for synthesis of nanoparticles and their applications. *International Journal of Nanoscience*, 16(4), 1750023.

8. Corrêa, R. C. G., Brugnari, T., Bracht, A., Peralta, R. M., & Ferreira, I. C. F. R. (2016). Biotechnological, nutritional, and therapeutic uses of *Pleurotus* spp. (oyster mushroom). *Food Research International*, 82, 19–30.
9. Gupta, S. (2012). Myconanotechnology: Emerging field of nanobiotechnology. *Journal of Nanotechnology*, 2012, Article ID 736951.
10. Llanaj, X., Lushaj, B., &Duriqi, H. (2023). Biotechnological applications of mushrooms: A review. *Journal of Fungi*, 9(3), 210.
11. Dhayalan, M., Denison, M. I. J., &Ayyaru, G. (2021). Biomedical applications of silver nanoparticles: A review. *Biotechnology Reports*, 31, e00630.
12. Skonieczna, M., et al. (2018). Biological activity of silver nanoparticles. *Journal of Applied Toxicology*, 38(3), 245–256.
13. Olawade, D. B., Ajayi, A. I., &Ojo, A. A. (2024). Nanoparticles for microbial control in water treatment: A review. *Environmental Nanotechnology, Monitoring & Management*, 21, 100812.
14. Dada, A. O., Adekola, F. A., &Odebunmi, E. O. (2018). Influence of operational parameters on nanoparticle synthesis. *Journal of Nanostructure in Chemistry*, 8(3), 255–266.
15. Mélinon, P., Begin-Colin, S., &Duvail, J. L. (2014). Engineered nanoparticles: A review of characterization techniques. *Journal of Physics D: Applied Physics*, 47(19), 193001.
16. Bankier, C., Matharu, R. K., & Cheong, Y. K. (2019). Synergistic antibacterial effects of nanoparticles. *Nanomaterials*, 9(10), 1447.
17. Brandt, O., Mildner, M., & Egger, A. (2012). Antimicrobial activity of silver nanoparticles. *Journal of Materials Chemistry*, 22(12), 12345–12350.
18. Chavez-Esquivel, G., et al. (2021). Antimicrobial activity of biosynthesized silver nanoparticles. *Applied Microbiology and Biotechnology*, 105(2), 567–578.
19. Naraian, R., &Abhishek. (2020). Mushroom-mediated synthesis of silver nanoparticles and their applications. *Biocatalysis and Agricultural Biotechnology*, 25, 101602.