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Research Article

Antibacterial Activities of Biosynthesized Silver-Nanoparticles from Three Species of Mushroom

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ABSTRACT

Nanoparticles are particles of less than 100 nm in diameter that exhibit new or enhanced size-dependent properties compared with bigger particles of similar material. In this study, AgNPs were synthesized using three mushroom species- *Lentinus squarrosulus*, *Ganoderma lucidum*, and *Pleurotus tuberregium*. The formation of AgNPs was observed using a UV-light spectrophotometer. The functional groups present were identified by Fourier transform infrared spectroscopy; while size and surface morphology were also analyzed using scanning electron microscopy. The study revealed that the AgNPs were highly aggregated crystalline spherical nanoparticles with most of them irregular in shape. The cubic structure of AgNPs was identified using the X-ray Diffraction analysis with peaks for *Lentinus squarrosulus* at 2θ = 28.5o and 21.8o, 8o and 44o for *Ganoderma lucidum*, and 39o and 26o for *Pleurotus tuberregium* (corresponding to the planes of silver 111, 200 respectively). Antibacterial analyses from the three mushrooms showed that the biosynthesized AgNPs possess antibacterial activity (against *Staphylococcus aureus*, *Escherichia coli*, Bacillus sp, and Pseudomonas aeruginosa). The antibacterial activities of each mushroom species were compared with three antibiotics- Chloramphenicol, Ciprofloxacin and Streptomycin. The study revealed that the AgNPs of *Pleurotus tuber-regium*exhibited a broader antibacterial activity compared to the *Lentinus squarrosulus* and *Ganoderma lucidum*.

Keywords: *Silver nanoparticles, Lentinus squarrosulus, Ganoderma lucidum, Pleurotus tuber-regium, Antibacterial activities*

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INTRODUCTION

Nanotechnology is the combination of two words-'nano' and 'technology'. The term 'nano' is derived from the Greek word 'Nanos', meaning dwarf. Nanotechnology is also referred to as Nanoscience which deals with the issues of nanometer-scale ranging from approximately of 1 to 100nm, which can be controlled at the atomic or molecular level (Abuayyash *et al*., 2018).

Nanotechnology is described as a quickly developing space of modern science because of its promising applications in different fields like medicine, technology, and agriculture; with respect to size, shape, chemical composition and expected use for human advantages. The most examined nanoparticles are those from the noble metals, which are Silver (Ag), Gold (Au), Platinum (Pt) and Palladium (Pd); among them, silver nanoparticles assume a critical part in biology and medicine (Numan *et al*., 2021).

The biosynthesis of these nanoparticles is the centre of research in nanotechnology. Not many mushrooms, for example, Volvariella volvacea, Pleurotus Sajor, Pleurotus Florida, *Ganoderma lucidum* and Micrporus xanthopus have been accounted for to be utilized in the creation of silver nanoparticles (AgNPs) (Numan et a;., 2021, Jigna *et al*., 2022).

Mushroom cultivation and use have a long history concerning human's health and food sources. They are extensively found in nature and remain the earliest type of fungi found by man. Mushroom is universally defined as the fleshy, spore-bearing fruiting body of a fungus that is distinctively produced over the ground, on soil or deteriorating wood. It has been described as a macro fungus with an individual fruiting body that can be epigeous or hypogeous, visible to the naked eye, and can be easily harvested belonging to either Ascomycetes or basidiomycetes under the Kingdom Fungi. (Samsudin *et al*., 2019). Various species of Mushrooms have been cultivated on industrial wastes, waste woods, agricultural wastes, or wastelands to foster rapid biological cleaning of the environment and create more arable farmlands for the cultivation of other economically important food crops (Victor and Clementina 2021).

Green synthesis of nanoparticles is a primary, conservative environmentally friendly and biological method of synthesizing silver nanoparticles. Green synthesis of silver nanoparticles has been utilized for different applications electronics, catalyst, energy, medicine, nonlinear optics, spectrally specific coating for sun-powered energy retention, bio-labelling, intercalation materials for electrical batteries, as optical receptors, and as antibacterial capacities (Muhammad *et al*., 2020). According to recent studies, nanoparticles such as silver nanoparticles (AgNPs) have been reported to inhibit the growth of microbes. (Loo *et al*., 2018, Mare *et al*., 2021)
The study aimed to synthesize, characterize silver nanoparticles from three species of mushroom, and determine their antibacterial activities.

MATERIALS AND METHODS

Materials: Preparation of Crude Extract of - *Lentinus squarrosulus*, *Ganoderma lucidum* and Pleurotus tuber-regium. The fruiting bodies of Fresh mushrooms (*Ganoderma spp*, *Pleurotus tuber-regium*and *Lentinus squarrosulus*) were harvested, washed and dried under a mild sun and after which were grinded using a blender separately and kept differently.
10g of each mushroom species was weighed into three beakers (500ml), and 100ml of distilled water was added to each beaker, heated at 60oC to enhance dissolution. After about 10minutes, each solution mixture was sieved using Whatman Filter Paper No 1 and the filtrate of each mushroom was collected in a different conical flask (Anyakorah *et al*., 2021).

Synthesis of Silver-nanoparticles of Fungi Species: 200ml of 0.01M of $AgNO_3$ prepared in the laboratory and stored in a volumetric flask. 50ml of $AgNO_3$ is then added to 50ml of each mushroom filtrate in a conical flask and also heated slightly for effective reaction; thus reducing Ag^+ to Ag^0**.** The resulting solution is then allowed to stay overnight in a dark cabinet and then stored for a further experiment under the temperature of -4.00C (Mbagwu *et al*., 2022).

Characterization of the Synthesized Silver Nanoparticles: This involves using instrumentation techniques to observe the structure, size, reaction between each mushroom filtrate and $AgNO_3$ and the nature of the synthesized nanoparticles. The instruments used were UV-Visible spectroscopy, Scanning Electron Microscope (SEM), X-ray Diffraction and Bomen Fourier Transform Infrared (FTIR) (Prabhu *et al*.,2019).
2.4 UV -Visible Spectroscopy Analysis

A resolution of 2.0 nm between 300 to 700 nm possessing a scanning speed of 300mm/min was used to carry out UV-visible spectroscopy analysis using a UV-Visible absorption spectrophotometer. The monitoring of the process of reaction between metal ions and each mushroom extract were done using UV–Visible spectra of silver nanoparticles in an aqueous solution.

Scanning Electron Microscopy (SEM): The size of silver nanoparticles was measured using Scanning electron microscope analysis. This was carried out by transporting the synthesized silver-nanoparticles to the University of Zaria, Department of Chemistry. The mushroom extract used to synthesize silver nanoparticles was allowed to dry entirely and grounded to powder for this technique. Complete dryness is a standard requirement for the SEM specimen since the specimen is at a high vacuum.

X-ray Diffraction Analysis (XRD): Silver nanoparticles solution obtained after bioreduction was purified by repeated centrifugation at 5000 rpm for 20min followed by redispersion of silver nanoparticles pellet into 10ml of sterile deionized water. After the freeze-drying of the purified silver particles, the structure and composition were analyzed by X-ray diffraction (XRD). X-ray diffractometer analysis was used to determine silver-nanoparticles after the dried mixture was collected. This was done to verify the results of the UV spectral studies; XRD examined the colloidal suspensions of AgNPs to observe their nature.

Fourier Transmission Infrared Spectroscopy (FTIR) Analysis: FTIR breakdown is performed to decide the functional groups present in a sample which reduces silver nitrate to metallic silver nanoparticles. For FTIR examination, pellets of crude mushroom samples containing nanoparticles were used.

Antibacterial Activities of Synthesized Silver-nanoparticles

Collection of microorganisms: The microbes selected for the present study were *Escherichia coli*, Pseudomonas aeruginosa, *Staphylococcus aureus* and *Bacillus spp.*

Preparation of innocula: Each organism was recovered for testing by sub-culturing on fresh Nutrient media. A loopful inoculum of each bacterium was suspended in 5 ml of nutrient broth and incubated overnight at 37°C. These overnight cultures were used as inoculums in all our experiments.

Preparation of media: The growth media employed in the present study included nutrient agar and nutrient broth. The medium was adjusted to pH 7 and sterilized by autoclaving at 15 lbs pressure (121°C) for 15 minutes for sterilization.

Sub-culturing of microorganisms: Frequent sub-culturing maintained the pure cultures of microorganisms on nutrient agar slant. These cultures were stored at 4˚C for further experiments.

Antibacterial activity: Bactericidal effects of Ag-NPs were studied against Gram-positive and Gram-negative bacteria. Antibacterial activity was demonstrated by using the Well-diffusion method. Eight plates were prepared, four allocated for the three mushroom species and the other four for the three antibiotics (Chloramphenicol, Streptomycin, and) to confirm the inhibition zones.

RESULTS

Biosynthesis of Silver Nanoparticles Using Mushrooms:: The colour change from light pale yellow to brown after adding AgNO3 to culture filtrate is the primary indication of silver nanoparticles synthesis.

In all the mushroom samples used, there were changes from light yellow to brown, indicating the reduction of aqueous Ag+ with culture filtrates by showing the production of silver nanoparticles (Plate 1, 2 &3).

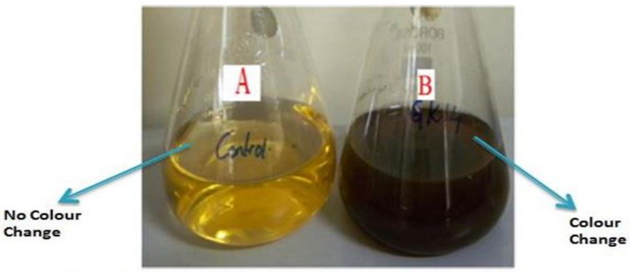

Plate 1:
Photograph Showing the Synthesized Silver Nanoparticles from *Lentinus squarrosulus*

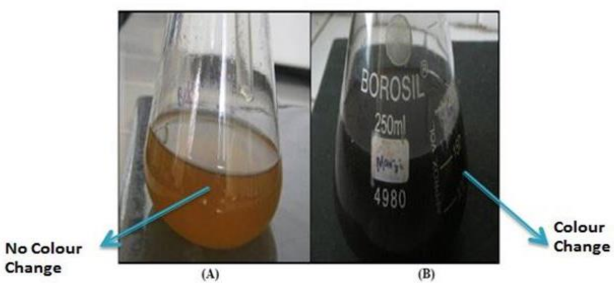

Plate 2:
Photograph Showing the Synthesized Silver Nanoparticles from *Ganoderma lucidum*

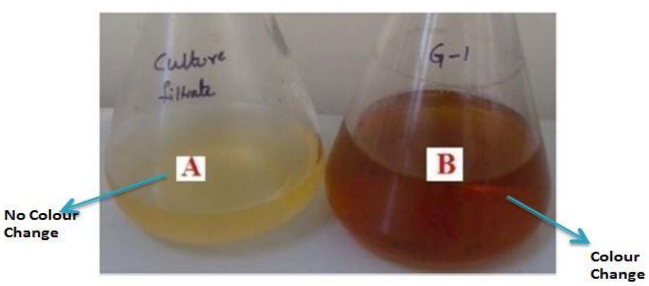

Plate 3:
Photograph Showing the Synthesized Silver Nanoparticles from Pleurotus tuber-regium
A-Filtrate of the Mushroom B Synthesized AgNPs

U.V Analysis of Silver nanoparticles Using Lentinus: squarrosulus, *Ganoderma lucidum* and *Pleurotus tuberregium* After 24hrs addition of mushroom extracts- *Lentinus squarrosulus*, *Ganoderma lucidum* and *Pleurotus tuberregium*; to Silver nitrate solution, UV-VIS scans were taken from 300 to 700nm. The sharp bands of silver nanoparticles from each mushroom extract were observed around 372nm, 376nm and 380nm, respectively (Fig. 2). The recorded bands suggest that the particles were well dispersed without aggregation (Jian-E *et al*., 2021).

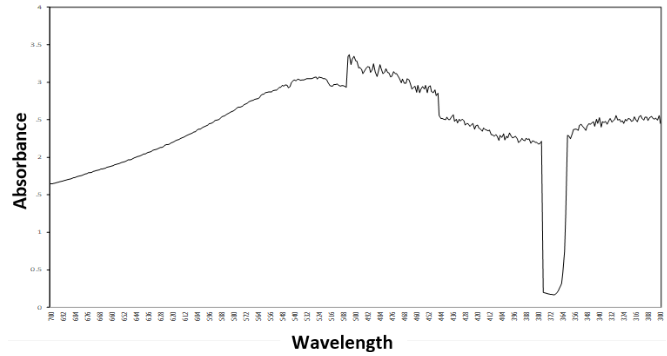

Figure 1:
UV-VIS spectra of silver nanoparticles using *Lentinus squarrosulus*

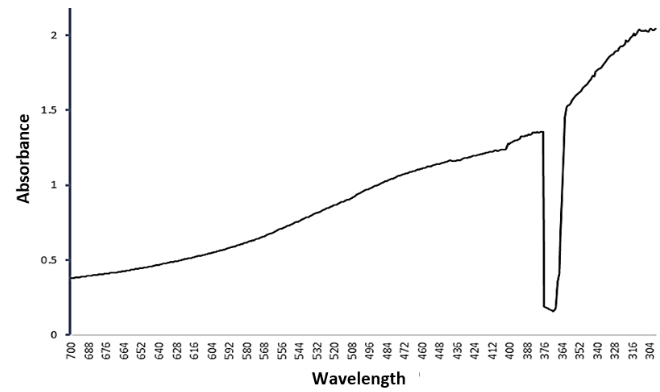

Figure 2:
Graph Showing the UV-VIS spectra of silver nanoparticles using *Ganoderma lucidum*

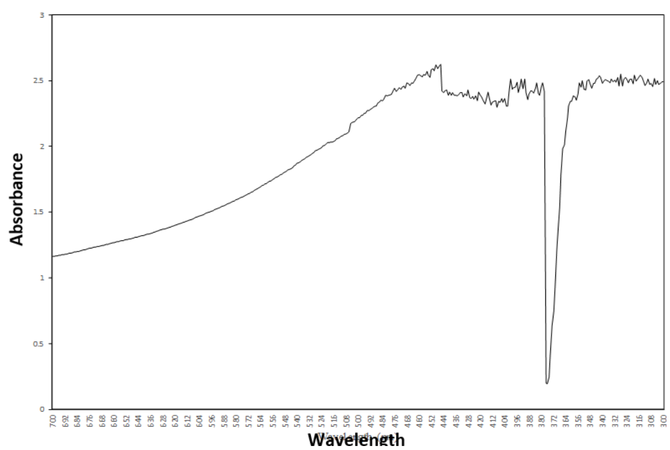

Figure 3:
Graph Showing the UV-VIS spectra of silver nanoparticles using *Pleurotus tuberregium*

Fourier Transmission Infrared (FTIR) Analysis of Silver nanoparticles

Fourier Transmission Infrared (FTIR) Analysis of *Lentinus squarrosulus*: The FTIR spectrum of aqueous extract of *Lentinus squarrosulus* showed five prominent peaks, which are 3854.76cm-1, 3443.57cm-1, 2075.87cm-1, and 1633.70cm-1, as shown in figure 4.

Fourier Transmission Infrared (FTIR) Analysis of *Ganoderma lucidum*: The FTIR spectrum of aqueous extract of *Ganoderma lucidum* showed five prominent peaks, which are 3854.67cm-1, 3443.57cm-1, 2079.34cm-1, and 708.90cm-1 as shown in Fig 5.

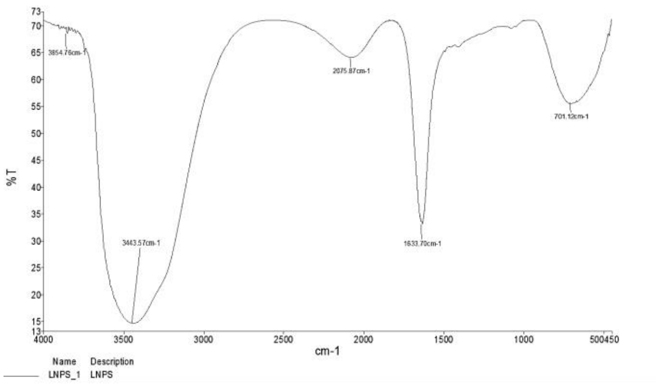

Figure 4:
FTIR Photograph of AgNPs synthesized from *Lentinus squarrosulus*

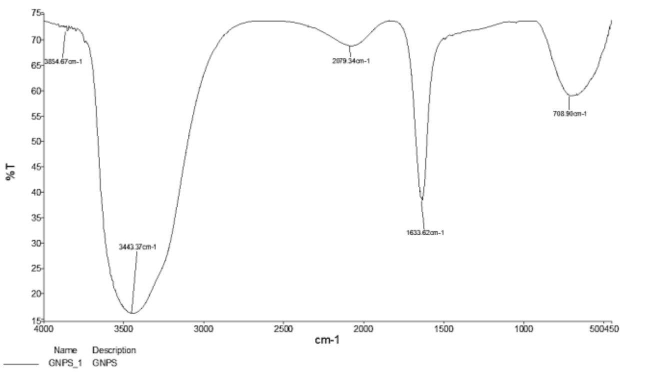

Figure 5:
FTIR Photograph of AgNPs synthesized from *Ganoderma lucidum*

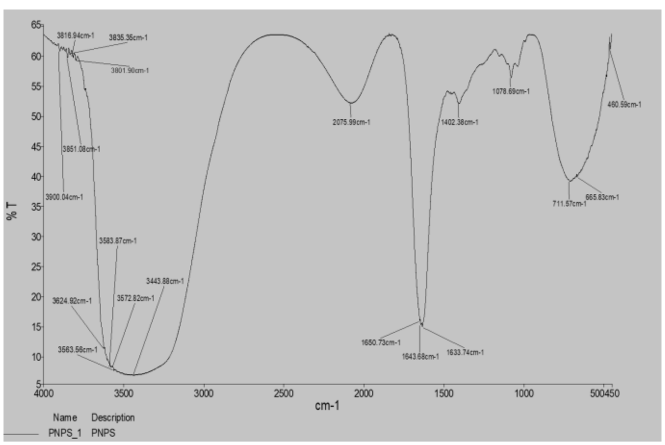

Figure 6:
FTIR Photograph of AgNPs synthesized from Pleurotus tuberregium

Fourier Transmission Infrared (FTIR) Analysis of *Pleurotus tuberregium*: The FTIR spectrum of aqueous extract of *Pleurotus tuberregium* showed sixteen prominent peaks 3816.94cm-1, 3835.35cm-1, 3801.90cm-1, 3851.08cm-1, 3900.04cm-1, 3624.92cm-1, 3563.58cm-1, 3583.87cm-1, 3572.82cm-1, 3443.88cm-1, 2075.99cm-1, 1650.73cm-1, 1643.68cm-1, 1633.74cm-1, 1402.36cm-1, 1078.69cm-1, as shown in Figure 6.

SEM ANALYSIS: The mcyo-synthesized silver Nanoparticles from *Lentinus squarrosulus*, *Ganoderma lucidum* and *Pleurotus tuberregium*; were subjected to the SEM analysis for the SEM photos of Silver nanoparticles were observed.

The Nanostructure and surface morphological studies of SEM images of silver nanoparticles using three species of mushroom were observed show highly aggregated crystalline spherical nanoparticles with most of them irregular in shape, as shown (Plates 4 – 6)..

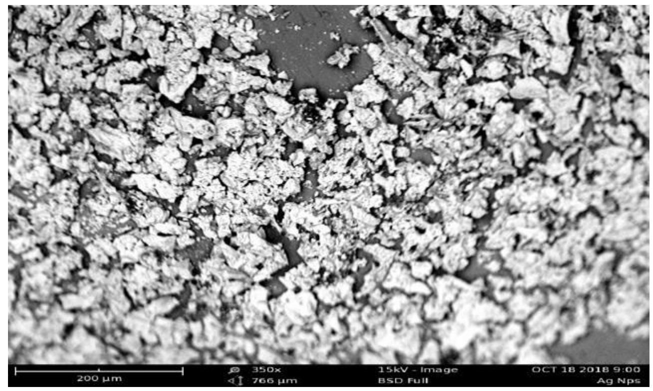

Plate 4:
Scanning electron microscopy image of silver nanoparticles from *Lentinus squarrosulus*

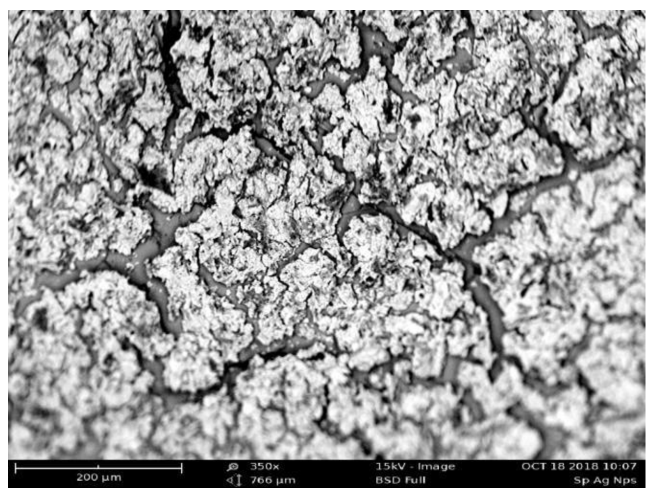

Plate 5:
Scanning electron microscopy image silver nanoparticles from *Ganoderma lucidum*

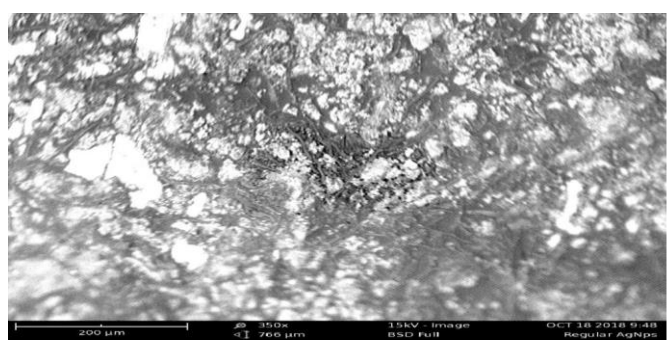

Plate 6:
Scanning electron microscopy image of silver nanoparticles from *Pleurotus tuberregium*

X-ray diffraction analysis (XRD) of synthesized silver-nanoparticles using *Lentinus squarrosulus*: The Braggs reflections were observed in the XRD pattern at 2θ were 39.77◦ and 43.71◦ in the whole spectrum of values ranging from 10 to 90 and indicated that the structure of AgNPs is face-centred cubic (fcc). These correspond to [111] and [200] planes for silver.

The estimated size of the synthesized silver nanoparticles was calculated using the Debye Scherrer Equation.

Table 1:
Size Estimate of synthesized silver nanoparticles using *Lentinus squarrosulus*

s/n	hkl	2Theta	Theta	FWHM (º)	β(radian)	Size (nm)
1.	111	39.77	19.89	**0.5515**	0.009625	28.48
2.	200	43.71	21.86	0.3676	0.006416	21.80

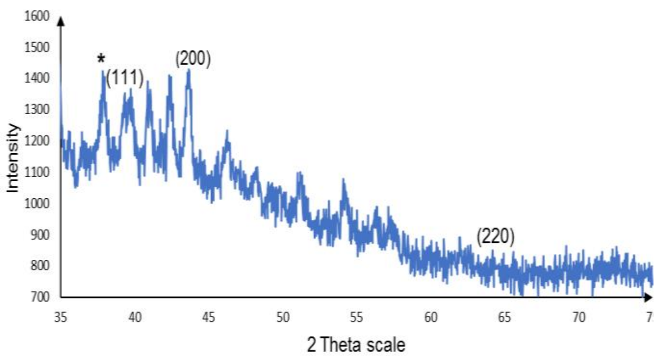

Figure 7:
XRD pattern of silver nanoparticles formed after reaction of *Lentinus squarrosulus*

X-ray diffraction analysis (XRD) of synthesized silver-nanoparticles using *Ganoderma lucidum*: The Braggs reflections were observed in the XRD pattern at 2θ were 38.01◦ and 46.31◦ in the whole spectrum of values ranging from 10 to 90 and indicated that the structure of AgNPs is face-centred cubic (fcc). These correspond to [111] and [200] planes for silver. The estimated size of the synthesized silver nanoparticles was calculated using Debye Scherrer Equation, as shown in Table 3.

Table 2:
Size Estimate of synthesized silver nanoparticles using *Ganoderma lucidum*

s/n	hkl	2Theta	Theta	FWHM (º)	β(radian)	Size (nm)
1.	111	38.01	19.00	0.6040	0.01745	8.04
2.	200	46.31	23.17	0.4727	0.008250	44.0

X-ray diffraction analysis (XRD) of synthesized silver-nanoparticles using *Pleurotus tuberregium*:
The Braggs reflections were observed in the XRD pattern at 2θ were 37.15◦ , 45.15◦ and 63.90◦ in the whole spectrum of value ranging from 10 to 90 and indicated that the structure of AgNPs is face-centred cubic (fcc).

These correspond to [111], [200] and [220] planes for silver. The estimated size of the synthesized silver nanoparticles was calculated using Debye Scherrer Equation, as shown in Table 3.

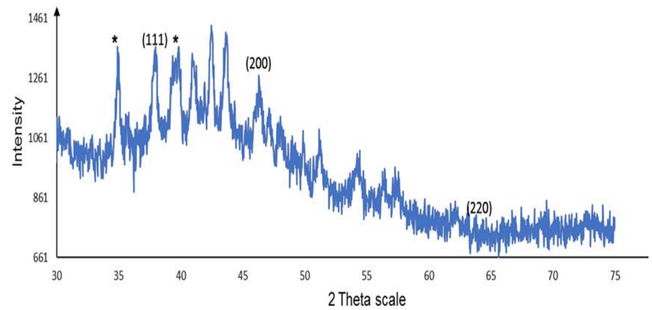

Figure 8:
XRD pattern of silver nanoparticles formed after reaction of *Ganoderma lucidum*

Table 3:
Size Estimate of synthesized silver nanoparticles using *Pleurotus tuberregium*

s/n	Hkl	2Theta	Theta	FWHM (º)	β(radian)	Size (nm)
1.	111	37.15	18.58	0.2364	0.004130	34.83
2.	200	45.15	22.58	0.3677	0.006418	24.48
3.	220	63.90	31.95	0.1314	0.002293	70.24

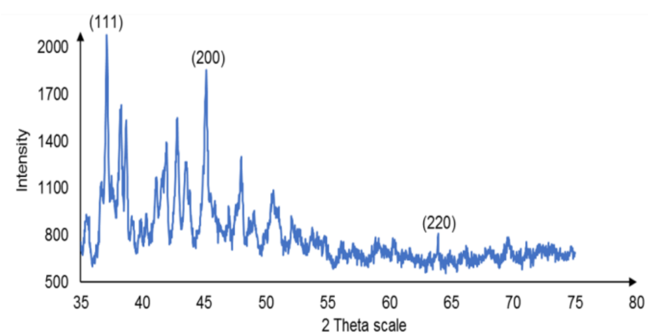

Figure 9:
XRD pattern of silver nanoparticles formed after reaction of *Pleurotus tuber-regium.*

Antibacterial activities of the silver nanoparticles using Agar well-diffusion Method*:* Synthesized silver nanoparticles have shown superior antibacterial activities against all the tested clinically isolated pathogens. The effect of Ag-NPs alone and associated with the antibiotics, Chloramphenicol(C), Ciprofloxacin (CIP) and Streptomycin (S), were investigated against pathogenic bacteria as shown in Table 4.

The XRD pattern of the synthesized silver nanoparticles from Lentinus squarrosulus, showed diffraction patterns at 2θvalues 39.77o and 43.71o, which corresponded with the 111and 200 reflections of metallic silver as seen in Figure 7.

Table 4:
Antibacterial Activities of Fungi against Synthesized Nanoparticles and Antibiotics

Test Microorganisms	L. squarrosulus (mm)	*G. lucidum* (mm)	*P. tuber-regium* (mm)	C (mm)	CIP (mm)	CP (mm)
Bacillus sp	16.50 ± 1.00	16.50 ± 0.50	22.00± 2.00	24.50± 0.50	26.00 ± 1.00	26.00± 0.00
Escherichia coli	10.00 ± 0.00	19.00 ± 1.00	15.00 ± 0.00	34.00± 0.00	34.00± 0.00	20.00± 0.00
Staphylococcus aureus	*16.50 ± 0.50*	*15.50± 0.50*	*17.50 ± 0.50*	*35.00 ± 0.00*	*30.00 ± 0.00*	*22.00± 0.00*
Pseudomonas aeruginosa	17.00 ± 0.00	20.00 ± 0.00	25.00± 0.00	NIL	30.00 ± 2.00	10.00± 0.00

In contrast, the synthesized silver nanoparticles from *Ganoderma lucidum* showed diffraction patterns at 2θ values 38.01o and 46.31°, which also meant 111 and 200 reflections as demonstrated in Figure 8 while the synthesized silver nanoparticles from *Pleurotus tuberregium* showed diffraction patterns at 2θ values 37.51o , 45.15o and 63.90o, these indicated 111, 200 and 220 reflections of metallic silver as described in Figure 9.

DISCUSSION

Three selected Mushrooms, *Lentinus squarrosulus, Ganoderma lucidum* and *Pleurotus tuberregium* were used to synthesize silver nanoparticles using a bio-synthetic method. The extracts of the selected species of mushroom were treated with silver nitrate solution, the mixtures were then isolated separately at room temperature. A visible colour change was observed in each mixture; from pale yellow to reddish-brown colour. This confirmed the complete synthesis of silverD nanoparticles. The colour change in each mixture of mushroom extracts demonstrated the Surface Plasmon Resonance, which is a result of the combined vibration of electrons of silver nanoparticles in resonance with lightwave. It is shown from a study by Habeeb (2022) that the use of medicinal plant extracts for the synthesis of silver nanoparticles enhances the reduction and stabilization of silver nitrates in an aqueous medium for the formation of silver nanoparticles SNPs) which resulted in colour changes as a result of the vibrations produced by surface plasmon resonance caused by the reduction of silver ions to silver nanoparticles. In addition, these outcomes were comparable with works of Dinesh (2022) and Adebayo (2021).

The silver nanoparticles fromed from the three mushroom extracts were subjected to a UV; various absorption peaks were recorded at 480nm, 390 nm and 430nm, in *Lentinus squarrosulus, Ganoderma lucidum* and *Pleurotus tuberregium*, respectively. It was denoted that the absorption spectra formation from the SNPs was due to the formation of Surface Plasmon Resonance (SPR) in the SNPs. Also, the recorded absorption spectra confirmed the presence of the synthesized mushroom silver nanoparticles; this demonstration agreed with reports by Pascale *et al*., (2023) and Fatemeh *et al*., (2020).

The presence of active biomolecules of free supernatant was further observed in the mushroom extracts which were recorded using the FT-IR. It was recorded that *Lentinus squarrosulus* and *Ganoderma lucidum*; have five prominent peaks while *Pleurotus tuberregium* has sixteen (16) prominent bands. Most of the bands represented similar compounds; bands between 3200-3900cm-1 represents the strong stretching vibrations of the hydroxyl (OH) functional group, which arose due to carbonyl stretch; while the bands between 2000-2250cm-1 represented C=C Alkyne (stretch), 1500-1700cm-1for C=C amide (stretch), and 700-1000cm-1 for C-O Alcohols, Ethers, Esters, and Carboxylic acid (stretch). The infra-red spectra confirmed the presence of the different functional groups after synthesis which included: NH, H-O, C-N, C-H, C-C, C-O, and the amide linkages and nitro compounds that may be present between the silver nanoparticles stabilizing caps along with the proteins and amino acid residues. The results obtained from the present study were comparatively similar to reports of Suba *et al*., (2022).

Therefore, it was later demonstrated that the synthesized nanoparticles were surrounded by proteins and metabolites such as terpenoids, and bioflavonoids having functional groups of alcohols, ketones, aldehydes, and carboxylic acids. The XRD pattern of the synthesized silver nanoparticles from *Lentinus squarrosulus*, showed diffraction patterns at 2θ values 39.77o and 43.71o, which corresponded with the 111 and 200 reflections of metallic silver. In contrast, the synthesized silver nanoparticles from *Ganoderma lucidum* showed diffraction patterns at 2θ values 38.01o and 46.31o, which also meant 111 and 200 reflections while the synthesized silver nanoparticles from *Pleurotus tuberregium* showed diffraction patterns at 2θ values 37.51o , 45.15o and 63.90o, these indicated 111, 200 and 220 reflections of metallic silver and the data were found to match with the database of the Joint Committee on Powder Diffraction Standards (JCPDS File No.04-0783). Also, the peak positions indicated the formation of silver nanoparticles, similar diffraction patterns were also reported by Okocha *et al*., (2023). The corresponding sizes of the synthesized silver nanoparticles were determined using the Debye Scherrer Equation, the AgNPs from *Lentinus squarrosulus* have an estimated size range of 22-29nm, while that of *Ganoderma lucidum* have an estimated size range of 8-44nm and *Pleurotus tuberregium* have estimated size range of 24-70nm. The shape and the distribution of the synthesized silver nanoparticles from the three selected mushrooms were analyzed using the SEM. The SEM results showed that the synthesized AgNPs were crystalline in nature, had the ability to slide over each other. It also revealed the spherical and irregular shapes of some of the synthesized nanoparticles. This result is similar to a report by Abbas *et al*.,(2017).

One of the most remarkable and peculiar properties of silver nanoparticles is their antibacterial activities compared to AgNO3. The silver nanoparticles biosynthesized from extracts of *Lentinus squarrosulus* and *Ganoderma lucidum*, and *Pleurotus tuberregium* revealed a very potent and effective activity of the nanoparticles against all the test organisms in this study. The three synthesized silver nanoparticles were then demonstrated for various inhibitory activities against four test microorganisms, which were Bacillus sp, *Escherichia coli*, Streptococcus aureus, and Pseudomonas arginosa compared with three other selected antibiotics-Chloramphenicol, Ciprofloxacin and Streptomycin. The antimicrobial activities varied among the three selected mushrooms. P.tuberrogium exhibited the highest antimicrobial activity against three of the test organisms and was relatively similar to the inhibitory activity of ciprofloxacin. Although, G. lucidum was observed to have the highest activity against E.coli compared to the other mushroom SNPs. One of the most remarkable and peculiar properties of silver nanoparticles is their antibacterial activities.

In conclusion, the green synthesis of silver nanoparticles has attracted the attention of researchers because it is eco-friendly, safer, and cheaper than the chemical method. Nanoparticles possess unique properties and are applicable in areas such as medicine, biotechnology, catalysis, electronics, optics, and wastewater treatment. It is evident th.at incorporating silver molecules into extracts of mushrooms improved the metabolites' performance. Therefore, synthesized silver nanoparticles from the three mushrooms demonstrated a high potential for antibacterial activities.

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