Original Research Article

Green synthesis of silver nanoparticles using *Pleurotus ostreatus*

ABSTRACT

Aims: The aim of the study was to synthesize silver nanoparticles from aqueous $AgNO_3$ using phytochemicals present in *Pleurotus ostreatus* and assess the antibacterial activity on *Bacillus subtilis* and *Providencia rettgeri.*

Study design: Experimental study design.

Place and Duration of Study: Bells University of Technology between December 2020 and August 2021.

Methodology: The mushroom was washed, dried, pulverized and 5g stirred into 100ml deionized water. The solution was sonicated using ultrasonic cleaner at 40 ^oC for 40 min, centrifuged at 4000 rpm for 10 min. The supernatant was filtered, and 1ml filtrate mixed with 9 ml of 10mM $AgNO_3$. After the reaction period the mixture was centrifuged at 15,000 rpm for 15 min. The residues were washed thrice with deionized water and dried.

Synthesis of AgNPs was monitored by UV–Vis spectrophotometer, characterized by Fourier transform infrared spectroscopy, scanning electron microscopy and X-ray diffraction analysis. Antibacterial analysis was by agar well diffusion using gentamicin as control.

Results: A dark brown colour change and UV visible spectroscopy peak at 400 nm confirmed the formation of AgNPs. Fourier transform infrared spectroscopy analysis showed the presence of functional groups involved in reduction of $AgNO_3$. GCMS performed on the methanolic extract of *Pleurotus ostreatus* showed the presence of 37 organic compounds, among them were cathecol, hydroquinone and phenols etc. Antimicrobial activity revealed the AgNPs inhibited the growth of *B. subtilis* and *P. rettgeri*.

Conclusion: The study revealed that *Pleurotus ostreatus* effectively synthesized AgNPs and the AgNPs inhibited the growth of *Providencia. rettgeri* and *Bacillus subtilis* and can play major roles in the field of medical and pharmaceutical nanotechnology.

Keywords: Antimicrobial activity, Bacillus subtilis, Pleurotus ostreatus. Providencia rettgeri, Silver nanoparticles

1. INTRODUCTION

Nanotechnology is a promising field of research that is generating nanomaterials with unique properties different from bulk materials. Nanoparticles are particles of very small sizes in the range of 1-100 nm and have found vast applications in advanced biomedical and industrial ventures. Recently, silver nanoparticles (AgNPs) have gained much attention because of their characteristic physical and chemical properties, high electrical conductivity and antibacterial potential [1-3]. Hence, AgNPs have been used extensively as antibacterial

agents in the health industry [4], food storage [5], textile coating [6], and a number of environmental [1,3,7] and biomedical applications, including utility as antiangiogenic [8] and anticancer agents [9]. Nanoparticles have been produced using chemical (vapour deposition, irradiation and chemical reduction of metal salts) and physical methods [10] but most of these processes give rise to harmful byproducts and pollutants [11].

Biological or green synthesis of nanoparticles has gained a lot of attention as it offers an environmentally, ecofriendly alternative to chemical and physical strategies. Microbial assisted biosynthesis is a rapidly growing area of nanotechnology. Biomolecules present in microorganisms act as reducing and capping agents and form stable nanoparticles. Mushrooms are known to possess phytochemicals with anti-inflammatory, antioxidant, antibacterial, antiviral and anti-tumour activities which could serve as reductants for nanoparticles synthesis. Apart from being environmentally friendly, the nanoparticles synthesized using mushrooms are reported to exhibit higher stability, longer shelf life and enhanced biological activities [12]. A variety of oyster mushrooms have been tried for synthesis of metal (Au, Ag, Fe, Pt etc.) and non-metal (Se, CdS etc.) nanoparticles [4,5]. *Pleurotus ostreatus,* an edible oyster mushroom, commercially cultivated in Nigeria is known for its high content of nutrients and vast array of chemical compounds with antioxidant and antibacterial potentials which can act as reductant for synthesis of silver nanoparticle. An oyster synthesized AgNP was reported to be inhibitory against pathogenic bacteria with MIC value in the range of 13-27 µg/ml [13]. In another study, AgNPs synthesized using *P. platypus* was shown to have a wider antibacterial activity than those synthesized with *Agaricus bisporus, Calocybe indica* and *Pleurotus florida* [10].

Antibiotics and other chemotherapeutics have revolutionized human health by offering easy cure for diseases, but widespread antibiotic usage and abuse have led to the emergence of

multiple drug-resistant infectious organisms which is posing a threat to global public health. The advent of drug-resistant bacteria that are resistant to widely used antibiotics necessitated the development of new drugs and/or materials to fight pathogenic bacteria. As a result, the hunt for new antibacterial agents has intensified [14]. The use of metallic nanoparticles could be the most promising method to mitigate the effect of multidrug resistance since such drugs have enhanced antibacterial properties [15]. Therefore, the aim of this research is to synthesize silver nanoparticle using *Pleurotus ostreatus* mushroom and testing it against two organisms, *Providencia rettgeri* and *Bacillus subtilis*.

2. MATERIAL AND METHODS

2.1 Materials

The mushroom, *Pleurotus ostreatus* used for the study was procured from Federal Institute of Industrial Research Oshodi, Lagos, Nigeria, while the bacterial strains, *Bacillus subtilis* and *Providencia rettgeri* were collected from the Microbiology laboratory of Bells University of Technology, Ota, Ogun State, Nigeria and identified conventionally and with Analytical Profile Index kits (API, bioMerieux, Inc) 50CHB and API 20E V4.0 respectively. Silver nitrate ($AgNO_3$) was purchased from Sigma Aldrich.

2.2 Preparation of *Pleurotus ostreatus* aqueous extract

The preparation was according to the method of Mohanta *et al*. [17]. The mushroom was initially washed with tap water and rinsed thoroughly with deionized water to remove particulate matter. They were dried in the oven at 45^{o}C for 72 h and pulverized into fine powder to obtain a large surface area for absorption. The dried mushroom powder (5g) was

stirred in 100 ml deionized water in a 500 mL capacity conical flask. The mixture was sealed and sonicated in an ultrasonic cleaner (CLEAN-120HD) at 40 ^{o}C for 40 min, then centrifuged at 4000 rpm for 10 min. The supernatant was filtered to obtain the aqueous extract and stored in a refrigerator between 5 - 10^{o}C for further use.

2.3 Synthesis of AgNPs

The aqueous mushroom extract (1 ml) was mixed with 9ml of 10mM $AgNO_3$ and kept in the dark at room temperature overnight. After the reaction period, the mixture was centrifuged at 15,000 rpm for 15 min to separate the AgNPs. The supernatant was discarded, residues washed with deionized water to remove residual organic compounds. This was repeated thrice to increase the purity of the particles. The wet particles were dried in an oven at 60^{o}C for 2 h. Finally, the particles were stirred in absolute ethanol to reduce aggregation and then dried at 60 °C for 30 min in an oven to expel the solvent [18].

2.4 Characterization of synthesized AgNPs

2.4.1 Phytochemical analysis of *Pleurotus ostreatus*

The mushroom phytochemicals responsible for the reduction and stabilization of AgNPs were identified using a gas chromatography-mass spectrometer (GC-MS) (Shimadzu QP2010SE) analyzer. A 1:1 ratio of methanolic solution and the mushroom extract was injected in to the GC-MS machine and the phytochemicals identified based on their fragmentation patterns via a database incorporated into the GC-MS machine.

2.4.2 UV-Visible

The synthesis of AgNP was monitored by UV-Visible absorption spectrophotometer (Uniscope SM 7504) within the wavelength ranges of 300 nm - 800 nm operated at a

resolution of 1 nm at room temperature (25^{o}C) to monitor the formation of AgNPs based on surface plasmon resonance (SPR) phenomenon.

2.4.3 Scanning electron microscopy (SEM)

A morphological evaluation to determine the microstructure, particle distribution and elemental composition of the AgNPs was performed in a scanning electron microscope (SEM) having an energy dispersive X-ray analyzer (EDX) unit (SEM: JEOL JSM 7660F). The sample was analyzed using an accelerating voltage of 15 kV.

2.4.4 Fourier transform infrared (FTIR) spectroscopy

The type of bonds present in the mushroom aqueous extract and AgNPs were assessed using Fourier transform infrared (FTIR) spectroscopy (FTIR: Nicolet iS10) in the wavenumber range of 650 – 4000 $\cdot$ cm^{-1} to confirm the formation of AgNPs and detect bonds from the biomolecules that were involved in the capping reaction.

2.5 Antibacterial studies

The preliminary antimicrobial activity of the biosynthesized AgNPs was assessed by agar well diffusion method [19]. The turbidity of test organisms *Bacillus subtilis* and *Providencia rettgeri* was adjusted to 0.5 MacFarland standard and the organisms inoculated onto Mueller Hinton agar using a sterile cotton swab. Wells of 6 mm diameter were made with a sterile cork borer. A solution of the AgNPs was made by sonicating AgNPs (10mg/ml) in deionized water at 40^{o}C for 5 h. Then 100 µl of the AgNPs and gentamicin (10 µg/ml) respectively were dispensed in the wells. Incubation of the plates was at 37^{o}C for 24 h. Formation of clear zone of inhibition around the well is an indication of antimicrobial sensitivity. The zone of inhibition was measured in mm.

3. RESULTS AND DISCUSSION

3.1 GC-MS analysis of *Pleurotus ostreatus* extract

Mushrooms are recognized as important food sources and are well known for production of biologically active compounds with anti-inflammatory, anti-oxidant, antibacterial and anti-tumour potentials. The result of GC-MS analysis of methanolic extract of *Pleurotus ostreatus* presented in (Figure 1), revealed the presence of 37 organic compounds, among them were acids, phenols, hydroquinone, sugars, glucopyranoside, flavour and fragrances agents and unsaturated fatty acids. The most abundant compound (as shown in Table 1) was catechol, an organic phenol with percentage area of 23.59% at peak 6, followed by 1-cyclohexane-1-carboxyldehyde, 2,6,6-tri with percentage area of 8.54% at peak 9, and propanal 2,3-dihydroxy- (S)- with percentage area of 7.27% at peak 4. Phenolic compounds are used as antiseptics or disinfectants as they denature and coagulate proteins and at low concentration are active against a wide range of microorganisms [20]. Ramos *et al* [21] in a study reported the presence of 30 different acids, alcohols, aldehydes, heterocyclic-compounds and certain esters, ketones and aldehydes in methanolic extract of *Pleurotus ostreatus* and most abundant was glycerin with 23.36% area. In a similar study, the hydroethanolic extract of *P ostreatus* revealed the presence of acids, methyl and ethyl esters [22].

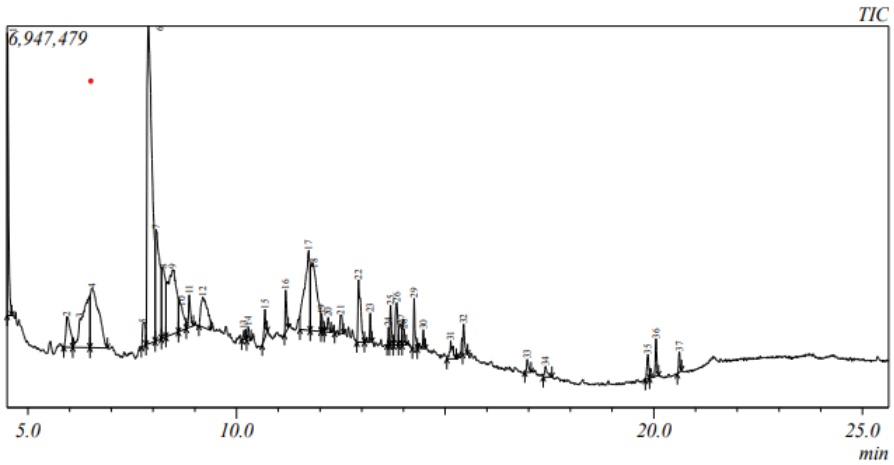


Fig. 1. Gas chromatography-mass spectrophotometer analysis of methanolic extract of *Pleurotus ostreatus*

Table 1. Phytochemicals present in methanolic extract of *Pleurotus ostreatus* identified by Gas chromatography-mass spectrophotometer.

Peak	Retention time (min)	Peak area (%)	Name of compound
1	4.511	5.26	Acetic acid
2	5.937	1.97	Benzeneacetic acid, butyl ester
3	6.250	6.17	3-Allyloxy-1,2 propanediol
4	6.539	7.27	Propanal, 2,3-dihydroxy-(S)-
5	7.758	1.08	1,4,3,6-Dianhydro-alpha-d-glucopyranose
6	7.888	23.59	Catecol
7	8.077	7.14	1-Silacyclo-3-pentene

8	8.250	3.75	Hydroquinone
9	8.472	8.54	1-Cyclohexene-1-carboxaldehyde-2,6,6-trimethyl
10	8.692	1.96	Isosorbide
11	8.868	0.99	Pyrazine-25.-dimethyl-1-propyl
12	9.192	2.93	Hydroquinone
13	10.182	0.18	1-Cyclohexene-1-ethanol, 2,6,6-trimethyl
14	10.285	0.28	Cyclohexane-1,2-dimethyl-3,5-bis(1-methyl
15	10.682	0.66	Phenol-2.6-bis(1,1-dimethylethyl)-
16	11.179	0.91	3-Methyl-4-phenyl-1H-pyrrole
17	11.731	6.40	Acetate, 2-cylohexacehyl-3-[1-(2-oxopropyl
18	11.858	6.43	alpha-D-Galactopyranoside, methyl
19	12.042	0.44	9,10-secochelesta-5,7,10(19)-triene-3,24
20	12.198	0.51	3-Methyl-1,4-diazabicyclo[4,3,0]hexan-2,5
21	12.495	0.67	5H-Inden-5-one, octylhydro-1-hydroxy-7a
22	12.925	2.47	Cyclohexanecaboxaldehyde, 3,3-dimethyl
23	13.205	0.53	Cyclohexanol, 3-(acetyloxymethyl)-2,2,4
24	13.645	0.26	Spiro[5,5undec-2-ene, 3,7,7-trimethyl-11
25	13.691	0.87	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahyd
26	13.840	1.53	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahyd
27	13.942	0.81	Pyrrolo[1,2-a]pyrazine-1,4-dione, hexahyd
28	14.017	0.59	D-Arabino-Hexopyranoside, methyl-2,6-dione
29	14.257	0.98	n-hexadecanoic acid
30	14.477	0.44	7a-Isopropenyl-4,5-dimethyloctahydroind
31	15.1334	0.75	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethyl

32	15.442	0.71	2,4-Undecadien-1-ol
33	16.968	0.39	9-Octadecenamide, (Z),
34	17.406	0.41	(-)-Isolongifolol, acetate
35	19.858	0.70	Azulene,1,2,3,4,5,6,7,8,8a-octahydro-1,4-dione
36	20.053	0.99	(7a-Isopropynyl-4,5-dimethyloctahydroind
37	20.511	0.42	2-Furoic acid, 2-methyl-5-yn-4yl ester

3.2 Synthesis of silver nanoparticles

During the biosynthesis the change in colour from yellow to deep brown strongly suggested the formation of silver nanoparticles. It has been reported that silver nitrate when mixed with extracts of biological origin, is reduced to silver nanoparticles after an incubation period of time; the solution gradually changes colour from yellow to dark brown [23, 24]. The colour change correlates with reduction potential of silver ions by the biological extract as observed by these studies. Mushrooms are known to be rich in quality protein; fresh *Pleurotus ostreatus*, an edible mushroom contained up to 28% protein [25] with abundant essential and non-essential amino acid contents [26].

The UV-vis spectroscopy analysis, is a primary tool in nanotechnology for monitoring the formation and stability nanoparticles. The result of UV-visible absorption spectrum presented in (Fig. 2) showed a peak at 400nm which corresponds to Plasmon absorbance of AgNPs and an indication of the formation of AgNPs. This result is close to the values obtained in previous studies; AgNPs synthesized using *Ganoderma* extracts exhibited a band at 421 nm [17] and 432 nm [27] while silver nanoparticles synthesized with *Pleurotus florida* showed 435 nm [28]. The sizes and shapes of the nanoparticles usually influence the absorbance spectra (surface plasmon bands) as do the dielectric constants of the encompassing media.

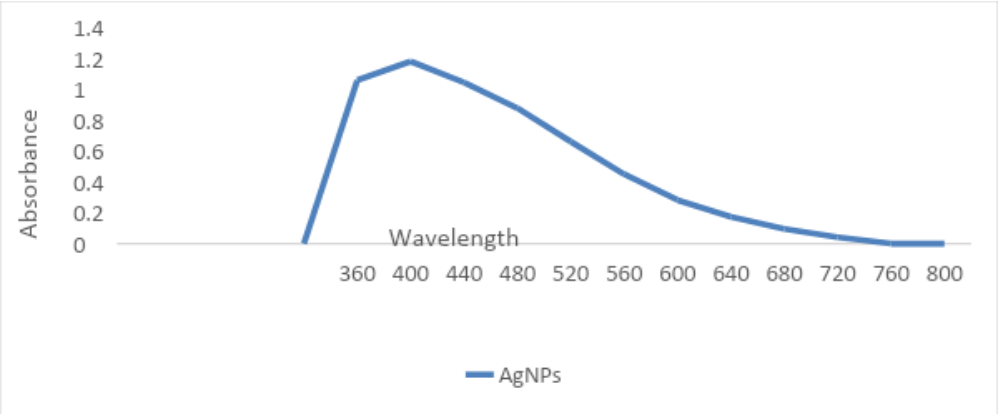
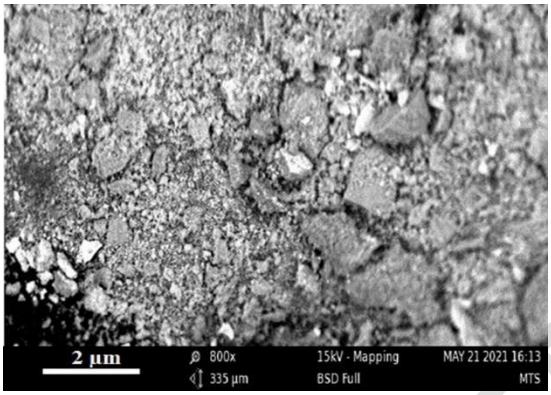


Fig. 2 UV–Vis spectrum of the colloidal solution of *Pleurotus ostreatus* extract and $AgNO_3$ showing maximum absorption at 400 nm due to the presence of AgNPs

3.3 SEM observation

The scanning electron micrograph showing the morphology of AgNPs is displayed in (Fig. 2) As observed, the microstructure of AgNPs showed that the particles were heterogeneous in morphology and composed of various sizes. Some were spherical in shape whereas others were hexagonal. Nonetheless, they were well-distributed on the surface of the sample to provide a large surface area which was of agglomeration to a reasonable extent. The size ranged between 18-82 nm as determined by the software, IMAGEJ. A large surface area is desirable for nanoparticles to serve as antimicrobial agents [29, 30, 31]. *Pleurotus tuber-regium* extract synthesized AgNPs that were cubical and spherical in shape with average size of 50 nm [32]. Other researchers synthesized spherical AgNPs with sizes ranging from 2-100 nm [33, 34, 35].

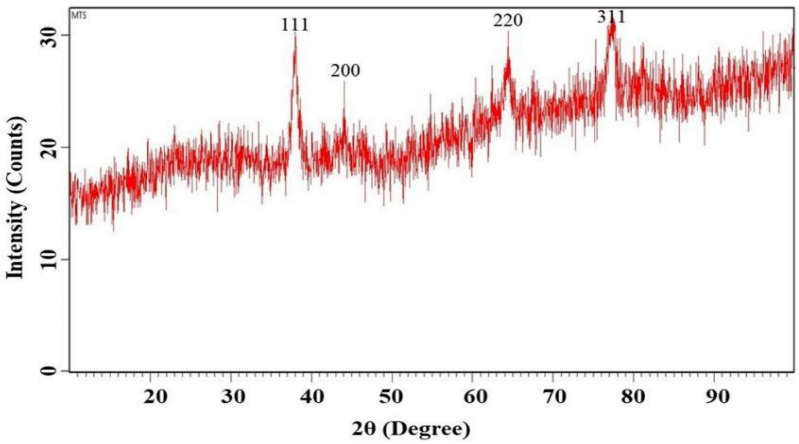
Fig. 3 Scanning Electron Micrograph (SEM) of AgNPs showing the morphology of the AgNPs



XRD pattern

XRD evaluated the diffraction pattern crystal structure of biosynthesized AgNPs between 2θ angles 20 – 90^{o}. The diffraction pattern presented in (Fig. 3) showed that various peaks were obtained at 2θ angles 38.2^{o}, 44.35^{oC}, 64.5, 77.4^{o}, corresponding to the reflection plane indices 111, 200, 220, and 311, of AgNPs [36].

Fig. 4 (XRD) pattern of the AgNP indicating the presence of AgNPs in the sample



3.4 (FTIR) of the AgNPs

FTIR spectroscopy indicated the vibrational modes of the bond in the biomolecules involved in the capping reaction to generate the AgNPs as well as the obtained AgNPs. It showed major transmission peaks at 3186 cm^{-1} corresponding to N-H or O-H stretching vibration of aromatic compounds [37, 18], 2918 cm^{-1} stretch ascribed to C-H stretch of alkanes [18].

Additional peaks are observed at 2117 cm^{-1} considered for the C=C stretching vibration of alkyne, the one near 1596 cm^{-1} is attributed to N=N bond while that around 1030 cm^{-1} is the bending vibration of C-O bond. These bond vibrational modes support the phytochemicals identified earlier in the GC-MS result. Furthermore, the presence of AgNPs in the sample is confirmed by the small peak appearing as a shoulder around 778 cm^{-1}.

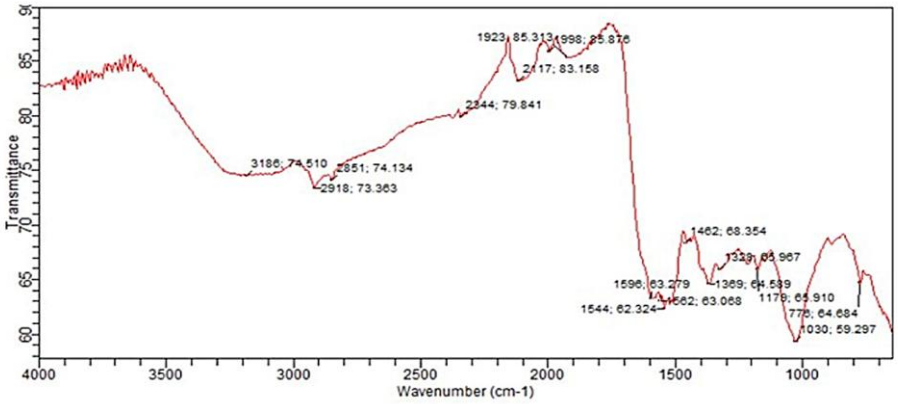


Fig. 5 (FT-IR) spectrum of the AgNPs showing bonds related to the phytochemicals present in the *Pleurotus ostreatus* aqueous extract

3.5 Antibacterial studies of Silver nanoparticles

The antibacterial studies of the AgNPs on *Providencia rettgeri* and *Bacillus subtilis* using gentamicin antibiotic as control is presented in (Fig. 5). The biosynthesized AgNPs inhibited the growth of *Providencia rettgeri* and *Bacillus subtilis* respectively. Metal NPs derived from mushrooms have been reported in literature to inhibit the growth of numerous foodborne pathogenic bacteria and fungi. The inhibition of AgNPs on the bacterial cells has been attributed to direct physical contact of metal nanoparticles with bacterial membranes which caused the release of intercellular materials, loss of cell membrane integrity and cell death [39]. Other researchers reported that the subsequent release of Ag^{+} ions by the

nanoparticles resulted in DNA damage, protein denaturation and enzyme inhibition [40]. Biosynthesized AgNPs using *Pleurotus florida* was shown to be active against *Staphylococcus aureus*, *Salmonella typhi, Providencia alcalifaciens* and *Proteus mirabilis* though a higher activity was observed against Gram positive than the Gram-negative bacteria [28]. In a similar study, Acay and Baran [38], using vanomycin, colistin and

fluconazole as control tested the antimicrobial activity of AgNPs from *Pleurotus eryngii* extract against *Esherichia coli, Staphylococcus aureus, Streptococcus pyogenes*, *Pseudomonas aeruginosa* and *Candida albicans* and observed MIC values between 0.035 – 0.07 mg/l and claimed that the AgNPs could be a better alternative. The research is contrary to the observation made in this study as gentamicin, an aminoglycoside antibiotic with broad spectrum activity had a higher inhibition compared to the mushroom extract. Aminoglycosides are known to disrupt the ability of bacteria to make proteins, a vital biomolecule in living things. Vanomycin is a glycopeptide that prevents cell wall synthesis in mostly Gram-positive bacteria while colistin, a polymyxin antibiotics breaks down the cytoplasmic membrane in susceptible Gram-negative bacteria. Due to abuse of antibiotics, most bacteria have evolved mechanisms of bypassing such barriers and have developed resistance against most of these antibiotics. This could be the reason vanomycin and colistin had lower inhibition unlike gentamicin.

4 Conclusion

Silver nanoparticles were successfully synthesized using the phytochemicals present in *Pleurotus ostreatus* as a reducing agent. The UV-Vis spectroscopy confirmed the formation of AgNPS at 400 nm while the SEM revealed the nanoparticles were heterogenous in morphology and in the size range of 18-82 nm. The GC-MS identified inherent phytochemicals present in *Pleurotus ostreatus* that reduced the $AgNO_3$ and stabilized the AgNPs. The XRD and FTIR further supported the involvement of these organic molecules in the reduction and capping reactions. Antibacterial studies revealed the potential of the biosynthesized AgNPs for inhibition of growth of *Providencia rettgeri* (Gram-negative) and *Bacillus subtilis* (Gram-positive) bacteria. The study has demonstrated that *P. ostreatus*

possessed the necessary phytochemicals for the reduction of $AgNO_3$ and the formation of stable AgNPs and the biosynthesized AgNPs significantly inhibited the growth of *P. rettgeri* and *B. subtilis.* The implication of the study is that *P. ostreatus* has potential application in Nano therapy for treatment of bacterial infections. In addition, *Pleurotus ostreatus* is a tropical mushroom that grows very fast on cheap agricultural residues in the environment and is commercially readily available for use. Also, the method adopted is ecofriendly, devoid of chemicals and expensive equipment.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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