

Green Synthesis of Silver Nanoparticles Using *Pleurotus ostreatus*

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Authors' contributions

This work was carried out in collaboration among all authors Author ACI designed the study, wrote the protocol, supervised the work and corrected the first draft of manuscript. Authors OM and EER managed the biosynthesis of AgNPs and characterization of AgNPs. Author OM wrote the draft of the manuscript and Author IUE managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The study aimed to synthesize silver nanoparticles (AgNPs) from aqueous AgNO₃ using phytochemicals present in *Pleurotus ostreatus* and assess the AgNPs antibacterial activity on *Bacillus subtilis* and *Providencia rettgeri*.

Study design: Experimental/Analytical.

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 was mixed with 9 ml of 10mM AgNO₃. 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.

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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 done 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 the reduction of AgNO₃. GCMS performed on the methanolic extract of *Pleurotus ostreatus* showed the presence of 37 organic compounds, among them were catechol, hydroquinone and phenols etc. Antimicrobial activity revealed that 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 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 and biomolecules present in these 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 environment friendly, the nanoparticles synthesized using mushrooms

are reported to exhibit higher stability, longer shelf life and enhanced biological activities [12]. A variety of oyster mushroom 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 an 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 have 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 was to synthesize silver nanoparticles using *Pleurotus ostreatus* mushroom and testing the antibacterial activity

against two organisms, *Providencia rettgeri* and *Bacillus subtilis* [16].

2. MATERIALS AND METHODS

2.1 Materials

The mushroom, *Pleurotus ostreatus* used for the study was procured from the 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°C for 72 h and pulverized into fine powder to obtain a large surface area for absorption. The dried mushroom powder (5 g) was stirred in 100 ml deionized water in a 500 mL capacity conical flask. The mixture was sealed, sonicated in an ultrasonic cleaner (CLEAN-120HD) at 40°C for 40 min and centrifuged at 4000 rpm for 10 min. The supernatant was filtered to obtain the aqueous extract and stored in a refrigerator between 5 - 10°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 overnight in the dark, at room temperature. After the reaction period, the mixture was centrifuged at 15,000 rpm for 15 min to separate the AgNPs. The supernatant was discarded and the 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°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 into the GC-MS machine and the phytochemicals were 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 (Uniscop SM 7504) within the wavelength ranges of 300 nm - 800 nm operated at a resolution of 1 nm at room temperature (25°C). The formation of AgNPs was 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 wave number range of 650 – 4000 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 were 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°C for 5 h. Then 100 µl of the AgNPs and gentamicin (10 µg/ml) respectively were dispensed in to the wells. Incubation of the plates was at 37°C for 24 h. The formation of a clear zone of inhibition around the well was 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 the production of biologically active compounds with anti-inflammatory, anti-oxidant, anti-bacterial and anti-tumour potentials. The result of GC-MS analysis of methanolic extract of *Pleurotus ostreatus* presented in (Fig. 1), revealed the presence of 37 organic compounds, among them were acids, phenols, hydroquinone, sugars, glucopyranoside, unsaturated fatty acids, flavour and fragrances agents. The most abundant compounds (as shown in Table 1) were catechol, an organic phenol with a 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 a 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 concentrations 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 the most abundant compound 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].

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 the reduction potential of silver ions by the biological extract as observed by the 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 [26].

The UV-vis spectroscopy analysis, is a primary tool in nanotechnology for monitoring the formation and stability of nanoparticles. The result of the 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 a peak at 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.

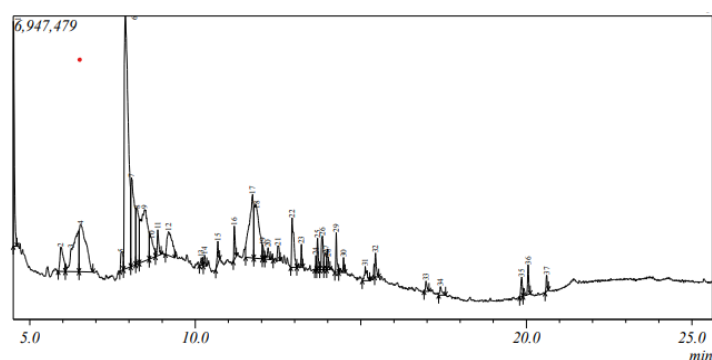
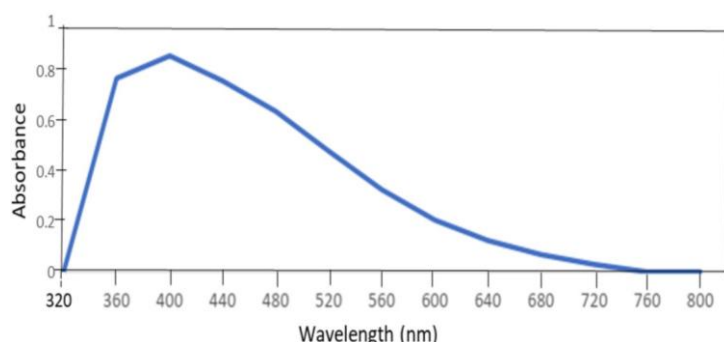


Fig. 1. Gas chromatography-mass spectrophotometer analysis of methanolic extract of *Pleurotus ostreatus*

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

**Fig. 2. UV-Vis spectrum of the colloidal solution of *Pleurotus ostreatus* extract and AgNO₃ 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. 3) As observed, the microstructure of AgNPs showed that the particles were heterogeneous in morphology and composed of various sizes. Some were spherical in shape while others were hexagonal. Nonetheless, they were well-distributed on the surface of the sample to provide a large surface area which was free of agglomeration to a reasonable extent.. The size ranged between 18-82 nm as determined by the software, Image J. A large surface area is desirable for nanoparticles to serve as

antimicrobial agents [29,30,31]. *Pleurotus tuber-regium* extract was used for synthesis of 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].

XRD evaluated the diffraction pattern crystal structure of biosynthesized AgNPs between 2θ angles 20 – 90°. The diffraction pattern presented in (Fig. 4) showed that various peaks were obtained at 2θ angles 38.2°, 44.35°, 64.5, 77.4°, corresponding to the reflection plane indices 111, 200, 220, and 311, of AgNPs [36].

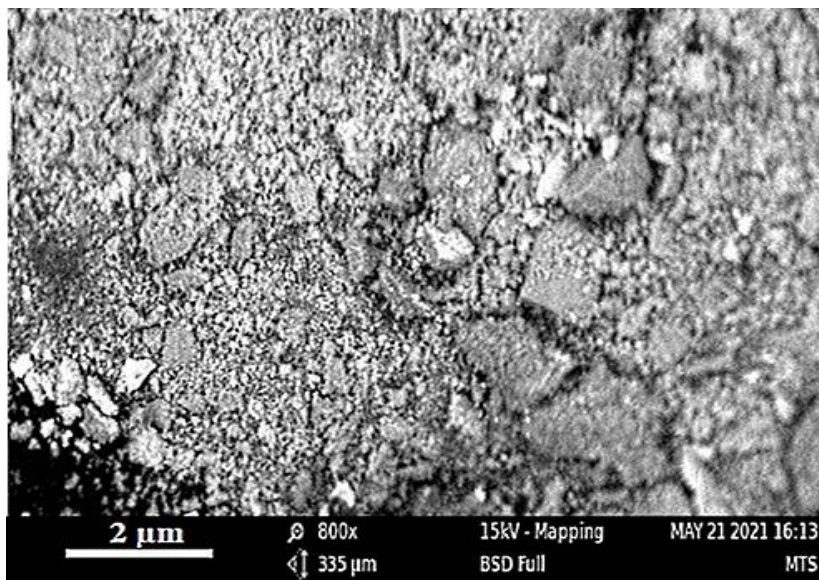


Fig. 3. Scanning Electron Micrograph (SEM) of AgNPs showing the morphology of the AgNP XRD pattern

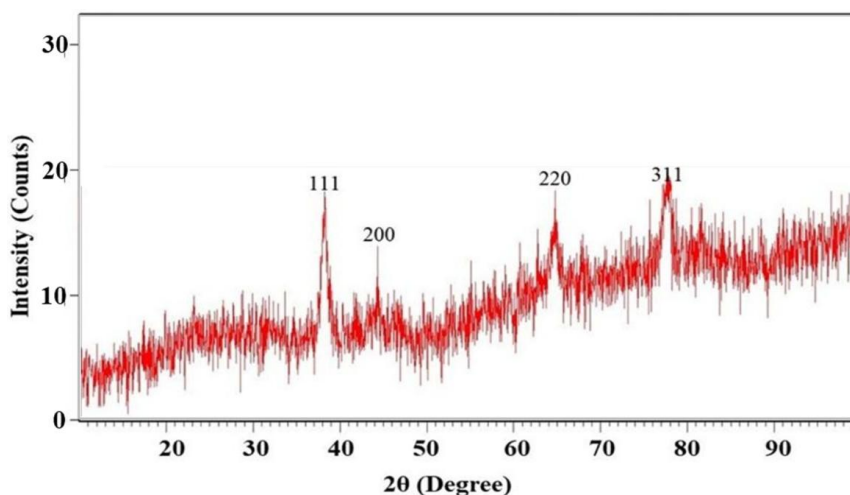


Fig. 4. (XRD) pattern of the AgNP indicating the presence of AgNPs in the sample

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 (Fig. 5). It showed major transmission peaks at 3186 cm^{-1} corresponding to N-H or O-H stretching vibration of aromatic compounds [37,38], 2918 cm^{-1} stretch ascribed to C-H stretch of alkanes [18]. Additional peaks were observed at 2117 cm^{-1} considered for the C=C stretching vibration of alkyne, the one near 1596 cm^{-1} was attributed to N=N bond while that around 1030 cm^{-1} was 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 was confirmed by the small peak appearing as a shoulder around 778 cm^{-1} .

3.5 Antibacterial Studies of Silver Nanoparticles

The antibacterial studies of the AgNPs on *Providencia rettgeri* and *Bacillus subtilis* using gentamicin antibiotic as control showed. 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*. He observed MIC values were between $0.035 - 0.07\text{ mg/l}$ and concluded 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 antibiotic breaks down the cytoplasmic membrane in susceptible Gram-negative bacteria. Due to the 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.

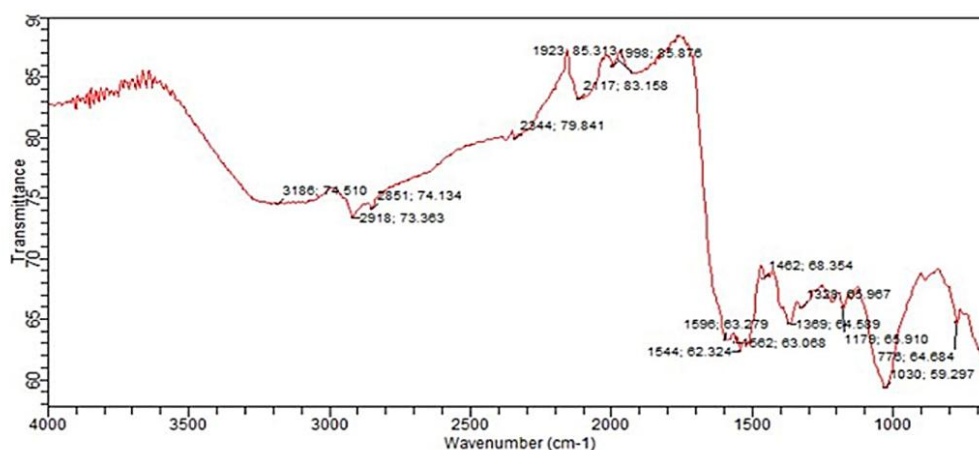


Fig. 5. (FT-IR) spectrum of the AgNPs showing bonds related to the phytochemicals present in the *Pleurotus ostreatus* aqueous extract

4. CONCLUSION

Results obtained from the study revealed that silver nanoparticles in the size range of 18 - 82 nm were successfully synthesized using the phytochemicals present in *Pleurotus ostreatus* as a reducing agent. The biosynthesized AgNPs significantly inhibited the growth of *Providencia rettgeri* (Gram-negative) and *Bacillus subtilis* (Gram-positive) bacteria. . The study implies that *P. ostreatus* has potential application in Nano therapy for the treatment of bacterial infections. .

DISCLAIMER

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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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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