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FUNCTIONAL ASSESSMENT OF SILVER NANOPARTICLES OF WILD MUSHROOMS MORCHELLA ESCULENTA AND LYCOPERDON PYRIFORME

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Abstract

Mushrooms have had a culinary role for more than two thousand years. Mushrooms are an excellent resource of important nutrients since they are high in proteins, vitamins, and minerals. Furthermore, there are more than 100 known health advantages associated with mushrooms and fungi. Antioxidant, anticancer, antidiabetic, antiallergic, immunomodulatory, cardiovascular protective, anticholesterolemic, antiviral, antibacterial, antiparasitic, antifungal, detoxifying, and hepatic protective properties are merely some of these health benefits. In order to assess the possible antibacterial and antioxidant qualities of ethanolic extracts made from native wild mushrooms, this research project involves the careful collection and subsequent investigation of these extracts. Morchella esculenta and Lycoperdon pyriforme are the two different wild mushroom species examined here in order to identify and clarify their innate antibacterial and antioxidant properties. The results of our study show no detectable antibacterial activity against three particular bacterial strains: Salmonella typhi, Enterobacter cloacae, and Pseudomonas aeruginosa. However, using DPPH scavenging tests, it was determined that the ethanolic extracts from Lycoperdon pyriforme and Morchella esculenta both have strong antioxidant efficacy. This significant antioxidative activity highlights the extracts' possible use in treating illnesses linked to oxidative stress. Furthermore, we started synthesizing nanoparticles as part of our study, which also encompasses the field of nanotechnology. Following careful characterization using Scanning Electron Microscopy (SEM) and Energy-Dispersive X-ray Spectroscopy (EDS) investigations, these nanoparticles provide information about their elemental composition and morphological characteristics. In Morchella esculenta, the characterization study revealed positive results for nanoparticle production; however, in the case of Lycoperdon pyriforme, the results showed a less favorable reaction. This multifaceted investigation highlights the thoroughness of our study, covering various aspects of extracts from mushrooms and the synthesis of nanoparticles, which together advance our understanding of natural product pharmacology and nanomaterial science.

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INTRODUCTION

The field of science and engineering known as nanotechnology is devoted to investigating the properties of materials with sizes of 100 nanometers or less. The discipline of nanobiotechnology is defined as a subfield of biotechnology that focuses on the production, organization, and stability of various nanoscale particles through biological processes. This includes the efficient application of particles of sizes less than 100 nm (LATEEF and ADEEYO, 2015). Humanity has benefited greatly from the astounding advancements in nanotechnology, including significant advances in medicinal applications (Liu *et al.*, 2010), catalysis, microelectronics, and bio sensing devices as well as its contributions to air and water purification and advancements in paints (Shin *et al.*, 2009). AgNPs, or silver nanoparticles, are known to be strong nanoweapons with strong antibacterial qualities that can successfully fight off bacteria that are resistant to many drugs (Rai *et al.*, 2012). The silver nanoparticle's numerous antibacterial processes, such as slow oxidation and the release of Ag+ ions into the environment, are responsible for its exceptional bactericidal qualities. Furthermore, significant interactions with the environment are made possible by its enormous surface area. A thorough assessment of both intracellular and membrane-associated silver nanoparticles is necessary to fully comprehend their biocidal effects. By rupturing their cell membranes and allowing silver nanoparticles to diffuse inside the cells, bacteria may be successfully eliminated as microorganisms (Ravindran *et al.*, 2013).

Numerous aspects of the medical sector, including treatments, innovative biomaterial designs, active implants, in vitro diagnostics, fast drug delivery systems, and bioimaging, have been greatly improved by nanoparticles. At the moment, there is strong evidence supporting the use and growth of medications that are nano sized (Lou *et al.*, 2025). First of all, the application of nanoparticles presents encouraging ways to tackle issues pertaining to the solubility and chemical stability of bioactive elements. Second, the use of nanoparticles may improve the pharmacokinetic profiles of medicinal resources by reducing waste and speeding up the compounds' biodegradation. Thirdly, the application of nanotechnology has the potential to greatly improve the localization and targeted administration of substances that combat cancer (Lou *et al.*, 2025).

Like other fungi, mushrooms are important in nanobiotechnology because they produce a large number of vital biomolecules needed for the development of nanoparticles (Anthony *et al.*, 2014). Biomolecules generated from mushrooms are essential to nanobiotechnology, mainly because they help stabilize metal ions and reduce them to nanoparticles, which frequently requires the action of reductase enzymes. The various proteins, essential amino acids, and polysaccharides found in mushroom mycelia and fruiting bodies may be useful in the creation of intracellular or extracellular nanoparticles of silver, gold, iron, and selenium, among other metallic elements (Owaid *et al.*, 2017). *Morchella esculenta*, a member of the Ascomycota fungus group, is a flavorful and healthful mushroom that is also known as morel. Numerous earlier research has found that Morchella contains a variety of physiologically advantageous components, including proteins, dietary fibers, vitamins, and polysaccharides. Because of its remarkable antioxidant properties, *M. esculenta* has drawn interest in earlier research. *M. esculenta* has a wide range of active substances with strong antioxidant qualities. Its remarkable antioxidant activity is attributed to the abundance of beta-carotene and linoleic acid in its mycelia (Tietel and Masaphy, 2018).

Often referred to as stump puffball or pear-shaped puffball, *Lycoperdon pyriforme* is a saprobic fungus that grows all over the world. A high amount of lipids, carbohydrates, proteins, and fatty acids, including linoleic acid (37% of all fatty acids), oleic acid (24%), and palmitic acid (14.5%), may be found in the fruiting body of *L. pyriforme*. The phytochemical components of *L. pyriforme* have potent antibacterial and antioxidant properties. Based on their application in ethnomedicine, this plant's antioxidant, antibacterial, and phytochemical properties were assessed (Colak *et al.*, 2009).

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MATERIALS AND METHODS

Sample Collection

Mushroom species were collected from different areas of Khyber Pakhtunkhwa during the months of May and July 2023. *Morchella esculenta* was collected from Abbottabad Sarban hill and *Lycoperdon pyriforme* from Havelian district farms.

Fig 1. *Morchella esculenta* collected from Abbottabad Sarban hill

Fig 2. *Lycoperdon pyriforme* collected from Havelian farmland

Sample processing

Double-distilled water was used to wash the mushrooms, and they were then allowed to air dry for three days in a shady area. Following drying, samples were crushed in a traditional grinder to a powder. For two weeks, 10 grams of powdered materials were soaked in 70% ethanol in a falcon tube and kept in a shaking incubator set at 37°C. The resultant extracts were then filtered through a syringe filter following this incubation time, and they were then placed in a water bath set at 62°C for two hours to produce the concentrated extracts (Adeyemi *et al.,* 2022).

Media preparation

The current study employed culture media, specifically Mueller Hinton agar (MHA) medium and Luria Broth (LB) medium. A precise mixture of certain materials is required to prepare 100ml of LB agar: 0.5g of sodium chloride (NaCl), 1g of tryptone, 0.5g of yeast extract, and 1.7g of bacteriological agar. 100ml of distilled water was used to dissolve 3.8g of Mueller Hinton agar for the MHA medium preparation. After that, the solid ingredients are completely submerged in water and autoclaved for 20 minutes at 121°C. The prepared medium is ready for use right away in the experimental methods once the flask has been autoclaved, cooled, and gently swirled to ensure the uniform dispersion of the ingredients.

Antimicrobial Activity

To test the antibacterial properties of both extracts, three strains were chosen. *Salmonella typhi*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* were the strains used for testing. We cultured strains in Luria Broth medium and incubated the tubes at 37°C for 24 hours in order to prepare the microbial culture (Hudzicki, 2009).

Agar well Diffusion Method

The agar well diffusion technique was used to assess both extracts' antibacterial activity. To achieve pure colonies, prepared bacterial solutions containing

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bacteria in the exponential growth phase were spread out over LB agar. Following a 24-hour incubation period, two to three colonies were selected and combined with five milliliters of autoclave water. The absorbance of each strain was measured at 600 nm using a UV-visible spectrophotometer. The strains' measured absorbance ranged from 0.08 to 0.1, with water serving as the blank. Then, using sterile cotton buds, they were spread out on Mueller-Hinton agar medium. 50 µl of the extracts were added to wells formed with 1 ml pipette tips, and once they had dried, the petri dishes were incubated for 24 hours at 37°C. After 24 hours, the width of the inhibition zone was measured in millimeters (mm) to determine the amount of antimicrobial activity (Hudzicki, 2009).

Antioxidant Activity

Both extracts' antioxidant capacity was evaluated by means of the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging test. After obtaining different amounts of the mushroom extracts (50, 100, 115, 125, 150, and 200 µl/ml) from different test tubes, 1 ml of a 0.1 mM DPPH solution was added to each test tube. A color change was noted after 30 minutes of incubation, and a UV-Vis spectrophotometer was used to measure the decrease in absorbance at 517 nm. Additionally, a control with two milliliters of extract-free DPPH solution was made. Increased free radical scavenging activity is shown by a decrease in absorption. The following formula was used to determine the reduction in absorption:

% Inhibition = Absorption of control- absorption of sample/ Absorption of control.

Nano-particles Synthesis

AgNPs were created by combining 8 milliliters of concentrated extracts of each mushrooms with 72 milliliters of AgNO_3. The transition from silver ions to silver nanoparticles was carefully monitored by analyzing color changes. Following this step, the solution was centrifuged for 20 minutes at a rate of 15,000 rpm in order to separate the precipitate from the supernatant. The precipitate that resulted from this process was then dried in order to produce desiccated silver nanoparticles (Singh *et al.,* 2016).

Characterization of nano-particles

After the nanoparticles have been successfully synthesized, the next step is to assess their characteristics. Advanced methods like Energy-Dispersive X-ray Spectroscopy (EDS) and Scanning Electron Microscopy (SEM) are used to perform this important investigation (Singh *et al.,* 2016).

Scanning Electron Microscopy

Another technique used to characterize the morphology of nanoparticles (NPs) is scanning electron microscopy (SEM). Based on the basic ideas of electron microscopy, this method has unique advantages for assessing the size and shape of NPs. However, it also has limitations, such as only giving a limited amount of information on the size distribution, mean dimension, and actual number of particles (Pal *et al.,* 2011). The equipment consists of a vacuum environment, condenser lenses, and an electron emission source (electron gun). Three main forms of imaging are produced by scanning electron microscopy (SEM): surface X-ray maps, backscattered electron pictures, and secondary electron images (Gupta *et al.,* 2013).

A particulate substance is formed by desiccating the colloidal NP solution during the nano-particle (NP) characterization procedure. A conductive metallic substance is then applied to this material after it has been adhered to a specimen mount (in the case of non-conductive materials). Well-known options for this use include metals like palladium, gold, alloys, platinum, tungsten, chromium, graphite iridium, or osmium, which are deposited via a sputter coating process. The specimen is then carefully exposed to a beam of high-energy electrons, which causes the sample to produce a signal (Jores *et al.,* 2004). The resulting signals are obtained using specialized detectors and processed to produce formats that provide detailed information about the samples, including their surface properties (external morphology), crystal structure, atomic orientation, and chemical makeup (Rai *et al.,* 2009). To enable a thorough investigation, the nanoparticles (NPs) need to be able to withstand environments of vacuum pressure. It is important to remember, nevertheless, that the electron beam has the potential to harm the nanopolymer substance. The morphological characteristics of several metallic nanoparticles (NPs),

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including gold, (Narayanan *et al.*, 2015) and silver are determined using this technique (Kumar *et al.*, 2013).

Energy dispersive X-ray (EDX)

One technique used to ascertain the elemental makeup of a particular specimen is energy dispersive X-ray (EDX) analysis. The application of an electron beam onto the specimen, which causes the emission of X-rays, is the fundamental idea behind EDX. Both qualitative and quantitative information is obtained by methodically collecting and closely examining the released X-rays. In quantitative analysis, the intensities of spectral peaks are used to determine the amounts of various elemental elements in the sample (Ruparelia *et al.*, 2008).

At the same time, characteristic X-ray peaks that are identified by certain locations in the spectrum are determined in qualitative analysis. It is noteworthy that this method is effective in identifying elements with atomic numbers between 4 and 92. The resulting X-ray spectrum provides important information about the identification and compositional characteristics of the elements present in the sample because the emitted X-ray quantities from each constituent within the specimen are directly proportional to their respective concentrations. A feature X-rays are produced by all elements except lithium, helium, and hydrogen. Even though X-rays from all elements are easily measurable, it is important to remember that they cannot detect lower energy levels, such lithium (0.052 keV) (Narayanan *et al.*, 2015). The use of beryllium (Be) windows with energy levels higher than 2 keV is the cause of this occurrence. As a result, lower energy X-rays cannot pass through these windows and are thus not detected. This method is widely used in the physical, chemical, and electronic sciences. At present, an analytical electron microscope (AEM) is utilized, in which Transmission Electron Microscopy (TEM) is combined with an Energy Dispersive X-ray (EDX) system. Additionally, to facilitate trace element identification and quantification, EDX is utilized in conjunction with Scanning Electron Microscopy (SEM). It is important to keep in mind that EDX has a limitation in that it is unable to differentiate between compounds with atomic numbers lower than sodium (Na) and X-rays (Joshi *et al.*, 2008). Moreover, spectral peaks may be displaced to incorrect energy levels by a device that is misaligned or improperly calibrated. The EDX method was used to examine gold nanoparticles (Au) and silver nanoparticles (Ag). In a different study, copper nanoparticles (Cu-NPs) showed an oxide layer on their surface, whereas Ag nanoparticles were shown to have a pure composition (Ruparelia *et al.*, 2008).

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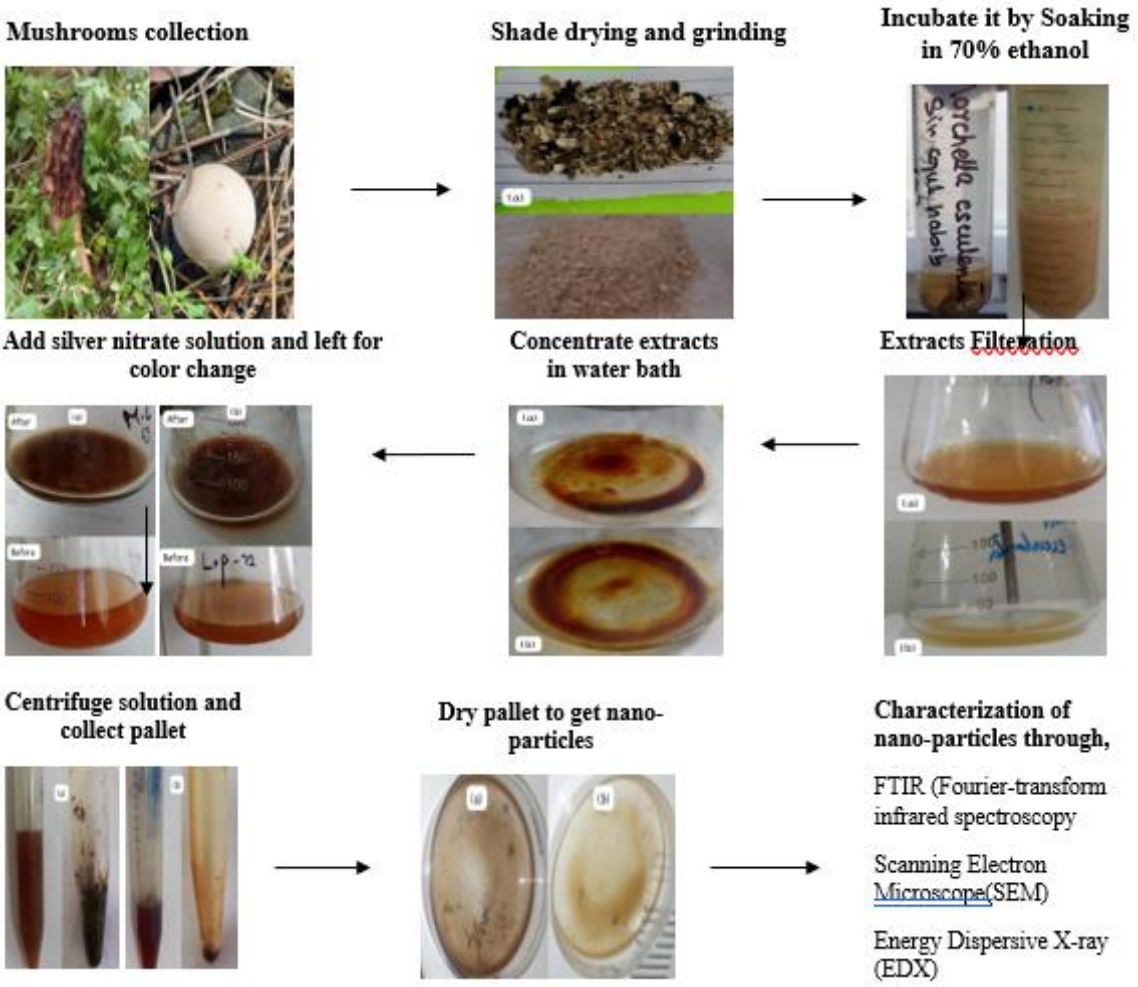
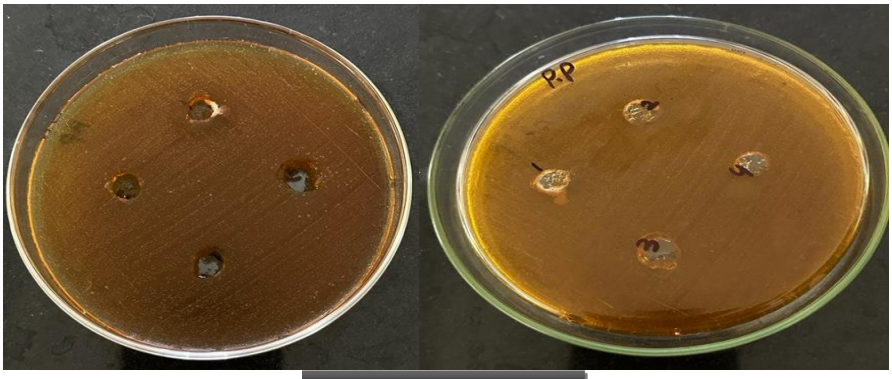


Evaluation of antimicrobial activity

During this study, we recruited three different types of bacteria: *Salmonella typhi*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa*. On Muller-Hinton agar plates with wells, several bacterial strains were grown. 50 µl of the corresponding extract was then precisely added to each of these wells. After a 24-hour incubation period, an evaluation was carried out to ascertain the size of the zone of inhibition. The extracts showed no discernible inhibitory zones, with the exception of a small zone that appeared to target *Salmonella typhi*.



Pseudomonas aeruginosa

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Pseudomonas aeruginosa **plate**
Well 1 (*Flacourtia indica*)
Well 2 (*Caralluma tuberculata*)
Well 3 (*Morchella esculenta*)
Well 4 (*Lycoperdon pyriforme*)

Enterobacter cloacae **plate**
Well 1 (*Alkanna tinctoria*) zone of inhibition is present
Well 2(*Lycoperdon pyriforme)*
Well 3(*Morchella esculenta*)
Well 4(*Berberis lycium*)

Salmonella typhi **plate**
Morchella esculenta **extract showing little activity against *S.typhi***

Fig 3: Evaluation of antimicrobial activity

Evaluation of Antioxidant activity

1, 1-Diphenyl-2-picrylhydrazyl (DPPH) is a free radical that changes from a dark violet to a pale yellow color when it reacts with an active antioxidant by transferring electrons or hydrogen. There are noticeable differences between BHT (butylated hydroxytoluene) and plant-derived extractions when the antioxidant activity is compared. Separate test tubes were used to obtain the mushroom extracts at concentrations of 50, 100, 115, 125, 150, and 200 µl/ml. After the addition of DPPH, a noticeable variation in color intensity was observed across various dilutions within a period of thirty minutes in both extracts.

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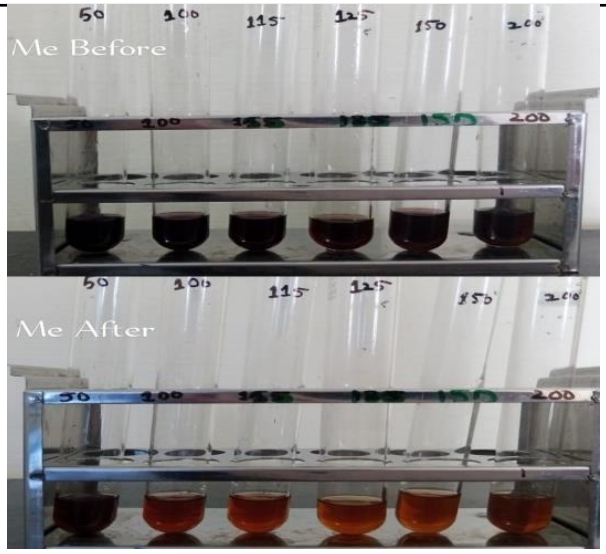

Fig 4. *Morchella esculenta* Color Change in Response to Various Dilutions upon Addition of DPPH

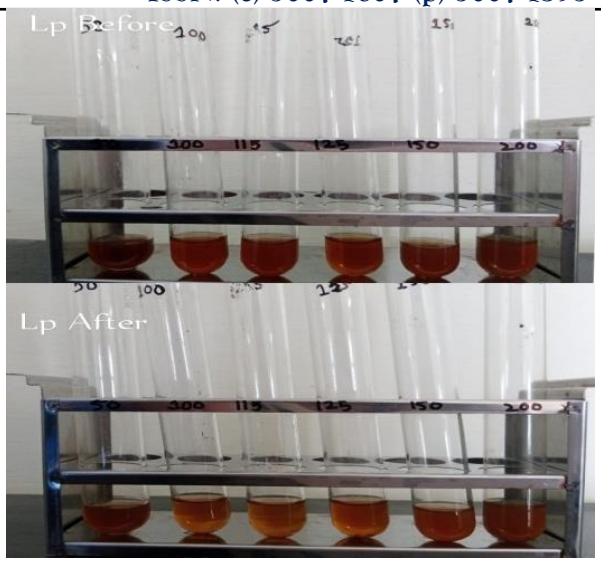

Fig 5. *Lycoperdon pyriforme* Color Change in Response to Various Dilutions upon Addition of DPPH

After 30 minutes, the optical absorbance at a wavelength of 517 nm was measured with a UV-Vis spectrophotometer, producing the following results for *M. esculenta*,

Optical Density(OD) of control	OD of 50%	OD of 100%	OD of 115%	OD of 125%	OD of 150%	OD of 200%
2.16	1.76	1.71	1.37	1.30	1.45	1.86

Now, decrease in absorption is measured by using formula,

$$\% \textbf{ Inhibition} = \frac{\textbf{Absorptionofcontrol} - \textbf{absorptionofsample}}{\textbf{Absorptionofcontrol}}$$

% Inhibition	50%	100%	115%	125%	150%	200%
	18.5%%	20.8%	36.5%	39.8%	32.8%	13.8%

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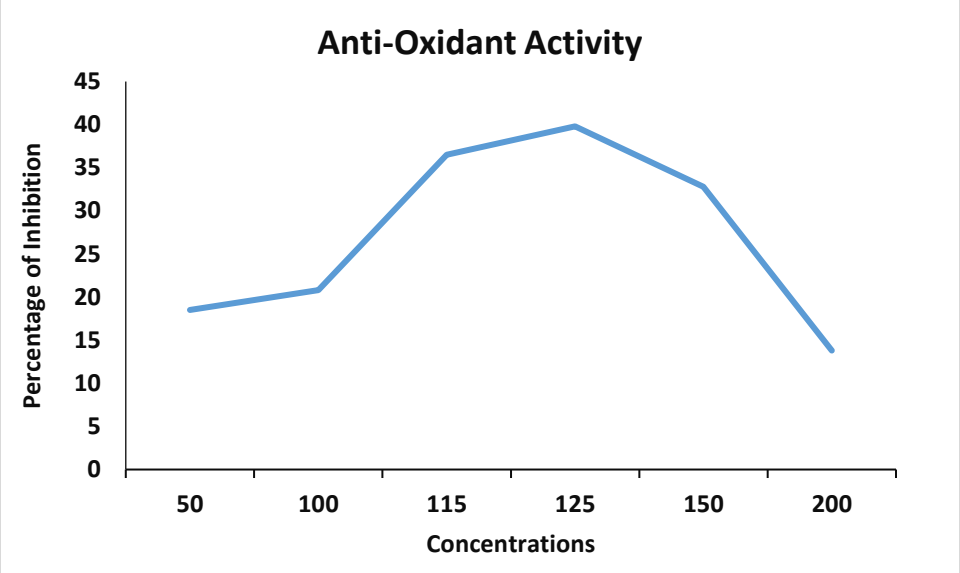

Fig 6: Antioxidant Potential of Ethanolic Extracts

Derived from *Morchella esculenta*

The scavenging effectiveness of 1,1-diphenyl-2-picrylhydrazyl radicals increases with concentration and subsequently declines with further concentration increments after reaching a peak value of 39.8%.

A UV-Vis spectrophotometer was used to measure the optical absorbance of Lycoperdon pyriforme at a wavelength of 517 nm after 30 minutes. The results were as follows:

Optical Density (OD) of control	OD of 50%	OD of 100%	OD of 115%	OD of 125%	OD of 150%	OD of 200%
2.16	1.83	1.69	1.41	1.44	1.46	1.88

Now, decrease in absorption is measured by using formula,

$$\% \textbf{ Inhibition} = \frac{\textbf{Absorptionofcontrol} - \textbf{absorptionofsample}}{\textbf{Absorptionofcontrol}}$$

% Inhibition	50%	100%	115%	125%	150%	200%
	15.2%	21.7%	34.7%	33.3%	32.4%	12.9%

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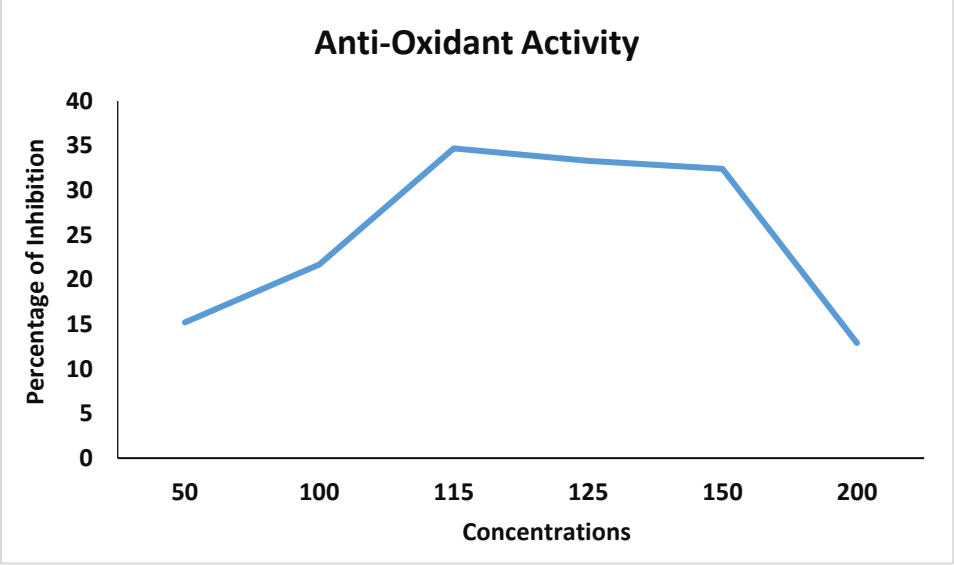

Fig 7: Antioxidant Potential of Ethanolic Extracts

Derived from *Lycoperdon pyriforme*

Potential Antioxidants in Ethanolic Extracts This compound, which is derived from Lycoperdon pyriforme, scavenges 1,1-diphenyl-2-picrylhydrazyl radicals with increasing effectiveness as concentration increases. It peaks at 34.7% and subsequently declines as concentration increases.

Nano-particles characterization

To ensure that nanoparticles are suitable for a wide range of applications, it is essential to confirm their formation through examination. In this study, energy-dispersive X-ray spectroscopy (EDX) and scanning electron microscopy (SEM) were used to analyze the characteristics of biologically generated ME-AgNPs and LP-AgNPs. The creation of nanoparticles is facilitated by *Morchella esculenta*, but Lycoperdon pyriforme does not exhibit nanoparticle formation.

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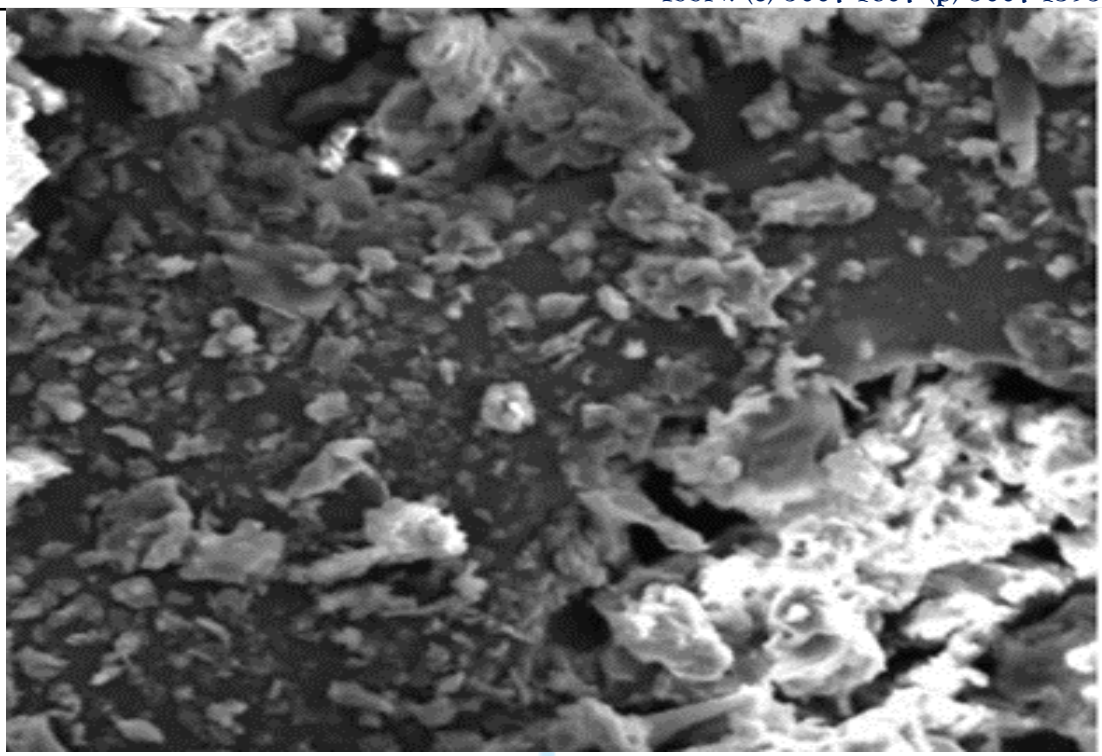

Fig 8: SEM analysis of silver nano-particles of *M. esculenta*

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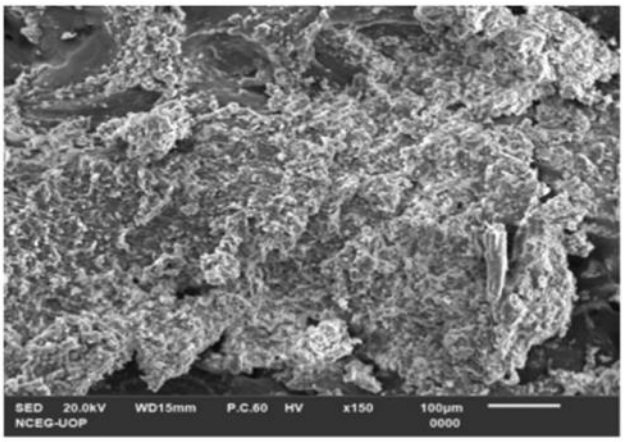
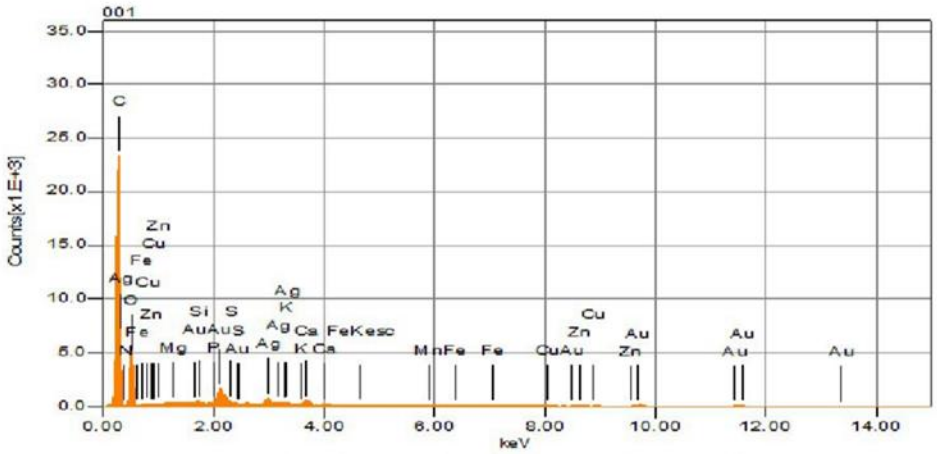


Acquisition Condition
Instrument : IT100LA
Volt : 20.00 kV
Current : ---
Process Time : T4
Live time : 60.00 sec.
Real Time : 68.75 sec.
DeadTime : 12.00 %
Count Rate : 7835.00 CPS



Formula	mass%	Atom%	Sigma	Net	K ratio	Line
C	57.03	67.61	0.01	591151	0.0745633	K
N*	0.75	0.76	0.01	7629	0.0130547	K
O	34.17	30.41	0.06	132284	0.0762479	K
Mg*	0.06	0.04	0.01	2105	0.0004739	K
Si*	0.11	0.05	0.01	5053	0.0008780	K
P*	0.13	0.06	0.01	6184	0.0013262	K
S*	0.08	0.04	0.01	4088	0.0008394	K
K	0.12	0.04	0.01	5072	0.0013499	K
Ca	0.52	0.18	0.01	21624	0.0060496	K
Mn*	0.01	0.00	0.01	114	0.0000609	K
Fe*	0.02	0.00	0.01	326	0.0001938	K
Cu	0.87	0.19	0.02	8449	0.0082662	K
Zn*	0.57	0.12	0.02	4648	0.0054246	K
Ag	1.35	0.18	0.02	38079	0.0119290	L
Au	4.21	0.30	0.04	94190	0.0314047	M
Total	100.00	100.00				

JEOL EDS System JEOL

Fig 9: EDS analysis of silver nano-particles of *M. esculenta*

DISCUSSION

Mushrooms have a powerful ability to help the body heal from dangerous and serious illnesses including diabetes, heart disease, AIDS, cancer, and more. Consequently, mushrooms are now considered a prospective and possible medicinal resource (Sultana and Qureshi, 2007). In considering this, the main focus of this research is on the synthesis and characterization of nanoparticles as well as the exploration of the potential present in wild mushrooms, with a particular focus on their antibacterial and antioxidant qualities. Through

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plant extraction, L. pyriforme's antibacterial potential was shown. *L. pyriforme* showed inhibitory effects on a variety of bacteria at a dose of 100µg/ml. *Bacillus subtilis, Candida albicans, Escherichia coli*, and *Staphylococcus aureus* all had their inhibitory zones assessed. Additionally, methanol extracts from *L. pyriforme* were shown to have substantial activity against the experimental microorganisms for *Candida albicans, Escherichia coli, Bacillus subtilis, and Staphylococcus aureus*, as indicated by their minimum inhibitory concentration (MIC) values. These findings underscore the strong antimicrobial activity of *L. pyriforme* extracts (Asfaw, 2022).

Using a standardized Agar well diffusion technique, we assessed the antibacterial activity of *M. esculenta* and *L. pyriforme* extracts in order to compare their antimicrobial potential. *Salmonella typhi*, *Enterobacter cloacae*, and *Pseudomonas aeruginosa* were the strains used for testing. To guarantee pristine colonies, we used bacterial suspensions in the exponential growth phase and disseminated them over LB agar, much like in other investigations. Colonies were chosen and combined for a UV-visible spectrophotometer absorbance measurement at 600 nm after a 24-hour incubation period, using water as the blank. Interestingly, the strains' measured ODs were consistent across the testing conditions, falling between 0.08 and 0.1. Sterile cotton buds were then used to make wells with 1 ml pipette tips and disseminate the bacterial suspensions onto Mueller-Hinton agar medium. Following the addition of extracts to the wells and subsequent drying, the Petri dishes were incubated for 24 hours at 37°C. In order to determine the antimicrobial efficiency after the previously specified 24-hour period, the inhibition zone width in millimeters (mm) was measured after this incubation time.

Both our *M. esculenta* and *L. pyriforme* extracts, however, showed no appreciable antibacterial action against the tested bacterial strains, which was contrary to our predictions and the results of earlier investigations. This finding suggests that neither extract had any discernible impact in our experimental design. This departure from earlier research emphasizes the inherent unpredictability of natural product studies and the significance of taking into account variables like sample source, extraction techniques, and experimental circumstances, all of which might have an impact on the results that are obtained. To determine the precise causes of our experiment's lack of antimicrobial effects in comparison to earlier findings, more research and analysis could be required. Jeng-Leun Mau, Chieh-No Chang, Shih-Jeng Huang, and Chin-Chu Chen previously investigated the antioxidant efficacy of three distinct mushroom extracts (Shimada *et al.*, 1992). Among the three extracts, *M. esculenta's* extract had the strongest DPPH radical scavenging activity, especially at doses below 10 mg/ml. The scavenging effects of *T. albuminosus* and G. *frondosa* extracts were comparable despite their modest levels. These results highlight the extract from *M. esculenta's* strong antioxidant ability to efficiently counteract DPPH radicals. This demonstrates how promising it is as a natural antioxidant for a range of use (Mau *et al.*, 2004).

Using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging test, we assessed the antioxidant capacity of the M. esculenta extract in our study. After preparing the mushroom extracts at different quantities (50, 100, 115, 125, 150, and 200 µl/ml), they were combined with 1 ml of a 0.1 mM DPPH solution. Color changes were noted after 30 minutes of incubation, and a UV-Vis spectrophotometer was used to measure the decrease in absorbance at 517 nm. For comparison, a control sample that included only the DPPH solution was also developed.

Increased free radical scavenging activity is shown by the reduction in absorption. The absorbance values for *M. esculenta* were measured at various concentrations; the control absorbance was 2.16, and the extract concentrations showed decreasing values between 1.76 and 1.30. Using the formula % Inhibition = (Absorption of control - absorption of sample) / (Absorption of control), the percentage inhibition was determined to be 18.5%, 20.8%, 36.5%, 39.8%, 32.8%, and 13.8% at 50%, 100%, 115%, 125%, 150%, and 200% concentrations, respectively. These tests show that the extract has the potential to be an antioxidant by scavenging free radicals. The DPPH test was used in both investigations to examine the antioxidant capacity of *M. esculenta's* extract.

Our research confirmed *M. esculenta's* capacity to scavenge free radicals at a variety of concentrations,

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while the previous study observed its strong scavenging impact on DPPH radicals, particularly at lower concentrations. This consistent result implies that the extract from M. esculenta has strong antioxidant qualities. Our study's % inhibition findings, which show significant scavenging effects across the concentration range, further support the extract's antioxidant potential. All things considered, our results support and enhance the earlier research, demonstrating the powerful antioxidant capacity of the extract from *M. esculenta*.

The test reagent used in the previous study to assess the antioxidative effectiveness of *L. pyriforme* was 1,1-Diphenyl-2-picrylhydrazyl (DPPH). Comparing plant-derived extracts with the traditional synthetic antioxidant Butylated Hydroxytoluene (BHT) revealed a clear difference in antioxidant efficacy. BHT at a standard dose of 100 µg/L showed strong inhibition (98.3 ± 0.3%), followed closely by the *L. pyriforme* extract (95.1 ± 0.19%), indicating a significant difference in inhibition percentages. Additionally, as its concentration increased, the *L. pyriforme* extract showed an expanding and proportional tendency in neutralizing free DPPH radicals. The *L. pyriforme* plant extract's IC50 value, which indicates the concentration at which the antioxidant activity decreases the DPPH radicals by 50%, was found to be 22.8µg/mL, whereas BHT's was 12.2µg/mL. The observed DPPH detoxifying activity seems to be influenced by the amount of flavonoids and phenolic compounds, as measured by mass determination (Asfaw, 2022).

After a 30-minute incubation period, we used a UV-Vis spectrophotometer to measure the absorbance values at a wavelength of 517 nm in order to evaluate the antioxidant activity of *L. pyriforme*. The following absorbance values were measured at various concentrations: 50%, 100%, 115%, 125%, 150%, and 200% concentrations showed matching absorbance values of 1.83, 1.69, 1.41, 1.44, 1.46, and 1.88, whereas the control showed an absorbance of 2.16. The percentage inhibition values were calculated using the formula (% Inhibition = (Absorption of control - absorption of sample) / (Absorption of control) and were 15.2%, 21.7%, 34.7%, 33.3%, 32.4%, and 12.9% for 50%, 100%, 115%, 125%, 150%, and 200% concentrations, respectively. The extract's promising antioxidant capability was highlighted by its strong DPPH radical scavenging ability. All of these findings demonstrate how well *L. pyriforme* scavenges DPPH radicals, suggesting that it has significant potential as an antioxidant agent at different doses. The comparison shows that our result supports the antioxidant ability of the extract from *L. pyriforme* and is consistent with the previous findings.

Previously, published research on the characterization of gold nanoparticles from *M. esculenta* was available. AuNPs were synthesized in that study by incubating a HAuCl4·3H2O solution with an extract from *M. esculenta* When the gold solution and the fungal extract interacted, a color shift from yellow to brown was seen during the reaction, indicating the creation of nanoparticles. When surface plasmon oscillations inside the gold nanoparticles (AuNPs) were activated, the brown hue emerged. It was evident from the UV-Vis spectroscopic investigation that AuNPs were forming in the reaction mixture since a peak nanoparticle production was seen at 511 nm. Utilizing SEM, the surface morphology of the AuNPs produced using *M. esculenta* extract was investigated. The SEM findings revealed the presence of cubic-shaped nano-particles (Acay, 2021). This analysis aimed to elucidate the size and morphological attributes of the synthesized AuNPs (Chellapandian *et al.*, 2019); (Balalakshmi *et al.*, 2017).

After a thorough field study, fifteen different kinds of mushrooms were collected from Jammu & Kashmir's Bandipora area. These species included *Tremates versicolor*, *Pleurotus florida*, *Ganoderma applanatum*, *Agaricus bisporus*, *Helvella lacunosa*, *Fomes fomentarius*, and unidentified species with code numbers VM-3, VM-4, VM-6, VM-7, VM-8, VM-9, VM-10, VM-11, VM-12, and VM-1. Samples of mushrooms were handled and transported with extreme care to maintain their qualities for simple identification and the subsequent manufacture of silver nanoparticles (AgNPs). To get rid of any adhering particles, 20 grams of dried mushrooms were thoroughly cleaned with double-distilled water demonstrating unequivocally that AuNPs were formed in the reaction mixture. The mushrooms were allowed to air dry in shady areas before being broken up into tiny bits, ground into a powder, and

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then suspended in 100 milliliters of sterile distilled water. The extract was produced by heating the mushroom extract to 55°C for 10 minutes. It was then dual-filtered through Whatman filter paper No. 1. The filtrate that was produced was then kept in storage at 4°C. This filtrate was used as a reducing agent for 1 mM AgNO3, and after 24 hours, there was a noticeable change in color from yellow to deep brown. The early creation of silver nanoparticles (AgNPs) was suggested by this color shift, which was followed by characterization using UV-visible spectroscopy.

In the UV-Vis Spectroscopy Analysis, five distinct species of the collected mushrooms *Agaricus bisporus*, *Helvella lacunosa*, *Fomes fomentarius*, *Pleurotus florida*, and *Ganoderma applanatum* showed positive peaks. VM-3, which was identified as *Morchella esculenta*, and the other mushrooms, on the other hand, did not show any peaks, suggesting that the synthesis of AgNPs was unsuccessful (Manzoor-Ul-Haq *et al.*, 2014). Although our extraction process for the mushroom extract was different from this study's, a relatively comparable strategy was used for the synthesis of AgNPs, which produced a successful result as validated by color change, SEM, and EDX analysis.

Conclusion

In conclusion, because of their nutritional value and potential for medicinal use, mushrooms have evolved from being regarded as fine foods to being essential parts of Pakistani diets. Their many health advantages, which range from preventing chronic illnesses to strengthening the immune system, demonstrate their adaptability. The significance of mushrooms is becoming more widely acknowledged, underscoring its function in preventing and enhancing general health in addition to their use in culinary traditions. In particular, this study focuses on wild edible types with the goal of identifying their potentially beneficial qualities for humans. There is still much to learn in this field of study, though.

REFERENCES

ACAY, H. 2021. Utilization of Morchella esculenta-mediated green synthesis golden nanoparticles in biomedicine applications. *Preparative Biochemistry & Biotechnology,* 51**,** 127-136.

ADEYEMI, J. O., ORIOLA, A. O., ONWUDIWE, D. C. & OYEDEJI, A. O. 2022. Plant extracts mediated metal-based nanoparticles: synthesis and biological applications. *Biomolecules,* 12**,** 627.

ANTHONY, K. J. P., MURUGAN, M., JEYARAJ, M., RATHINAM, N. K. & SANGILIYANDI, G. 2014. Synthesis of silver nanoparticles using pine mushroom extract: A potential antimicrobial agent against E. coli and B. subtilis. *Journal of Industrial and Engineering Chemistry,* 20**,** 2325-2331.

ASFAW, M. D. 2022. Antioxidant and Antimicrobial activity of Lycoperdon pyriforme fruiting body.

BALALAKSHMI, C., GOPINATH, K., GOVINDARAJAN, M., LOKESH, R., ARUMUGAM, A., ALHARBI, N. S., KADAIKUNNAN, S., KHALED, J. M. & BENELLI, G. 2017. Green synthesis of gold nanoparticles using a cheap Sphaeranthus indicus extract: impact on plant cells and the aquatic crustacean Artemia nauplii. *Journal of Photochemistry and Photobiology B: Biology,* 173**,** 598-605.

CHELLAPANDIAN, C., RAMKUMAR, B., PUJA, P., SHANMUGANATHAN, R., PUGAZHENDHI, A. & KUMAR, P. 2019. Gold nanoparticles using red seaweed Gracilaria verrucosa: green synthesis, characterization and biocompatibility studies. *Process Biochemistry,* 80**,** 58-63.

COLAK, A., FAIZ, O. & SESLI, E. 2009. Nutritional composition of some wild edible mushrooms. *Turkish Journal of Biochemistry,* 34**,** 25-31.

GUPTA, V., GUPTA, A. R. & KANT, V. 2013. Synthesis, characterization and biomedical application of nanoparticles. *Science International,* 1**,** 167-174.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

HUDZICKI, J. 2009. Kirby-Bauer disk diffusion susceptibility test protocol. *American society for microbiology,* 15**,** 1-23.

JORES, K., MEHNERT, W., DRECHSLER, M., BUNJES, H., JOHANN, C. & MÄDER, K. 2004. Investigations on the structure of solid lipid nanoparticles (SLN) and oil-loaded solid lipid nanoparticles by photon correlation spectroscopy, field-flow fractionation and transmission electron microscopy. *Journal of Controlled Release,* 95**,** 217-227.

JOSHI, M., BHATTACHARYYA, A. & ALI, S. W. 2008. Characterization techniques for nanotechnology applications in textiles.

KUMAR, S., SAINI, J., KASHYAP, D., BATRA, B., GREWAL, A., MALIK, D. K. & KUMAR, R. 2013. Green synthesis of plant-mediated silver nanoparticles using mangifera indica and syzygium cumini leaf extract. *International Journal of Pharmaceutical Sciences and Research,* 4**,** 3189.

LATEEF, A. & ADEEYO, A. O. 2015. Green synthesis and antibacterial activities of silver nanoparticles using extracellular laccase of Lentinus edodes. *Notulae Scientia Biologicae,* 7**,** 405-411.

LIU, H.-L., HUA, M.-Y., YANG, H.-W., HUANG, C.-Y., CHU, P.-C., WU, J.-S., TSENG, I.-C., WANG, J.-J., YEN, T.-C. & CHEN, P.-Y. 2010. Magnetic resonance monitoring of focused ultrasound/magnetic nanoparticle targeting delivery of therapeutic agents to the brain. *Proceedings of the National Academy of Sciences,* 107**,** 15205-15210.

LOU, X., ZHOU, Q., JIANG, Q., LIN, L., ZHU, W., MEI, X., XIONG, J. & GAO, Y. 2025. Inhibitory effect and mechanism of violacein on planktonic growth, spore germination, biofilm formation and toxin production of Bacillus cereus and its application in grass carp preservation. *International Journal of Food Microbiology,* 426**,** 110917.

MANZOOR-UL-HAQ, R. V., PATIL, S., SINGH, D. & KRISHNAVENI, R. 2014. Isolation and screening of mushrooms for potent silver nanoparticles production from Bandipora District (Jammu and Kashmir) and their characterization. *Int. J. Curr. Microbiol. Appl. Sci,* 3**,** 704-714.

MAU, J.-L., CHANG, C.-N., HUANG, S.-J. & CHEN, C.-C. 2004. Antioxidant properties of methanolic extracts from Grifola frondosa, Morchella esculenta and Termitomyces albuminosus mycelia. *Food chemistry,* 87**,** 111-118.

NARAYANAN, K. B., PARK, H. H. & HAN, S. S. 2015. Synthesis and characterization of biomatrixed-gold nanoparticles by the mushroom Flammulina velutipes and its heterogeneous catalytic potential. *Chemosphere,* 141**,** 169-175.

OWAID, M. N., BARISH, A. & ALI SHARIATI, M. 2017. Cultivation of Agaricus bisporus (button mushroom) and its usages in the biosynthesis of nanoparticles. *Open Agriculture,* 2**,** 537-543.

PAL, S. L., JANA, U., MANNA, P. K., MOHANTA, G. P. & MANAVALAN, R. 2011. Nanoparticle: An overview of preparation and characterization. *Journal of applied pharmaceutical science***,** 228-234.

RAI, M., YADAV, A. & GADE, A. 2009. Silver nanoparticles as a new generation of antimicrobials. *Biotechnology advances,* 27**,** 76-83.

RAI, M. K., DESHMUKH, S., INGLE, A. & GADE, A. 2012. Silver nanoparticles: the powerful nanoweapon against multidrug-resistant bacteria. *Journal of applied microbiology,* 112**,** 841-852.

RAVINDRAN, A., CHANDRAN, P. & KHAN, S. S. 2013. Biofunctionalized silver nanoparticles: advances and prospects. *Colloids and Surfaces B: Biointerfaces,* 105**,** 342-352.

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

RUPARELIA, J. P., CHATTERJEE, A. K., DUTTAGUPTA, S. P. & MUKHERJI, S. 2008. Strain specificity in antimicrobial activity of silver and copper nanoparticles. *Acta biomaterialia*, **4**, 707-716.

SHIMADA, K., FUJIKAWA, K., YAHARA, K. & NAKAMURA, T. 1992. Antioxidative properties of xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of agricultural and food chemistry*, 40**,** 945-948.

SHIN, K. S., CHOI, J.-Y., PARK, C. S., JANG, H. J. & KIM, K. 2009. Facile synthesis and catalytic application of silver-deposited magnetic nanoparticles. *Catalysis letters*, 133**,** 1-7.

SINGH, P., KIM, Y.-J., ZHANG, D. & YANG, D.-C. 2016. Biological synthesis of nanoparticles from plants and microorganisms. *Trends in biotechnology*, 34**,** 588-599.

SULTANA, K. & QURESHI, R. A. 2007. Distribution of medicinally important mushrooms of mountainous/Northern areas of Pakistan. *Plant Pathology Journal*.

TIETEL, Z. & MASAPHY, S. 2018. Aroma-volatile profile of black morel (Morchella importuna) grown in Israel. *Journal of the Science of Food and Agriculture*, 98**,** 346-353.