

Research Article

Mycosynthesis of Noble Metal Nanoparticle Using *Laetiporus versisporus* Mushroom and Analysis of Antioxidant Activity

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Nanoparticles being the backbone of nanotechnology gain more attention nowadays as they are entirely different from the colloids and also exhibit unique electrochemical, physical, and optical properties corresponding to their dimensions and shape. The core concept of getting nanoparticles from metal colloids lies on the traditional approach that is applied in both conventional and mechanical methods these days. Among those, the techniques that rely on the biological systems for the generation of nanoparticles have more merits and precedence over other environmental mechanisms as this mitigates the expense as well as toxicity. Considering both nutritional and medicinal values of mushrooms, they were properly managed, adopted, and accustomed in distinct skills to get desired products of nanosizes. Gold is commonly known as a noble metal, and it owns enormous medicinal values since long time in history. Here, a mycosynthesis approach was applied; by the way, the mushroom *Laetiporus versisporus* and gold ions collaborate to bring out gold nanoparticles (AuNPs) were synthesized by the involvement of *Laetiporus versisporus*. Mycosynthesis deals with an environmentally friendly process that leads to the extracellular synthesis of AuNPs. The *Laetiporus versisporus* were collected from hills of Kodaikanal, Tamil Nadu. The gold source turned into ultrafiner particles which were then computed and monitored through UV-Vis spectrophotometer and Fourier-transform infrared (FTIR) spectroscopy, and the texture of particles was affirmed with the help of peaks originated in X-ray diffraction (XRD). Transmission electron microscopy (TEM) techniques and scanning electron microscope (SEM) unveiled spherical AuNPs, and the overall scale of which was figured as 10 nm. Various concentrations of AuNPs by the concentration of extracted *Laetiporus versisporus* were evaluated for antioxidant activity using the standard in vitro methods like ferrous ion chelating effect, nitric oxide (NO) scavenging assay, and DPPH assay activity.

1. Introduction

Mushrooms, an omnipresent creature, are macrofungi, which can be found in the woodland and be cultivated on farmland. Some types of mushrooms like *Lentinus edodes*, *Lentinus polychrous*, *Pleurotus ostreatus*, *Pleurotus florida*, and *Cordyceps militaris* have medicinal benefits like targeting and defeating the cancer cells and proteins, antioxidant, antimicrobial, anti-diabetic, antihypercholesterolic, and antiarthritic activities [1].

Antioxidants have an extraordinary contribution in promoting the well-being of human by depleting the free radicals when they are supplemented every day. In recent days, there is an attentiveness towards the realization of antioxidant capacity of food stuffs. Edible mushrooms contain immense primary and secondary metabolites, which are responsible for their nutritional and medicinal properties. Antioxidants say an alkaloid, flavonoid, steroid, protein, or an amino acid prevent oxidative stress caused by the unstable compounds,

i.e., free radicals [2]. However, in recent years, interest in the biological and pharmacological activities of mushrooms is grown. Nanotechnology deals with the nanomaterials derived from natural resources and their impact with the same. It is a field that connects various networks of science and technology. Nanoparticles are the sole representatives in nanoworld, and their application in diverse fields makes them more influential. Many nanoparticles are presented, and among those nanoparticles, the gold nanoparticles (AuNPs) have many benefits. A present-day research finds the fact that the nanoparticles synthesized biologically are preferred as it is integrated with the phytoconstituents [3]. The nanoparticles have been widely used in high tech implementation [4]. Noble metal nanoparticles like silver, platinum, gold, and lead are explored and reviewed by various researches earlier [5, 6]. Among the abovesaid metal nanoparticles, the gold nanoparticle is considered to be vital due to the medicinal use ranging from cancer to arthritis dated back in history [7]. Also, their telescoping synthesis, with great biocompatibility and apoptosis with promising applications especially in the development of novel antibacterial agents, makes AuNPs more powerful [6, 8]. Not only in tumor targeting and imaging, gold nanoparticles (GNPs) made an impact in electronic applications, photothermal therapy, and photonics due to their unique surface plasmon resonance effect [9]. This individuality makes them more enhancing in the fields of medical science, bioengineering, and industrial applications [10]. AuNPs extend their implementation in endocytosis, chemical catalysts, gene expression, pharmacokinetics, sensors, and fuel cells [11]. Above all, gold is time honored as it has uses as remedial, restorative, and ornamental roles since primitive days. "Soluble gold" is a phrase which was pioneered in two different countries in different continents: Province of China during the 4th century and Egypt in the 5th century BC [12].

Gold nanoparticle (AuNP) obtained from the plants is a customary method that produces ultrafine particles, which have discrete benefits in the desired fields [13]. Physical and chemical methods of synthesizing metal nanoparticles often lead to some hazardous results such as the toxicity and excess time taken to get the end product, though the nanoparticles are almost pure and stable. To overcome the disadvantages of unconventional methods, living organisms, their components, and byproducts are being used for the production of nanoparticles that has steady and firm activity in an energy efficient way [14]. Exploiting natural resources to produce nanomaterials is quite common in today's world which is concise with the use of chemicals and that too are not dangerous [15]. The green mushroom nanoparticles were investigated as antimicrobial, antioxidant, and antitumor agents [16]. Using mushrooms in the formation of metallic nanoparticles, lean on the fruiting bodies, mycelia, and enzymes that are produced and nurtured in massive quantity and also in prescribed laboratory conditions [17]. As the nanoparticles were reared by some chemical and physical means, their usage in health science is limited due to the ill effects given out by them [18]. Several studies examined the antioxidant activity raised by the gold nanoparticles proved to be evident in different concentrations and at varied environmental factors [19]. This research

study presented the mycosynthesis of gold nanoparticles and a comparative analysis of different antioxidants.

Balakumaran et al. [20] described the antimicrobial properties of gold and silver nanoparticles from *Aspergillus terreus* (soil and food mold) harvested from hills located in the southern region of India. The reaction conditions were optimized, for the mushroom *Aspergillus terreus* strain Bios PTK 6 to develop nanoparticles that are highly firm and sturdy in a day or less. FTIR analyses gave a detailed idea about the proteins that were bound and capped during the reaction. The nanoparticles were responsible for the deterioration of bacterial cells which was portrayed in SEM image showing the damage of bacterial membrane of human pathogens *Staphylococcus* and *Bacillus* species [21] and concluded that the obtained nanoparticles have antimicrobial activity as that of the antibiotic which was already used against the growth of those pathogens.

Madakka et al. [22] reported *Fusarium* and *Aspergillus* species were indulged in the generation of silver nanoparticles extracellularly. Maximum absorption spectra were obtained between 420 and 450 nm in UV-visible spectroscopy, whereas the nonclustered spherical conformation was illustrated in SEM image. Nanoparticles obtained from fungi inhibit the growth of bacteria like *E.coli* and *Staphylococcus* species and *Pseudomonas* species with a great pharmacokinetic activity thereby promoting the silver nanoparticles to the next level in pharmacology.

A perennial plant, *Coleus forskohlii*, was utilized by [23] to show its yield of gold and silver nanoparticles in one pot synthesis method so as to avoid the drawback of synthetic resources. The crystal structure, size, and shape of the newly formed gold (AuNPs) and silver nanoparticles (AgNPs) were studied characteristically. UV-visible spectrophotometer followed the principle of SPR which then leads to the display of maximum band. High-resolution transmission electron microscopy (HR-TEM) and particle size analysis (PSA) led out the size of particles. Fourier-transform infrared spectroscopy (FTIR) exposed the active molecules behind the reduction of colloids and ions to nanoparticles. GC-MS analysis performed in the sample gave a clear picture to understand the phytoconstituents present in the plant. Moreover, DPPH assay deduced the antioxidant ability. Nanoparticles showed apoptotic effect by resisting the growth of liver cancer cells that was determined by formation of formazan crystals via MTT assay.

Certain polymers were also used by some researchers to undergo the synthesis of metal nanoparticles. Shuai et al. [24] carried out a procedure which supports the production of gold nanoparticles from chitosan being the reducer. Deacetylation and molecular weight were the major factors during the course of the reaction. At 0.1% of concentration of reaction mixture, they tend to give polygonal nanoparticles. Assays like ABTS, DPPH, and FRAP were done in order to find the capability of antioxidants in the sample and the novel nanoparticles generated from chitosan. It was understood that the antioxidant activity and the shape of the nanoparticles were correlated with each other. Spherical gold nanoparticles and gold colloids with 0.3% of concentration exhibited maximum antioxidant activity rather than the others.

Cornus mas, an ornamental and adaptable shrub, has the potential of serving as a reducer and stabilizer in the process of getting metallic nanoparticles at room temperature. Filip et al. [25] investigated the relative analysis of polyphenols from the shrub. Size and shape of the nanomaterials were examined by individual medium for characterization. Anti-inflammatory level of the coinage metal nanoparticles is inspected in paw tissues of Wistar rats at different intervals: 2 hours, 24 hours, and 48 hours. A partial response was observed by the nanoparticles, and the anti-inflammatory activity was similar to that of the carrageenan injected into the tissues.

Saraschandra and Li [26] found that *Actinidia deliciosa* (kiwi fruit) has profound bioactive compounds that reduce 4-nitrophenol and methylene blue during the formation of Au and Ag nanoparticles that are globular with the range of 7–20 nm particle size and 25–40 nm diameter, respectively. Reduction of chemicals was encouraged by the biomolecules present in the fruit that was confirmed by FTIR analysis. Face-centered cubic and crystalline structure was elucidated by the analytical techniques such as XRD, EDAX, and XPS for AgNPs as well as AuNPs.

2. Materials and Methods

The research study presented the mycosynthesis of AuNPs and a comparative analysis of different antioxidants. The pictorial representation for the present research is given in Figure 1.

2.1. Preparation of Mushroom Extract. Here, the mushrooms (*Laetiporus versisporus*) were freshly collected from the hills of Kodaikanal, Tamil Nadu. They are often wiped in times by double-purified water to get rid of the impurities present on the surface. The mushrooms were chopped into small chunks, shade dried, and powdered. It was then allowed to boil for a while, and the extract was filtered, separated, and allowed to cool at room temperature.

2.2. Biosynthesis of Gold Nanoparticles. The freshly prepared mushroom extract was added to the desired concentration of gold chloride solution and set aside in a dark place together with the control as it was the optimal condition for the reaction to occur. The color change was observed visually. The sample was then centrifuged at 8000 rpm for about 10 minutes. The water content in the resultant precipitate was evaporated when it was placed in a hot air oven for 24 hours, which leads to the formation of gold nanoparticles in powder form.

2.3. Evaluation of Antioxidant Potential. Here, the antioxidant activity is evaluated by the ferrous ion chelating effect, nitric oxide scavenging activity, and scavenging activity by 2,2'-diphenyl-1-picrylhydrazyl.

2.3.1. Ferrous Ion Chelating Effect. The chelating of ferrous ions was estimated by the method used by Puntel et al. [27]. 1 ml of the methanolic extract in a proportion of 50 to 250 $\mu\text{g/ml}$ with an increasing degree of 50 $\mu\text{g/ml}$ was formulated. To that, 0.2 ml of 2 mM FeCl_2 and 0.25 ml of 5 mM ferrozine were put on and kept undisturbed at normal temperature for 10 min to the incubated mixtures, 1.5 ml of

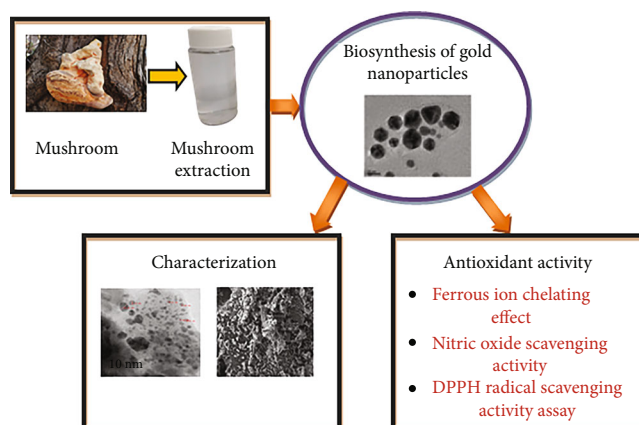


FIGURE 1: Pictorial representation for the present research study.

double distilled water was added, and the absorbance was measured at 562 nm. A triplicate of this assay was done parallel. EDTA was used as a standard to denote the changes in absorbance and chelation. The lowest OD values at 562 nm represent a greater chelation. Percent of chelation by the ferrous ion chelating assay is expressed by the following:

$$\left[1 - \left(\frac{\text{Absorbance of test sample}}{\text{Absorbance of blank}} \right) \right] \times 100. \quad (1)$$

2.3.2. Nitric Oxide Scavenging Activity. This method was proposed and inspected by Alderton et al. [28]. Sodium nitroprusside undergoes reaction in aqueous solution at pH of 7.4 to form nitric oxides. 1 ml of sodium nitroprusside at a concentration of 10 mM in phosphate-buffered saline was prepared, and it was mixed with increasing concentrations of mushroom extract (50, 100, 150, 200, and 250 $\mu\text{g/ml}$). For the next three hours, incubation occurred at 25°C. Meantime, an equal volume of 1% sulphanilamide, 0.1% naphthyl ethylenediamine dichloride, and 3% phosphoric acid were mixed to get Griess reagent (1 ml). Later, the incubated solution and Griess reagent were put together, and the optical density was studied at 546 nm. Similar procedure was followed for gold nanoparticles and standard ascorbic acid in which the mushroom sample was replaced by the nanoparticles and standard. The percentage of inhibition was determined using the formula:

$$\begin{aligned} &\text{Percentage of scavenging effect} \\ &= \left[\frac{(\text{Absorbance of control} - \text{Absorbance of sample})}{(\text{Absorbance of control})} \right] \times 100. \end{aligned} \quad (2)$$

2.3.3. DPPH Radical Scavenging Activity Assay. Brand-Williams and Berset [29] defined the standard procedure of DPPH analysis for determining the scavenging potential of mushroom samples in addition to the nanoparticles in a culture dish [29]. A stock solution of 24 mg DPPH in 100 ml of ethanol was prepared and preserved at 20°C for the consequent use. 3 ml aliquot of this solution was mixed with 1 ml of freshly prepared mushroom extract and gold nanoparticles in the order of 20 $\mu\text{g/ml}$ from 10 to 100 $\mu\text{g/ml}$. The test

samples along with the working solution were agitated thoroughly and placed at 37°C in an unilluminated area for about 15 minutes, and the OD was recorded at 517 nm. Likewise, a control tube was prepared with all ingredients except the test sample. Scavenging activity was evaluated depending on the rate of DPPH traced out which is calculated by the formula:

$$\begin{aligned} &\text{Percentage of inhibition} \\ &= \left[\frac{(\text{Optical density of control} - \text{Optical density of sample})}{(\text{Optical density of control})} \right] \times 100. \end{aligned} \quad (3)$$

3. Results and Discussion

In this section, the experimental analysis is carried out for this study. Here, the characterization of gold nanoparticles followed by the antioxidant activity is analyzed.

3.1. Characterization of Gold Nanoparticles. Current research connects the world of mushrooms with nanotechnology by the way of adapting mushroom *Laetiporus versisporus* in the formation of gold nanoparticles. Though the mushrooms are excellent source of nutrients and other metabolites, they support the biosynthesis perfectly. Appearance, physical model, and structural elucidation of the mushroom mediated gold nanoparticles are assessed by ultraviolet-visible (UV-Vis) spectroscopy, X-ray diffraction (XRD) patterns, Fourier-transform infrared (FTIR) spectroscopy, scanning electron microscope (SEM), and transmission electron microscope (TEM). Characterization of gold nanoparticles clearly says that they are crystalline with 10 nm in size. The size and composition of nanoparticles were characterized by different techniques and were reviewed by [30].

Figure 2 displays UV-visible spectroscopy of gold nanoparticles. The concrete evidence for the presence of gold nanoparticles was clearly understood by the colour change from yellow to purple and then deep purple that is further certified by UV-Vis spectroscopy studies. Colour variation is not concise, and some reactions result in an array of same colours [31]. Visual conformation was the first spotting in the current study followed by several other techniques that prop up the existing data. Transformation of colour from pale yellow to dark brown was observed in the mushroom *Inonotus obliquus* which showed the maximum spectra at 532 nm via UV-visible spectroscopy [32]. Some AuNps obtained from plants exhibit the surface plasmon which is greater than 570 nm [33]. Different proportions of ethanol used for the purpose of production of gold nanoparticles relate with the peak formed at different wavelengths, i.e., below 520 nm for shorter spectra and above 560 nm for a greater peak [34]. A change in the number of electrons possibly leads to a fluctuation in the plasmon resonance through which the fact of presence of nanoparticles comes true [35]. This spectroscopy helps to understand the maximum absorption spectra of gold nanoparticles. It was noted that it has high spectra at 566 nm.

Structural specification and characterization were executed by XRD analysis, and the outcome of the XRD pattern

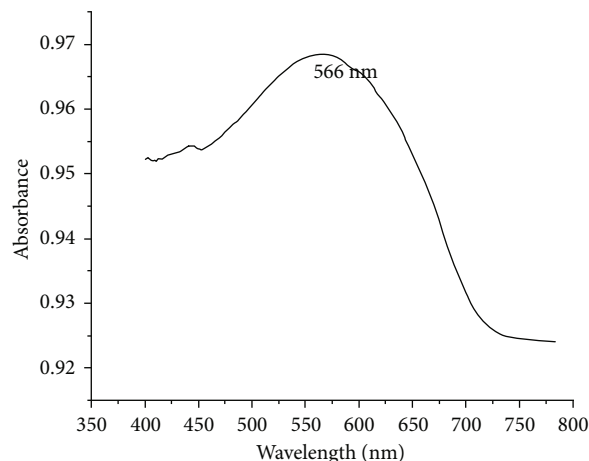


FIGURE 2: UV-Vis spectroscopy of mushroom-synthesized gold nanoparticles.

for gold nanoparticles synthesized from *L. versisporus* is displayed in Figure 3. Dehydrated and dried gold nanoparticles were examined by X-ray diffraction (XRD) patterns to ascertain and also confirm those peak intensity, corresponding position, and width of the peak. XRD analysis exhibits the diffraction peaks at 38.2° and 44.3° that correspond to the crystalline structure of gold (JCPDS no. 04 0784). X-ray diffraction studies provide information about the texture of the particles. The crystalline or amorphous nature can be easily detected with the help of XRD data. The XRD peaks obtained for *L. versisporus*-mediated AuNps are 38.2° and 44.3° that are quite similar to the crystalline nature of *Agaricus bisporus*-engaged gold nanoparticles studied by [36] where they found the 2 theta values at 38.18°, 44.38°, 64.56°, and 77.55°. The exact similar 2 theta values were observed in the study of gold nanoparticles obtained from aqueous extract of *Dolichos biflorus* [37]. Another four high peaks were observed in the XRD pattern of gold nanoparticles synthesized from marine organism [38]. Not only the mushrooms, AuNps extracted from actinomycetes [39], plants [40], and yeast [41] too which had the similar XRD patterns.

Figure 4 shows the FTIR analysis of gold nanoparticles. Fourier-transform infrared (FTIR) spectroscopy measurements are usually carried out to identify the functional groups of bioactive molecules that are accountable for capping and reducing gold ions to gold nanoparticles and ultimately maintaining the stability of them. FTIR bands were intense at 3444 cm⁻¹, which shows the O-H stretch of alcohols and phenols. Fusarium-mediated AuNps showed a band at the similar point which was noted as O-H stretch [12]. Band at 1634 cm⁻¹ conveys the C=O stretch of amide linkage of protein. This can be correlated with the work done in gold nanoparticles originated from *Pseudomonas species* [42] and citrate capped AuNps [43]. Also, the bands at 1557 cm⁻¹ and 1417 cm⁻¹ corresponds to the N-H bend of proteins which was discussed earlier for the nanoparticles got from penicillin [44]; thus, the entire spectra showing that the gold ions were capped majorly by the alcohols and phenols present in the mushroom that were reduced to gold nanoparticles. FTIR spectra delineate the bioactive compounds which were actively participated in

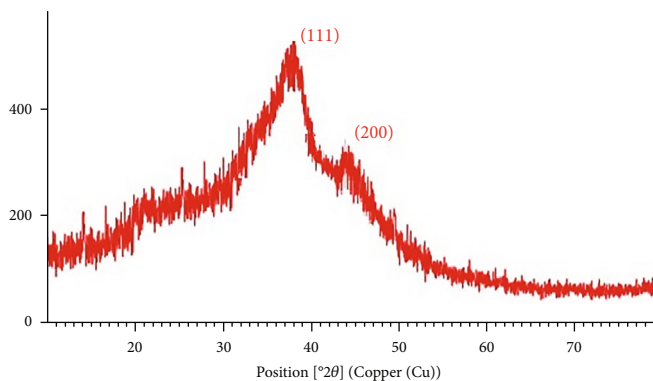


FIGURE 3: XRD of gold nanoparticles.

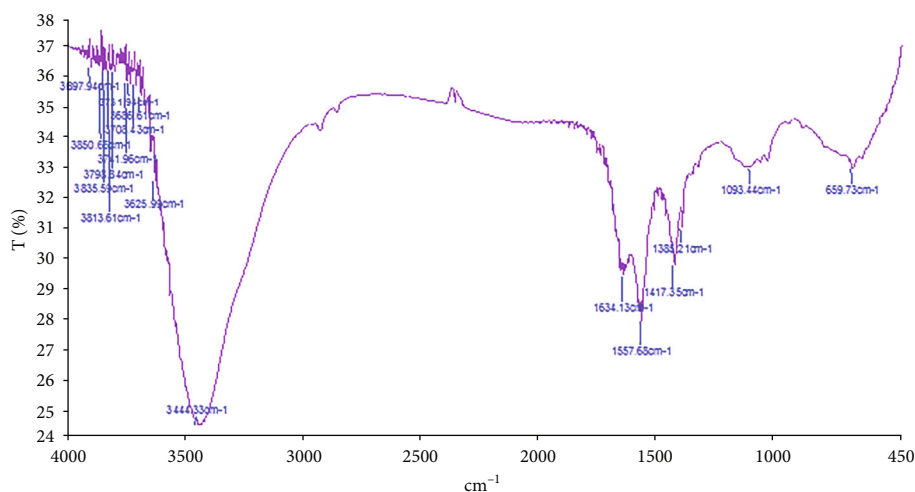


FIGURE 4: FTIR of gold nanoparticles.

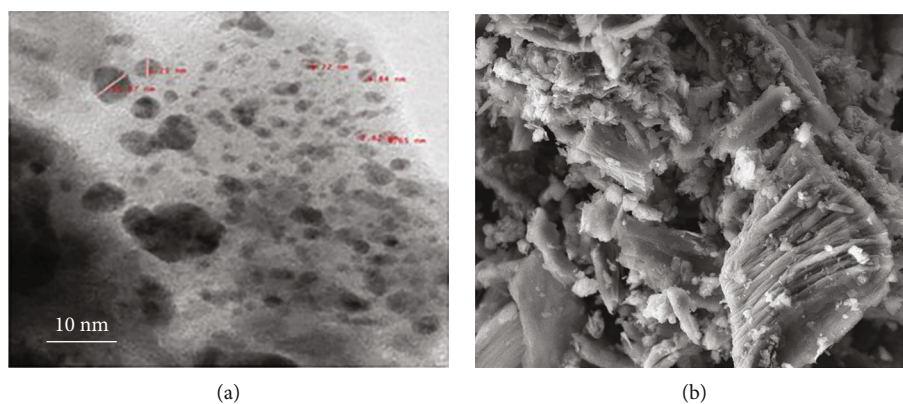


FIGURE 5: Analysis of gold nanoparticles by (a) TEM image and (b) SEM image.

the transmission process that resulted in the gold nanoparticles. Peaks appeared from 400 to 4000 cm^{-1} in which the peak at 3444 cm^{-1} shows OH stretching of alcohols and phenols that has an evidence of both N-H as well as the OH stretches of alcohol and phenol groups in aqueous extract of *Alpinia nigra*. The same showed peak at 1623 cm^{-1} which is in connection with 1634 cm^{-1} [35]. The nutraceutical study of *Laetiporus sulphureus* was reported by [45] indicating the presence of carbo-

hydrates, proteins, and fat in the decreasing order. A relevant study done by [46] explains the bioactive metabolites present in *Laetiporus versisporus*. The species of *Laetiporus* composing these metabolites are responsible for the biochemical conversion which took place in the current study.

Figure 5 displays the analysis of gold nanoparticles by (a) TEM image and (b) SEM image. SEM and TEM analyses of gold nanoparticles showed the size and shape as 10 nm on an

TABLE 1: Ferrous ion chelating assay.

Sample conc. ($\mu\text{g/ml}$)	Absorbance measurement data						
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous	AuNps	Standard (EDTA)
50	7.3	8.2	9.8	21.3	21.6	21.2	22
100	12	13.5	11.5	26.8	27.5	26.4	26.3
200	15.8	17.4	20.2	35.5	32	34.7	35.6

average of spherical particles. AuNps of size lesser than 10 nm were isolated via self-assembly [13]. Transmission and scanning electron microscopy generally conveys the size and shape of the particles. Gold nanoparticles obtained here are 10 nm in size, and most of them show a spherical shape. Not only in the ecofriendly mechanism but also the chemical and physical modes of obtaining AuNps were previously studied by nanotechnologists. Spherical gold nanoparticles of diameters range 7-35 nm were obtained by the use of sodium citrate and sodium citrate/borohydride in citrate pathway [47]. On the other hand, saprophytic fungi acted as good reducers in the formation of polydispersed gold nanoparticles in a size range of 20–40 nm [48].

3.2. Analysis of Antioxidant Potential. Here, the antioxidant potentials were analyzed by the ferrous ion chelating effect, nitric oxide scavenging activity, and DPPH radical scavenging assay. These are the routine methods which were used earlier and still in use for observing the antioxidant activity of different live systems such as plants [31] and mushrooms [49]. Antioxidant nature of mushroom was found out quantitatively along with the mushroom-mediated gold nanoparticles. Assays to estimate the quantity and percentage of activity done by the antioxidants to chase the free radicals were different, and some common assays [50] like ferrous ion chelating assay, nitric oxide scavenging assay, and DPPH assays in association with earlier works that carried out these assays in food stuffs [51] are done here.

3.2.1. Ferrous Ion Chelating Effect. Here, the ferrous ion chelating effect is analyzed. It measures the capability of test samples to chelate-free ferrous ions existing in the sample solution.

Table 1 shows the ferrous ion chelating assay. The metal chelating assay involves colour reduction, which determines the chelating ability of the synthesized nanoparticles for ferrous ions. Here, three sample concentrations considered are 50, 100, and 200 $\mu\text{g/ml}$. For each sample concentration, the percentage of chelation is varied. This is quite similar to the studied conducted in methanolic extracts of mushrooms: *Lactarius semisanguifluus*, *Lactarius deliciosus*, *Lactarius sanguifluus*, *Russula delica*, and *Suillus bellinii*. *Lactarius* and *Russula* species exhibited major chelation, i.e., above 40 μmol ferrous ions/100 gm of the source, whereas the *Suillus* species had a chelating range of less than 30 μmol ferrous ions [52]. The graphical plot for the ferrous ion chelating assay is shown in Figure 6.

Table 2 shows the average IC₅₀ obtained from ferrous ion chelating assay. Here, the average value is higher for the ethyl acetate and hexane sample, i.e., 10.08 and

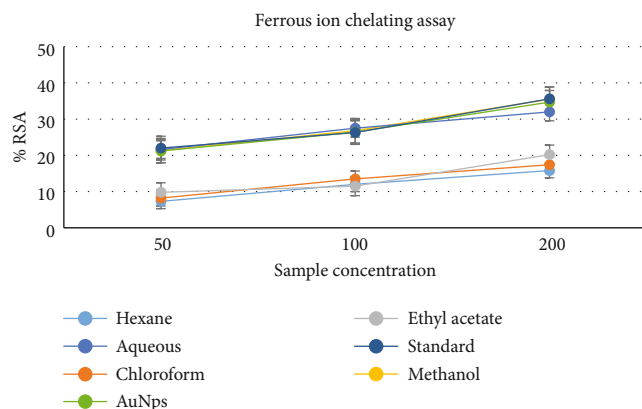
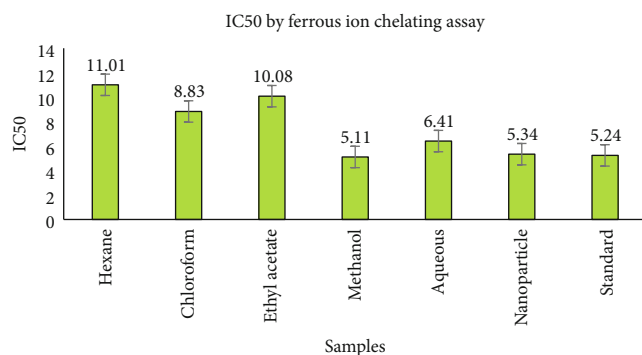


FIGURE 6: Analysis of ferrous ion chelating assay.

TABLE 2: Average IC₅₀ ferrous ion chelating assay.

Sl. no.	Sample	Average IC ₅₀ ($\mu\text{g/ml}$)
1	Hexane	11.01
2	Chloroform	8.83
3	Ethyl acetate	10.08
4	Methanol	5.11
5	Aqueous	6.41
6	Nanoparticle	5.34
7	Standard (EDTA)	5.24

FIGURE 7: Pictorial plot for the average IC₅₀ of ferrous ion chelating assay.

11.01 $\mu\text{g/ml}$, respectively. Thirdly, the chloroform sample has the IC₅₀ average of 8.83 $\mu\text{g/ml}$. The remaining samples such as methanol, aqueous, nanoparticle, and standard (EDTA) are lower than the above said samples which

TABLE 3: Nitric oxide scavenging assay.

Sample conc ($\mu\text{g/ml}$)	Absorbance measurement data						
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous	AuNps	Standard (ascorbic acid)
50	15.7	14.4	10	20.3	21.1	21.2	27.1
100	22.4	23.8	17.6	34.6	31.6	33.5	39.2
200	25.6	28.8	27.8	53.6	54.7	54.8	63.4

describes that they have higher antioxidant activity. The pictorial plot for the average IC₅₀ ferrous ion chelating assay is shown in Figure 7.

Ferrous ion chelating assay was considerably used for the estimation of varied antioxidants that are seen in both body fluids and edibles [53]. The activity of methanol extract is more or less similar to the standard of same concentration followed by AuNps and aqueous extract of *L. versisporus*. IC₅₀ values of those were low compared to the other extracts, and this was correlated with the IC₅₀ values of edible mushroom, and *Cantharellus cibarius* was evaluated as 4.324 $\mu\text{g/ml}$ [54].

3.2.2. Nitric Oxide Scavenging Activity. In this subsection, the nitric oxide scavenging activity is analyzed. Nitric oxide scavenging assay being one of the antioxidant tests is done in bioproducts by silver nanoparticles [55]. The nitric oxide emerged from sodium nitroprusside was computed by the Greiss reaction, and the absorbance was tabulated.

Table 3 shows the nitric acid scavenging assay. Here, the nitric acid scavenging activity of nanoparticles was directly proportional to the sample concentration. For the sample concentration of 50 $\mu\text{g/ml}$, the RSA value varies between 15 and 27 RSA. The percentage of scavenging NO at 200 $\mu\text{g/ml}$ of hexane, ethyl acetate, chloroform, methanol, aqueous, nanoparticles, and standard (ascorbic acid) was found to be 25.6%, 28.8%, 27.8%, 53.6%, 54.7%, 54.8%, and 63.4% which is conveyed in either way as standard (ascorbic acid) > nanoparticles > aqueous > methanol > hexane > ethyl acetate > chloroform for all the sample concentrations. 100 to 1000 $\mu\text{g/ml}$ of different mushrooms were tested for nitric oxide scavenging and found that *Pleurotus floridanus* made a greater percentage of scavenging (44-64%) comparatively. The graphical plot for the nitric acid scavenging assay is shown in Figure 8,

Table 4 displays the average IC₅₀ of nitric acid scavenging assay. Here, the order of the samples are chloroform > ethyl acetate > hexane > methanol > aqueous > nanoparticle > standard (ascorbic acid), and hence, it is understood that methanol, aqueous extract, and nanoparticles have an antioxidant activity equivalent to the ideal and default ascorbic acid that is uniformly used as a standard component. The pictorial representation of Table 3 is shown in Figure 9,

The prime active participants of all the samples via nitric oxide assay are same, whereas the AuNps showed much more scavenging as that of the standard rather than the others. IC₅₀ values are lesser than 10 $\mu\text{g/ml}$. Scavenging activity of *Boletus edulis* was done by three different assays, and the IC₅₀ value was noted as 10.74 $\mu\text{g/ml}$ in nitric oxide scavenging assay [56].

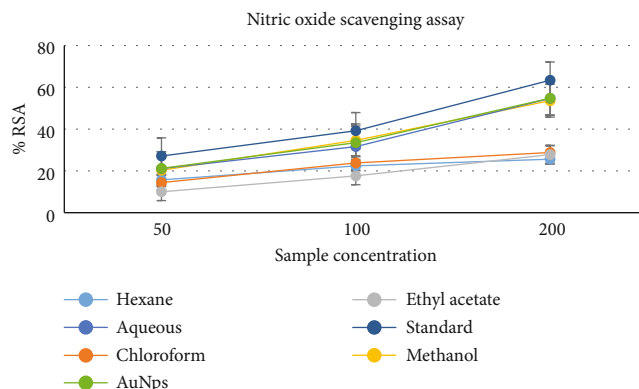
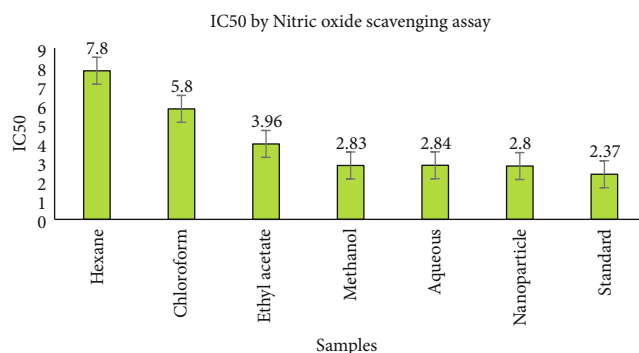


FIGURE 8: Graphical plot for the nitric acid scavenging assay.

TABLE 4: Average IC₅₀ nitric acid scavenging assay.

Sl. no.	Sample	Average IC ₅₀ ($\mu\text{g/ml}$)
1	Hexane	3.92
2	Chloroform	5.54
3	Ethyl acetate	3.96
4	Methanol	2.83
5	Aqueous	2.84
6	Nanoparticle	2.8
7	Standard (ascorbic acid)	2.37

FIGURE 9: Pictorial plot for the average IC₅₀ of nitric acid scavenging assay.

3.2.3. DPPH Radical Scavenging Activity Assay. Here, the DPPH radical scavenging activity of antioxidants is evaluated. Radical scavenging activities happen naturally in the human body to shut out the free radicals intervene in extensive amount of ailments and dreadful diseases. DPPH free

TABLE 5: DPPH radical scavenging activity assay.

Sample conc. ($\mu\text{g/ml}$)	Absorbance measurement data						
	Hexane	Chloroform	Ethyl acetate	Methanol	Aqueous	AuNps	Standard (ascorbic acid)
50	13.8	6	9.1	21.2	20.5	21.4	23.5
100	23.8	13	16.4	31.8	31.9	31.4	33
200	44.1	27.3	31.5	52.2	51.4	52.3	54.6

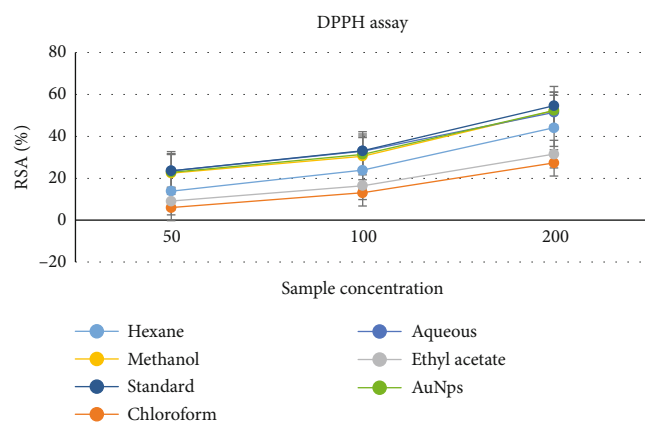


FIGURE 10: Analysis of DPPH assay.

radical scavenging is a customary technique for scanning the radicals that cause cell death.

Table 5 displays the RSA of different sample concentrations using DPPH radical scavenging activity assay. Here, the standard (ascorbic acid) is higher than the other samples that are 23.5% for 50 $\mu\text{g/ml}$, 33% for 100 $\mu\text{g/ml}$, and 54.6% for 200 $\mu\text{g/ml}$ which was similar to that of methanol, aqueous, and gold nanoparticles at lower concentrations. A similar activity was found in the mushrooms *Agaricus* species, *Lentinula* species, and *Flammulina* species, i.e., range from 13 to 50 $\mu\text{mol TE/g}$ [57]. The remaining samples have lesser DPPH activity than the standard sample. The graphical plot is represented in Figure 10.

The average IC₅₀ value by using the DPPH assay activity is displayed in Table 6. The order of the performance by average value is given as ethyl acetate > chloroform > hexane > methanol > aqueous > standard (ascorbic acid) > nanoparticle. Table 6 is presented as a figure format which is shown in Figure 11.

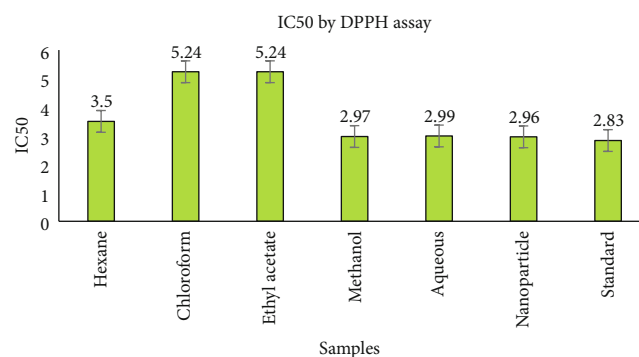
DPPH assay is used to explore the antioxidant ability of AuNps and methanol extract. IC₅₀ values extend from 2.8 to 5.4. *Trametes versicolor* exposed an IC₅₀ value of 5.6 $\mu\text{g/ml}$ by DPPH assay [30] (that is considerably similar to that of *L. versisporus* extracts, which means they possess better antioxidant activity).

Gold nanoparticles obtained from *Nerium oleander* leaf extract [58] showed remarkable increase in the antioxidant activity with the increase in concentration of the AuNps which is comparable to the current project that portrays the competent antioxidant activity of mushroom along with the gold nanoparticles.

Tables 1, 3, and 5 show a linear and steady correlation eventuated with the concentration of samples and radical

TABLE 6: Average IC₅₀ DPPH assay.

Sl. no.	Sample	Average IC ₅₀ ($\mu\text{g/ml}$)
1	Hexane	3.5
2	Chloroform	5.24
3	Ethyl acetate	5.24
4	Methanol	2.97
5	Aqueous	2.99
6	Nanoparticle	2.96
7	Standard (ascorbic acid)	2.83

FIGURE 11: Pictorial plot for the average IC₅₀ of DPPH assay.

scavenging in all the groups of ferrous ion chelating assay, nitric oxide scavenging assay, and DPPH assay. Furthermore, a remarkable and gradual rise ($p < 0.05$) of antioxidant activity of *L. versisporus* and nanoparticles is obtained from the mushroom along with the standard.

4. Conclusion

Although mycosynthesis of nanoparticles was implemented in the field of research involving living systems, an attempt with *Laetiporus versisporus* mushroom to get gold nanoparticles is unfamiliar. Comparatively, mushrooms make a suitable option for the generation of metallic nanoparticles as they are maneuverable and are loaded with various metabolites especially proteins thereby improving the productivity. The present study throws lights on the biological procedure for the production of AuNPs by using *Laetiporus versisporus* and the antioxidant activity. The gold nanoparticle of the current study undergone series of analytical studies including UV-Vis and FTIR spectroscopy. The size of particles was confirmed by XRD, TEM, techniques, and SEM. In UV-Vis spectrometer analysis, the maximum absorption

was found at 566 nm which lies on the standard range of gold nanoparticles, and FTIR gives a list of functional groups belonging to the biomolecules present in the mushroom. In the TEM and SEM analysis, the spherical structure was found and XRD analysis exhibits the diffraction peaks at 38.2° and 44.3°. In antioxidant analysis, six samples along with the standard were carried out that are hexane, chloroform, ethyl acetate, methanol, aqueous, nanoparticle, and standard (EDTA and ascorbic acid) to decipher the expected results. In all the antioxidant activity analyses, the standard attains higher RSA that was quite near with the methanol, aqueous, and gold nanoparticles. The lower IC50 values show a higher antioxidant activity. Thus, results conclude that the *Laetiporus versisporus* extraction and its synthesized AuNPs showed high antioxidant activity. Hence, it can be used in many biological and pharmacological applications.

Data Availability

All the experimental data are included in the manuscript. Hence, there are no other relevant data to be made available.

Conflicts of Interest

The authors declare no conflict of interest.

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