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Original Article

Antioxidant Assay of Gold and Silver Nanoparticles from Edible Basidiomycetes Mushroom Fungi

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ABSTRACT

Background: In recent years, nanotechnology research is emerging as cutting edge technology interdisciplinary with physics, chemistry, biology, material science and medicine. AuNPs and AgNPs are an important class of nanomaterial for a wide range of biomedical applications. Synthesis of AuNPs and AgNPs through biological route is preferred due to its environment-friendly and economic aspects. Mushrooms have been known for their nutritional values and used as medicines by humans for ages. In modern terms, they can be considered as functional foods which can provide health benefits beyond the traditional nutrients. The present study demonstrates an eco-friendly and low-cost method of biosynthesis of AuNPs and AgNPs using basidiomycetes mushroom fungal strains. **Materials and Methods:** The antioxidant potential of the basidiomycetes mushroom fungal strains was analysed by total flavonoid content, FRAP assay, ABTS assay, Metal chelating activity, Phosphomolybdenum assay, Assay of superoxide radical scavenging activity, Free radical scavenging activity on DPPH along with the determination of total phenolic and tannin contents in the mushroom mycelial extracts. **Results:** The synthesized gold nanoparticles (AuNPs) silver nanoparticles (AgNPs) were confirmed by the colour transformation and Ultraviolet-visible (UV-visible) spectroscopy. These biologically synthesized AuNPs and AgNPs were tested for antioxidant activities. The biosynthesized AuNPs and AgNPs showed significantly higher antioxidant activity. **Conclusion:** The present study explored that the mushrooms which are efficient producers of AuNPs and AgNPs, and could act as safe and cost-effective with potential antioxidant activities. These findings encourage studying these fungal strains further for their potential biological applications.

Key words: Flavonoid, Tannin, FRAP, ABTS, DPPH, Free Radical.

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INTRODUCTION

Nanotechnology (NT) is a rapidly progressing field. The emergence of nanotechnology platforms can enable development and commercialization of entirely new classes of bioactive macromolecules that need precise intracellular delivery for bioactivity. The ability to uncover the structure and function of biosystems at the nanoscale stimulates research leading to improvement in biology, biotechnology, medicine and healthcare. The size of nanomaterials is similar to that of most biological molecules and structures; therefore, nanomaterials can be useful for both *in vivo* and *in vitro* biomedical research and applications.[1] The integration of nanomaterials with biology has led to the development of diagnostic devices, contrast agents, analytical tools, physical therapy applications, and drug delivery vehicles.[2] Applications of nanotechnology to medicine and physiology imply materials and devices designed to interact with the body at subcellular (i.e., molecular) scales with a high degree of specificity. This can be potentially translated into targeted cellular and tissue-specific clinical applications designed to achieve maximal therapeutic efficacy with minimal side effects.[3]

Nanoparticles are gaining interest in the field of nanodrug delivery systems without harming the cells of the body organs. Hence, there is a need to develop green chemistry approaches in the synthesis for the nanomaterials. In this aspect, synthetic methods based on naturally occurring biomaterials are the alternative eco-friendly method.[4] The synthesis of noble metal nanoparticles and their description attracts an increasing interest in the field of nanotechnology because of their potential applications in various fields such as biotechnology, chemistry, physics and medicine. Among several nanoproducts, the most prominent nanoproducts are nanosilver and nanogold. They have been used for antimicrobial, antioxidant, anti-diabetic and anti-hemolytic effects.[5]

Silver nanoparticles (AgNPs or nanosilver) have attracted increasing interest due to their unique physical, chemical and biological properties compared to their macro-scaled counterparts.[6] Silver nanoparticles can be prepared using chemical, physical and biological methods but unlike biological synthesis for chemical and physical methods under high temperature, pressure, chemical solvent and capping agents are required for the preparation of nanoparticles.[7] In case of biologically synthesized nanoparticles microorganisms (bacteria and fungi) and plants are often used for synthesis of eco-friendly nanoparticles both in extracellular and intracellular process.[8]

Gold nanoparticles (AuNPs) provide a versatile surface chemistry and thus can be easily functionalized with ligands that specifically interact with receptors on the surface of target cells. Compared to other metals, metallic gold is highly inert and is regarded nontoxic in nature due to its low chemical reactivity. Synthesis of gold nanoparticles has gained great significance during the last few years due to biological properties. In biological methods, nanoparticles are synthesized using microorganisms (bacteria, fungi, algae) and plant extracts.[9] Biosynthetic processes have received much attention as a viable alternative for the development of gold nanoparticles where plant extract is used for the synthesis of nanoparticles without any chemical ingredients. Among microbes, fungi are considered as potent nano factories for the synthesis of extracellular gold nanoparticles because of its high metal tolerance, efficient secretion of soluble proteins and other reducing components, easy scale up, economic viability and easy handling. Extracellular synthesis of gold nanoparticles is reported using fungi such as[10] *F. oxysporum*,[11] *Colletotrichum sp*.,[12] *Trichothecium* sp.,[13] *Trichodermakoningii* and[14] *Penicillium sp*. has been reported.[15] Mushrooms are used for a variety of biotechnological applications, particularly for the production of food, enzymes, dietary supplements, pharmaceutical compounds, feed supplements. Basidiomycete medicinal properties originate from various cellular components and secondary metabolites that can be isolated and iden-

tified in the fruiting body, vegetative mycelia, or in the culture broth.[16] Reactive oxygen species (ROS) are generated in the body as by-products of several cellular metabolic reactions; they consist of radical and non-radical oxygen species formed by the partial reduction of oxygen. Low levels of ROS are necessary for cellular processes such as intracellular signaling, cell progression and cell defense. Conversely, high levels of the ROS or the inability of the antioxidant system to regulate ROS levels efficiently results in oxidative stress. Oxidative stress results in direct or indirect ROS-mediated damage of nucleic acids, proteins, and lipids. Consuming dietary antioxidant supplements to fight diseases, especially cancer, has become popular among the general public.[17]

Many studies have concluded that edible mushrooms possess potent antioxidants. It was found that the crude ethanol extract of 150 mushrooms shows the antioxidant activity. An extensive search for traditional plant treatments for diabetes has concluded that recognized edible mushrooms are an ideal food for the dietetic prevention of hyperglycemia.[18] Antioxidative materials are now thought to be prospective protective agents also against wood degrading organisms. Although natural antioxidants such as a-tocopherols and L-ascorbic acid are widely used, investigations are being carried out to discover more potent, safer antioxidants.[19] Antioxidants play a role in the treatment of hypertension and ischemic heart disease.[20] There are also reports on the role of flavonoid, a powerful antioxidant, in analgesic activity primarily by targeting prostaglandins.

In this work, nanoparticles were synthesized from mycelial extracts of the selected mushroom fungi (*Pleurotus citrinopileatus, Pleurotus eous, Pleurotus cystidiosis, Pleurotus ostreatus, Pleurotus eryngii, Pleuoruts flabellatus, Pleurotus florida, Pleurotus pulmonarius*, *Schizophyllum commune*). Antioxidant activities of selected mushroom mycelial filtrates and their nanoparticles were studied. This potentially can lead to novel therapeutic, imaging, and biomedical applications.

MATERIALS AND METHODS

Basidiomycetes Fungi

The pure cultures of basidiomycetes mushroom fungal strains used in the present study were *Pleurotus citrinopileatus, Pleurotus eous, Pleurotus cystidiosis, Pleurotus ostreatus, Pleurotus eryngii, Pleuoruts flabellatus, Pleurotus florida, Pleurotus pulmonarius*, *Schizophyllum commune* and pure cultures were maintained in the Centre for Research and PG studies in Biotechnology, M.G.R. College, Hosur, Tamilnadu, India.

Storage and maintenance of basidiomycetes fungi

Pure cultures of fungi were stored at 10°C. Sub-culturing was performed every month to ensure that the organism remained viable. The mycelial agar plugs (7 mm) from the stock cultures were aseptically transferred to fresh Potato Dextrose Agar medium and then incubated at room temperature (28±2°C)until confluent growth was achieved.

Synthesis of Silver nanoparticles

Aqueous solution (1mM) of silver nitrate was prepared using deionized water for the synthesis of silver nanoparticles. 5 ml of mushroom broth/ extract was added into 95 ml of aqueous solution of 1 mM silver nitrate for reduction into Ag^+ ions and heated on water bath at 75°C for 60 min. Reduction of silver nitrate to silver ions was confirmed by the color change from colorless to brown. The formation of silver nanoparticles was also confirmed by spectrophotometric determination. The fully reduced solution was centrifuged at 5000 rpm for 30 min. The supernatant liquid was discarded and the pellet obtained was redispersed in deionized water. The centrifugation process was repeated two to three times to wash off any absorbed substances on the surface of the silver nanoparticles.

Synthesis of Gold nanoparticles

Aqueous solution (1 mM) of Chloroauric acid ($H[AuCl_4]$) was prepared using deionized water for the synthesis of gold nanoparticles.1.5 ml of mushroom broth/ extract was added into 34 ml of aqueous solution of 1 mM chloroauric acid for reduction into Au^{3+} ions and incubated at 29°C for 24 hrs. Reduction of Chloroauric acid to Au^{3+} ions was confirmed by the color change. The formation of gold nanoparticles was also confirmed by spectrophotometric determination. The fully reduced solution was centrifuged at 6000 rpm for 11 min. The supernatant liquid was discarded and the pellet obtained was redispersed in deionized water. The centrifugation process was repeated two to three times to wash off any absorbed substances on the surface of the gold nanoparticles.

Antioxidant Assay

Estimation of total flavonoid content

The 0.5 ml extract was mixed with 2 ml of distilled water and subsequently with 0.15 ml of a 5% $NaNO_2$ solution. After 6min, 0.15 ml of a 10% AlCL3 solution was added and allowed to stand for 6min, then 2 ml of 4% NaOH solution was added to the mixture .Immediately distilled water was added to bring the final volume of 5 ml, and then the mixture is thoroughly mixed and allowed to stand for another 15 min.Absorbance of the mixture was determined at 510 nm versus a prepared water blank. Rutin was used as a standard compound for the quantification of total flavonoid. All the values were expressed as gram of rutin equivalent (RE) per 100 gram of extract.

Ferric-reducing/antioxidant power (FRAP) assay

FRAP reagent (900l), prepared freshly and incubated at 37°C, was mixed with 90l of distilled water and 30l of test sample or methanol (for the reagent blank).he test samples and reagent blank were incubated at 37°C for 30 in in a water bath. The final dilution of the test sample in the reaction mixture was 1/34.he FRAP reagent contained 2.5 ml of 20 m mol/l TPTZ solution in 40m mol/l HCl + 2.5 ml of 20mol/l $FeCl_3.6\ H_2O$ and 25l of 0.3ol/l acetate buffer (pH 3.6). At the end of incubation the absorbance readings were taken immediately at 593 nm, using a spectrophotometer. Methanolic solutions of known Fe (II) concentration, ranging from 100 to 2000 mmol/l, ($FeSO_4.7H_20$) were used for the preparation of the calibration curve. The parameter equivalent concentration (EC) was defined as the concentration of antioxidant having a ferric-TPTZ reducing ability equivalent to that of 1 m mol/l $FeSO_4.7H_2O$. EC was calculated as the concentration of antioxidant giving an absorbance increase in the FRAP assay equivalent to the theoretical absorbance value of a 1 m mol/l concentration of Fe (II) solution, determined using the corresponding regression equation.

Antioxidant activity by the ABTS assay

ABTS was produced by reacting 7 mM ABTS aqueous solution with 2.4 mM potassium per sulfate in the dark for 12-16 h at room temperature. Prior to assay, this solution was diluted in ethanol (about 1:89v/v) and equilibrated at 30°C to give an absorbance at 734 nm of 0.700±0.02.The stock solution of the sample extracts were diluted such that after introduction of 10 µl aliquots into the assay, they produced between 20% and 80% inhibition of the blank absorbance. After the addition of 1ml of diluted ABTS solution to 10 µl of sample or Trolex Standard (Final concentration 0-15 µ M) in ethanol, absorbance was measured at 30°C exactly 30 min after the initial mixing. Appropriate solvent blanks were also run in each assay. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition was calculated of the blank absorbance at 734 nm and then was plotted as a function of Trolox concentration. The unit of total antioxidant activity (TAA) is defined as the

concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts on dry matter.

Metal chelating activity

Briefly the extract samples (250 µl) were added to a solution of 2 mmol/l $FeCl_2$ (0.05 ml).The reaction was initiated by the addition of 5 mmol/l ferrozine (0.2 ml) and the mixture was shaken vigorously and left standing at room temperature for 10 min. Absorbance of the solution was then measured spectrophotometrically at 562 nm.The chelating activity of the extracts was evaluated using EDTA as standard. The results were expressed as 1 mg EDTA equivalent/g extract.

Phosphomolybdenum assay

An aliquot of 100 µl of sample solution (in 1 mM dimethyl sulphoxide) was combined with 1 ml of reagent solution (0.6 M Sulphuric acid, 28 mM sodium phosphate and 4mM ammonium molybdate) in a 4ml vial. The vials were capped and incubated in a water-bath at 95°C for 90 min. After the samples had cooled to room temperature; the absorbance of the mixture was measured at 695 nm against a blank. The results reported (Ascorbic acid equivalent activity) are mean values expressed as g of ascorbic acid equivalents /100 g extract.

Determination of total phenolic content

10 ml of aliquots of each extracts (10 mg/10ml) were taken in the test tubes and made upto the volume of 1ml with distilled water. Then 0.5 ml of folin-ciocalteu phenol reagent (1:1 with water) and 2.5 ml of sodium carbonate solution (20%) were added sequentially in each test tube. Soon after vortexing the reaction mixture, the test tubes were placed in dark for 40 min and the absorbance was recorded at 725nm against the reagent blank. The analysis was performed in triplicate and the results were expressed as the tannic acid equivalents.

Determination of total tannin content

Using the same extracts, the tannins were estimated after treatment with polyvinyl polypyrolidone (PVPP). 100 mg of PVPP was weighed in a test tube and to this 1.0 ml of distilled water and then 1.0 ml of tannin containing phenolic extract were added. The content was vortexed and kept in the test tube at 4°C for 4 hrs. Then the sample was centrifuged (3000 rpm for 10 min at room temperature) and the supernatant was collected .This supernatant has only simple phenolics other than tannins. The phenolic content of the supernatant was measured, as monitored above and expressed as the content of non-tannin phenolics on a dry matter from the above results, the tannin content of the sample was calculated as follows: Tannin (%) =Total phenolics (%) - Non-tannin phenolics (%).

Assay of superoxide radical scavenging activity

Each 3ml reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 µg riboflavin, and 12 mM EDTA, and 0.1 mg NBT and 100 µl sample solution. Reaction was started by illuminating the reaction mixture with sample extract for 90 seconds. Immediately after illumination; the absorbance was measured at 590 nm.The entire reaction assembly was enclosed in a box lined with aluminium foil. Identical tubes with reaction mixture kept in the dark served as blanks. The percentage inhibition of superoxide anion generation was calculated as: % inhibition = $[(A_0-A_1)/A_0] \times 100$, where A_0 is the absorbance of the control, and A_1 is the absorbance of the sample extract/standard.

Free radical scavenging activity on DPPH

Sample extract at various concentrations was taken and the volume was adjusted to 100 µl with methano l.5 ml of a 0.1 mM methanolic solution of DPPH was added and shaken vigorously. The tubes were allowed to stand for 20 min at 27°C. The absorbance of the sample was measured at 517 nm. Radical scavenging activity was expressed as the inhibition percentage of free radical by the sample and was calculated using the formula: % DPPH radical scavenging activity = (control OD-Sample OD /Control OD) × 100.

RESULTS

Synthesis of silver nanoparticles

As mushroom broth/ extracts were mixed into aqueous solution of the silver nitrate, it started to change the colour from watery to brown due to reduction of silver ions; which indicated the formation of silver nanoparticles.

Synthesis of gold nanoparticles

As mushroom broth/ extracts were mixed into aqueous solution of the chloroauric acid, it started to change the colour to red due to reduction of Au^{3+} ions; which indicated the formation of gold nanoparticles.

Antioxidant activity

Table 1 shows the total flavonoid content, Ferric-reducing/antioxidant power, ABTS assay, Metal chelating activity, Phosphomolybdenum assay, Assay of superoxide radical scavenging activity of synthesized gold nanoparticles and silver nanoparticles.

Estimation of total flavonoid content

Flavonoid compounds which were reported as RE equivalent by reference to standard curve (Y = 0.0036 X, R^2 = 0.9887). Among mushroom mycelial extracts it has been found that the scavenging effect of *Pleurotus pulmonaries* has the highest value of 5416.67 mg RE equivalent / g of extract in case of AuNPs and *Pleurotus eryngii* has the highest value of 45.56 mg RE equivalent / g of extract in case of AgNPs.

Ferric-reducing/antioxidant power (FRAP) assay

Ferric reducing antioxidant power which were reported as Fe (II) equivalent by reference to standard curve (Y = 0.456 X, R^2 = 0.9887). Among mushroom mycelial extracts it has been found that the scavenging effect of *Pleurotus eous and Pleurotus florida* has the highest value of 789.47 mg FE (II) equivalent / g of extract in case of AuNPs and *Pleurotus florida* has the highest value of 324.57 mg FE (II) equivalent / g of extract in case of AgNPs.

Antioxidant activity by the ABTS assay

ABTS assay were reported equivalent by reference to standard curve (Y = 0.702 X, R^2 = 0.9951). Among mushroom mycelial extracts it has been found that the scavenging effect of *Schizophyllum commune* has the highest value of 11684.18 mg equivalent /g of extract in case of AuNPs and *Pleurotus cystidiosis* has the highest value of 14154.67 mg equivalent /g of extract in case of AgNPs.

Metal chelating activity

Metal chelating activity which were reported as EDTA equivalent by reference to standard curve (Y = 0.0299 X, R^2 = 0.9887). Among mushroom mycelial extracts it has been found that the scavenging effect of *Pleurotus pulmonaries* has the highest value of 3.05 mg EDTA equivalent / g of extract in case of AuNPs and *Pleurotus citrinopileatus* has the highest value of 3.03 mg EDTA equivalent / g of extract in case of AgNPs.

Phosphomolybdenum assay

Phosphomolybdinum compounds were reported as ascorbic acid equivalent by reference to standard curve (Y = 0.187 X, R^2 = 0.9951). Among mushroom mycelial extracts it has been found that the scavenging effect of *Pleurotus flabellatus* has the highest value of 28.33 mg AAE equivalent / g of extract in case of AuNPs and *Pleurotus ostreatus* has the highest value of 17.78 mg AAE equivalent / g of extract in case of AgNPs.

Assay of superoxide radical scavenging activity

Superoxide Radical scavenging activity were reported equivalent by reference to standard curve (Y = 0.053 X, R^2 = 0.9951). Among mushroom mycelial extracts it has been found that the scavenging effect of *Pleurotus pulmonaries* has the highest value of 76.79 mg in case of AuNPs and *Pleurotus eous* has the highest value of 76.79 mg in case of AgNPs.

Determination of total phenolic content

Phenolic compounds which were determined by folin ciocaltaue's method were reported as Gallic acid equivalents by reference to standard curve (Y = 0.1075 X, R^2 = 0.9887). Among mushroom mycelial extracts it has been found that the scavenging effect of *Pleurotus pulmonaries* has the highest value of 29.3 mg Gallic acid equivalent / g of extract in case of AuNPs and *Pleurotus ostreatus* has the highest value of 137.68 mg Gallic acid equivalent / g of extract in case of AgNPs.

Determination of total tannin content

Tannin compounds which were determined by folin ciocaltaue's method were reported as Gallic acid equivalents by reference to standard curve (Y = 0.1075 X, R^2 = 0.9887). Among mushroom mycelial extracts it has been found that the scavenging effect of *Pleurotus citrinopileatus* has the highest value of 70.04 mg Gallic acid equivalent / g of extract in case of AuNPs and *Pleurotus ostreatus* has the highest value of 74.63 mg Gallic acid equivalent / g of extract in case of AgNPs.

Free radical scavenging activity on DPPH

Table 2 shows the DPPH assay of AuNPs and AgNPs of the selected mushroom fungal strains. Free radical scavenging activity on DPPH were reported equivalent by reference to standard curve (Y = 0.389 X, R^2 = 0.9951). Among mushroom mycelial extracts it has been found that the scavenging effect of *Pleurotus flabellatus* has the highest value of 415.93mg in case of AuNPs and *Pleurotus flabellatus* has the highest value of 148.85 mg in case of AgNPs.[21]

DISCUSSION

Nanoscience has been established recently as a new interdisciplinary science. It can be defined as a whole knowledge on fundamental properties of nano-size objects.[22] Nanoparticles are being viewed as fundamental building blocks of nanotechnology. Metallic nanoparticles exhibit unusual optical, thermal, chemical, and physical properties. The reduction of materials dimension has pronounced effects on the physical properties that may be significantly different from the corresponding bulk material.[23] In very recent years, many interesting methods are being applied currently to the green preparation of nanosized silver and gold nanoparticles such as biosynthesis of nanoparticles by starch, by plant leaf broth, by edible mushroom extract, by apiin, by latex of *Jatropha curcas*. Silver nanoparticles have been synthesized as a result of silver ions reduction from silver nitrate by basidiomycetes mushroom fungi species. Gold nanoparticles have been synthesized as a result of Au^{3+} ions reduction from chloroauric acid by basidiomycetes mushroom fungi species.[24]

Nanoparticles have a wide range of applications, as in combating microbes, biolabelling and in the treatment of cancer.[25] Biologically synthesized silver nanoparticles could have many applications: they might be used as spectrally-selective coatings for solar energy absorption and intercalation material for electrical batteries; they also find use as optical receptors and as catalysts in chemical reactions. The synthesized gold nanoparticles and silver nanoparticles were analysed by the total flavonoid content, Ferric-reducing/antioxidant power, ABTS assay, Metal chelating activity, Phosphomolybdenum assay, Assay of superoxide radical scavenging activity.[26]

Recent studies have demonstrated the efficacy of silver nanoparticles for various biomedical applications such as antimicrobial agents, in wound healing as well as substrates for surface enhanced Raman scattering (SERS) and metal enhanced fluorescence (MEF).[27] Phenolic compounds are considered to produce significant antioxidant, and anti- diabetic activity. There is an increasing interest in antioxidants, particularly in those intended to prevent the presumed deleterious effects of free radicals in the human body, and to prevent the deterioration of fats and other con-

Table 1: Assay of antioxidant activity of synthesized Gold nanoparticles and Silver nanoparticles

Name of the extract	Total Flavonoid Content		Ferric-Reducing/ Antioxidant Power		ABTS Assay		Metal Chelating Activity		Phospho molybdenum Assay		Assay of Superoxide Radical	
	Scavenging effect RE (mg/ml)		Scavenging effect Fe (II) (mg/ml)		Scavenging effect (mg/ml)		Scavenging effect EDTA (mg/ml)		Scavenging effect AAE (mg/ml)		Scavenging effect (%)	
	AuNPs	AgNPs	AuNPs	AgNPs	AuNPs	AgNPs	AuNPs	AgNPs	AuNPs	AgNPs	AuNPs	AgNPs
Pleurotus citrinopileatus	5166.66	11.67	359.64	317.99	6925.46	14012.92	2.43	3.03	5.55	1.67	3.61	17.82
Pleurotus eous	4462.96	26.12	789.47	293.86	10043.94	13688.92	1.65	2.89	20	3.89	62.93	76.79
Pleurotus cystidiosis	5000	17.23	574.56	322.37	10590.69	14154.67	2.97	2.73	2.22	2.24	47.52	20.49
Pleurotus ostreatus	4388.88	16.12	15.35	269.74	7269.70	14114.17	2.65	2.62	3.88	17.78	73.89	56.19
Pleurotus eryngii	4638.88	45.56	65.78	315.79	7897.45	13688.92	2.84	2.87	25	6.67	73.23	32.90
Pleuoruts flabellatus	4583.33	19.45	359.64	287.28	9517.44	14053.42	2.78	2.98	28.33	8.34	53.91	52.25
Pleurotus florida	4750	37.23	789.47	324.57	6358.46	12797.93	2.93	2.91	2.77	7.23	55.46	1.83
Pleurotus pulmonarius	5416.67	17.78	385.96	293.86	9659.19	13871.12	3.05	2.75	23.33	5.56	76.79	27.39
Schizophyllum commune	4527.77	2.78	385.96	293.86	11684.18	14114.17	2.38	2.73	13.33	10.56	40.44	66.64

Table 2: DPPH assay of AuNPs and AgNPs of the selected mushroom fungal strains

Concentration of the Sample used (µg/ml)	Pleurotus citrinopileatus		Pleurotus eous		Pleurotus cystidiosis		Pleurotus ostreatus		Pleurotus eryngii		Pleuoruts flabellatus		Pleurotus florida		Pleurotus pulmonarius		Schizophyllum commune	
	AuNPs	AgNPs	AuNPs	AgNPs	AuNPs	AgNPs	AuNPs	AgNPs	AuNPs	AgNPs	AuNPs	AgNPs	AuNPs	AgNPs	AuNPs	AgNPs	AuNPs	AgNPs
20	1.51 ± 0.01	0.78 ±0.26	1.108 ±0.01	5.39 ±0.26	1.76 ± 0.01	8.49 ±0.26	1.63 ± 0.01	-35.99 ±0.26	1.67 ± 0.01	-64.02 ±0.26	1.41 ± 0.01	19.02 ± 0.26	1.64 ± 0.01	-4.63 ±0.26	1.67 ± 0.01	16.97 ±0.26	1.105 ±0.01	6.95 ±0.26
40	1.77 ± 0.01	0.26 ±0.26	1.21 ± 0.01	7.97 ±0.26	1.82 ± 0.01	-4.89 ±0.26	1.75 ± 0.01	-7.72 ±0.26	1.83 ± 0.01	-89.47 ±0.26	1.69 ± 0.01	6.167 ± 0.26	1.85 ± 0.01	-4.12 ±0.26	1.92 ± 0.01	10.29 ±0.26	1.397 ±0.01	3.09 ±0.26
60	1.87 ± 0.01	7.97 ±0.26	1.78 ± 0.01	5.66 ±01.57	1.84 ± 0.01	-11.83 ±0.26	1.91 ± 0.01	-5.92 ±0.26	1.89 ± 0.01	-134.1 ±0.26	1.87 ± 0.01	- 47.04 ± 0.26	1.86 ± 0.01	-6.69 ±0.26	1.94 ± 0.01	13.12 ±0.26	1.66 ± 0.01	5.32 ±0.26
80	1.93 ± 0.01	1.79 ±0.26	1.93 ± 0.01	3.26 ±0.39	1.9 ± 0.01	1.79 ±0.26	1.95 ± 0.01	2.06 ±0.26	1.95 ± 0.01	-110.0 ±0.26	1.91 ± 0.01	- 118.0 ± 0.26	1.94 ± 0.01	-9.77 ±0.26	1.95 ± 0.01	-1.55 ± 0.26	1.77 ± 0.01	7.46 ±0.26
100	1.98 ± 0.01	-4.12 ±0.26	1.98 ± 0.01	-0.26 ±0.26	1.93 ± 0.01	-27.51 ±0.26	1.97 ± 0.01	2.32 ±0.26	1.96 ± 0.01	-148.8 ±0.26	1.97 ± 0.01	- 37.01 ± 0.26	1.98 ± 0.01	-11.83 ±0.26	1.98 ± 0.01	-1.03 ± 0.26	1.88 ± 0.01	0.25 ±0.26

stituents of foodstuffs. In both cases, there is a preference for antioxidants from natural rather than from synthetic sources. There is therefore a parallel increase in the use of methods for estimating the efficiency of such substances as antioxidants. The antioxidant properties of moringa leaf extract have been demonstrated by various ways.

The total flavonoid content has been found that the scavenging effect of *Pleurotus pulmonaries* has the highest value in case of AuNPs and *Pleurotus eryngii* has the highest value in case of AgNPs. Ferric reducing antioxidant power has been found that the scavenging effect of *Pleurotus eous and Pleurotus florida* has the highest value in case of AuNPs and *Pleurotus florida* has the highest value in case of AgNPs. ABTS assay has been found that the scavenging effect of *Schizophyllum commune* has the highest value in case of AuNPs and *Pleurotus cystidiosis* has the highest value in case of AgNPs. Metal chelating activity has been found that *Pleurotus pulmonaries* has the highest value in case of AuNPs and *Pleurotus citrinopileatus* has the highest value in case of AgNPs. *Phosphomolybdenum assay* has been found that the scavenging effect of *Pleurotus flabellatus* has the highest value in case of AuNPs and *Pleurotus ostreatus* has the highest value in case of AgNPs. Superoxide Radical scavenging activity has been found that the scavenging effect of *Pleurotus pulmonaries* has the highest value in case of AuNPs and *Pleurotus eous* has the highest value in case of AgNPs. Total phenolic content has been found that the scavenging effect of *Pleurotus pulmonaries* has the highest value in case of AuNPs and *Pleurotus ostreatus* has the highest value in case of AgNPs. Total tannin content has been found that the scavenging effect of *Pleurotus citrinopileatus* has the highest value in case of AuNPs and *Pleurotus ostreatus* has the highest value in case of AgNPs. Free radical scavenging activity on DPPH has been found that the scavenging effect of *Pleurotus flabellatus* has the highest value in case of AuNPs and *Pleurotus flabellatus* has the highest value in case of AgNPs. All the basidiomycetes mushroom fungi species showed the better antioxidant activity.

CONCLUSION

The present study demonstrates an eco-friendly and low-cost method of biosynthesis of AuNPs and AgNPs using basidiomycetes mushroom fungal strains *Pleurotus citrinopileatus, Pleurotus eous, Pleurotus cystidiosis, Pleurotus ostreatus, Pleurotus eryngii, Pleuoruts flabellatus, Pleurotus florida, Pleurotus pulmonarius* and *Schizophyllum commune* .The synthesized AuNPs and AgNPs were confirmed by color transformation and Ultraviolet-visible (UV-visible) spectroscopy. These biologically synthesized AuNPs and AgNPs were tested for antioxidant activities. The biosynthesized AuNPs and AgNPs showed significantly higher antioxidant activities. The present study explored that the mushrooms which are efficient producers of AuNPs and AgNPs, and could act as safe and cost-effective with potential antioxidant activities. These findings encourage studying these fungal strains further for their potential biological applications.

CONFLICT OF INTEREST

No conflict of interest are declared.

ABBREVIATIONS USED

AuNPs: Gold Nanoprticles; **AgNps:** Silver Nanoparticles; **ABTS:** 2,2′-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid); **FRAP:** Ferric Reducing Antioxidant Power; **DPPH:** 2,2-diphenyl-1-picrylhydrazyl.

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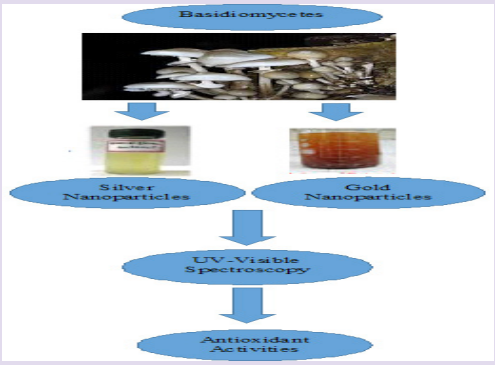
PICTORIAL ABSTRACT



SUMMARY

- The present study explored that the mushrooms are the efficient producers of gold nanoparticles and silver nanoparticles.
- These synthesized gold nanoparticles and silver nanoparticles from basidiomycetes mushroom fungi could act as safe, eco-friendly and cost-effective with potential antioxidant activities.
- These findings encourage studying these fungal strains further for their potential biological applications.

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