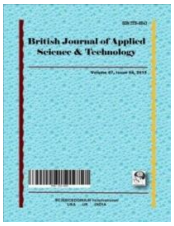

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Enhancement of Microbial Synthesis of Gold Nanoparticles by Gamma Radiation

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: An eco-friendly protocol for the synthesis of gold nanoparticles (GNPs) using submerged liquid fermentation of fungus *Pleurotus ostreatus* was established.
Methodology: Treatment of tetrachloro-auric acid ($HAuCl_4$) solution with the free cell filtrate (FCF) of the fungus led to the reduction of the $HAuCl_4$ ions and the formation of stable GNPs. These nanoparticles were characterized by Surface Plasmon Resonance (SPR) band at wavelength 550 nm for GNPs. The components of the media needed for the fungal growth were optimized using factorial design.
Results: The maximum SPR in UV-Vis spectra was recorded using a medium containing in (%); glucose (1), yeast (0.5), malt extract (0.5) and KNO_3 (0.2) and incubation period 8 days, the temperature and pH were kept at 30°C and 6, respectively. The particles were characterized by UV/Vis spectroscopy, Transmission electron microscope (TEM), Dynamic light scattering (DLS), Fourier Transform and Infrared Spectroscopy (FT-IR). Exposure of fungal strain or FCF after mixing with Au+ to Gamma radiation showed 36% increase in the SPR band intensity of the

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synthesized GNPs compared to un-irradiated strain and FCF irradiated before mixing with Au+. The synthesized GNPs exhibited antimicrobial activity against both Gram negative and Gram positive bacteria, measured by well diffusion assay. Moreover, it also showed good anticancer activity against human Breast carcinoma (T47D) cells, Prostate carcinoma (PC3) cells and hepatocellular (HEPG2) cells using Trypan blue exclusion and Sulfo-Rhodamine B assay.

Conclusion: The combined effect of both ϒ-radiation and proteins offers a highly efficient and inexpensive method for the synthesis of gold nanoparticles utilizing an edible mushroom, *P. ostreatus* for the bio- reduction of $HAuCl_4$.

Keywords: Pleurotus ostreatus; submerged fermentation; factorial design.

1. INTRODUCTION

In recent years, GNPs synthesis has been the focus of interest because of their applications in a number of areas such as biosensors, bioimaging and biomedicines [1,2]. Traditional synthetic methods of GNPs have involved a number of chemical methods [3]. However, these chemical procedures generate a large amount of hazardous by-products. Therefore, there is a need for 'green chemistry' that includes a nontoxic, clean and environment-friendly method of nanoparticle synthesis. Recently, studies have reported that some examples of microorganisms synthesizing inorganic GNPs include bacteria, fungi and algae [4].

In particular, fungi have advantages over bacteria and algae, because of their high metal tolerance, economic viability, low cost downstream processing, easy to scale-up and handling of biomass. Furthermore, fungi are extremely efficient secretor of extracellular enzymes and possible to easy large-scale production [5]. The cell free culture filtrates of different fungi were used for the synthesis of different nanoparticles like silver [6-8], selenium [9,10] and gold [11].

The genus *Pleurotus* is one of the most commercialized mushrooms in the world. *Pleurotus ostreatus*, famous for its delicious taste and high quantities of minerals, vitamins, proteins, carbohydrates as well as low fat, is the commercially important edible mushroom. The medicinally beneficial effects of *P. osteratus*, such as their immunomodulatory effects, antioxidant activities, antitumor activities, anti-inflammatory, antiviral and cholesterol-lowering activities are well known worldwide [12].

Biosynthetic method of GNPs can be divided into two categories according to the place where nanoparticles are synthesized as many microorganisms can provide inorganic materials, either intracellularly or extracellularly. The reductive pathway of extracellular synthesis of nanoparticles using microbe might include nitrate reductase-mediated synthesis. The reducing enzyme secreted by the fungi helps in the bioreduction of metal ions and synthesis of nanoparticles [13].

Gamma Irradiation synthesis of nanoparticles has been also employed as one of the most promising methods to synthesize GNPs. The process is clean, simple and harmless [14]. Compared to conventional chemical / photochemical methods, the radiochemical process can be performed to reduce gold Au+ ions at the ambient temperature without using excessive reducing agents or producing unwanted by-products of the reductant. Moreover, reducing agent can be uniformly distributed in the solution and GNPs are produced in highly stable and pure form [15].

This study demonstrates a method for the biological synthesis of gold nanoparticles through the reduction of aqueous tetrachloro-auric acid by using submerged culture filtrate of an edible mushroom, *Pleurotus ostreatus*. The effect of gamma irradiation on the microorganism and the free cell filtrate before and after mixing with gold ions was investigated and characterized by UV/Vis spectroscopy.

2. MATERIALS AND METHODS

2.1 Chemicals

All the media, chemicals reagents and tetrachloro-auric acid ($HAuCl_4$) used in the following experiments were of analytical grade and used without further purification. The human carcinoma Breast cells (T47D), prostate (PC3) hepatocellular (HEPG2) cells and normal amniotic (WISH) cells was procured from

National Cancer Institute (NCI). All the samples were prepared in deionized water.

2.2 Microorganism and Culture Media

White rot fungal strain *Pleurotus ostreatus* [CBS 411.71] was obtained from the Drug Radiation Research Department at National Center for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt. *Pleurotus ostreatus* was maintained after isolation on Potato dextrose agar (PDA) plates at 4°C. Prior use this strain was reactivated by subculture onto fresh PDA slants and cultured at 20 – 22°C for 8 d.

2.3 Preparation of Fungal Cell Filtrate

Fungal filtrate was prepared in erlenmeyer flask containing 100 ml medium composed of (g l^{-1}); Malt extract powder (5), glucose (10), yeast extract (5), KNO_3 (3) and the pH of the medium was adjusted to 6, The flasks were then inoculated with (10^6- 10^7 CFU ml^{-1}) of the mycelia of *P. ostreatus*. The flasks were incubated at 30°C under agitation at 120 rpm using (LAB-Line R Orbit Environ, USA) shaker for a period of 8 days. Eventually, the medium was filtered through whatman no.1 filter paper and the free cell filtrate kept for further use.

2.4 Biosynthesis of GNPs

Synthesis of GNPs was carried out using tetrachloro-auric acid (2.5 mM final concentration purity of gold 50%) mixed with FCF 1:10 (v/v) respectively. The reaction mixture was agitated for 24 h at 37°C, the yellow colored solution changed to pink then violet indicating the formation of GNPs. Simultaneously, only the FCF and only HAuCl4 solution were maintained under same conditions. The reaction mixture was routinely monitored by visual color change as well as periodic measuring of the absorption spectrum of the sample was recorded on JASCO V-560 UV-visible spectrophotometer operating at a resolution 1 nm [5].

2.5 Optimization of Media Components

Thirty two culture media were prepared by a 2^5 factorial designs to evaluate the effect of media composition on the production of GNPs using different sources and concentrations of organic nitrogen and carbon. (The experimental designs for the independent variables are presented in Table 1 against the dependent variable the SPR band intensity of the synthesized GNPs).

Table 1. Evaluated factors in the factorial design used for definition of a culture medium for the production of GNPS by FCF of *Pleurotus ostreatus*

Factors	Inferior level	Superior level
Incubation period	5 days	8 days
ME (g/100 ml)	0.5	1
YE (g/100 ml)	0.2	0.5
Glucose (g/100)	0.5	1
KNO3 (g/100 ml)	0.2	0.3

YE – Yeast extract (organic nitrogen source); ME-Malt extract (nitrogen source); KNO_3- Potassium nitrate (nitrogen source)

2.6 Characterization of GNPs

Initially, the UV/vis spectra of GNPs were recorded as a function of wavelength using (JASCO V-560 UV/ Vis, Japan) spectrophotometer in a range of 200-900 nm operated at a resolution of 1 nm. The average particle size and size distribution were assessed by PSS-NICOMP 380-ZLS particle sizing system St. Barbara, California, USA.

2.7 Fourier Transform Infra-Red Spectroscopy (FT-IR)

FT-IR measurements were carried out in order to obtain information about chemical groups present around GNPs for their stabilization and to understand the alteration of functional groups due to the reduction process. The measurements were carried out using (JASCO FT/IR-6300, Japan) infra-red spectrometer by employing KBr pellet technique.

2.8 Transmission Electron Microscope (TEM)

The morphology and size of the synthesized GNPs were recorded by using TEM model JEOL electron microscope (JEM-100 CX, Japan). TEM studies were prepared by drop coating GNPs onto carbon-coated TEM grids. The film on the TEM grids were allowed to dry and a blotting paper was used to remove the extra solution.

2.9 Bacterial Susceptibility to GNPs [Determination of Zone of Inhibition (ZOI)]

The antimicrobial activity of the synthesized GNPs was determined by agar well diffusion method [16] against different kinds of pathogenic bacteria isolated from clinical samples; MRSA *Staphylococcus aureus* (Gram positive bacteria), *Candida albicans* (yeast), *Pseudomonas aeruginosa* and *E. coli* (Gram negative bacteria). Standardized suspension of each tested strain (106 - 108 CFU ml-1) for bacteria and yeast respectively was swabbed uniformly onto sterile Muller-Hinton Agar (MHA, OXoid). An aliquot of 100 µl of the GNPs solution was added into each well (10 mm diameter). The plates were then incubated at 37°C for 24 h and then the zone of inhibition was measured and recorded.

2.10 Cytotoxicity Assays

2.10.1 Trypan blue exclusion method

The cytotoxicity of GNPs was tested on human Breast carcinoma (T47D) cells, human Prostate carcinoma (PC3) cells and human Hepatocellular carcinoma (HEPG2) cells using *Trypan blue exclusion* method [17]. Dilution several times with normal saline was used to balance the number of the cells ml^{-1}, where 0.8 ml saline mixed with 0.1 ml cell suspension and 0.1 ml of the synthesized GNPs solution. Cells were incubated for 2 h at 37°C under a constant overlay of 5% CO_2. 10 µl of Trypan blue (0.05%) was mixed with 10 µl of the cell suspension. Within 5 min, the mixture was spread onto haemocytometer, covered with a cover slip and then cells were examined under microscope. Dead cells were blue stained, while viable cells were not. Viable cells were counted by *Trypan blue exclusion* using haemocytometer. Control was conducted using the fungal cell filtrate without the GNPs. Cell surviving fraction (CSF) is calculated as follows; CSF= T/C where T and C represent the number of viable cells in a unit volume and the number of total (viable + dead) cells in the same unit volume respectively. Samples and control were assayed in triplicate.

2.10.2 Sulfo-Rhodamine-B assay (SRB assay)

Cytotoxicity of GNPs was determined by SRB assay. Briefly, Cells were plated in 96-multiwell plate (104 cells/well) for 24 h before treatment with the tested GNPs to allow attachment of cell to the wall of the plate. Different concentration of GNPs (10 folds serial dilutions, (0, 0.01, 0.1,1,10,100 µg ml-1) were added to the cell monolayer triplicate wells were prepared for each individual dose. Monolayer cells were incubated with GNPs for 48 h at 37°C and in atmosphere of 5% CO2. Cells were then fixed with 10% trichloroacetic acid 150 µl /well for 1 h at 4 oC, washed with water 3 times and stained [for 15 min at room temperature at dark place] with 0.4% Sulfo-Rhodamine-B dissolved in 1% acetic acid 70 µl/well. Excess stain was washed with 1% acetic acid to remove unbound dye and then the attached stain was recovered with Tris EDTA buffer. Colour intensity was measured in an ELISA microplate reader [18]. The percentage of cytotoxicity has been calculated in the following manner: % Viability = [viable cells in sample well/ viable cells in control well] × 100. The experiments were performed in triplicates. The cell viability percentage was plotted as percent of negative control group designated as 100%.

2.11 Gamma Exposure

The irradiation process was performed at NCRRT. Irradiations were performed within a gamma-irradiation system using Co-60 source (Gamma cell 4000-A-India) at dose rate 3.31 KGy h^{-1} at the time of the experiment. Targeted doses applied to the microorganism and the FCF before and after mixing with gold ions were 0.25, 0.5, 0.75, 1, 1.5, 2 and 3 kGy, respectively.

2.12 Statistical Analysis

The optimization results of the submerged liquid fermentation for the synthesis of GNPs were analyzed by using the software statistical version 6.0 (Minitab Inc., State College, PA, USA).The quality of fit of the first order model was expressed by the Coefficient of determination R2 and its statistical significance was determined by F-test. The data were expressed as mean ± standard deviation. The cell viability assays were analyzed by the SPSS/PC computer program was used for statistical analysis of the results. The data were expressed as mean ± standard error. Data were analyzed statistically using t-Test. Differences were considered significant at $P \leq 0.05$; highly significant at $P \leq 0.05$.

3. RESULTS AND DISCUSSION

3.1 GNPs Formation

The FCF mediated synthesis of GNPs was monitored visually by three test tubes containing a mixture of the FCF with $HAuCl_4$, only the FCF of *P. ostreatus* and $HAuCl_4$ solution. Only the mixture showed a time dependent color change, whereas the tetrachloroauric acid solution and the FCF retained their original color (Fig. 1b). The color was changed gradiently from yellow to red within 24 h. The appearance of the red color indicated the occurrence of the reaction and GNPs formation [5].

This change in color was due to the collective coherent oscillation of conduction electrons at the surface of GNPs when these particles interact with the oscillating electric field of the incident light, a phenomenon called Surface Plasmon Resonance (SPR). When this reaction was monitored with UV–vis spectroscopy, gold SPR bands were detected at wavelength 550 nm (Fig. 1a). Simultaneously in the same experimental conditions both the control sets (gold solution and FCF) showed no significant color change [5]. The UV-Vis spectra absorbance as a function of time, at a concentration of (2.5 mM) tetrachloroaurate solution and FCF by ratio 1:10 v/v, indicated that the reaction was finished after 24 h and further increase in time does not affect the formation of GNPs. Fig. 2 shows the SPR band intensity as a function of time after 0, 2, 4, 6 and 24 h.

3.2 Optimization of Medium Components for GNPs Production

The influence of fermentation physical factors on the formation of GNPs and its SPR band intensity was evaluated using full factorial two-level design (2^5) with a total of 32 runs. In the present study , the highest SPR band intensity of the formed GNPs (2.28) was observed in the 22^{th} run (Table 2) with optimized conditions (%) glucose: 1, yeast extract: 0.5, KNO_3:0.2, Malt extract: 0.5 and Incubation period 8 days. The 14^{th} run [(%) glucose: 1, yeast extract: 0.5, KNO_3:0.2, Malt extract: 1 and incubation period 5 days] was observed with SPR band intensity (2.00) which is close to 22^{th} with more malt extract and less incubation days. Both runs maybe used as a fermentation model for the synthesis of GNPs according to the cost-effectiveness of the media ingredients and electric consumption of the incubation conditions.

The experimental conditions and the results of SPR band intensity of the formed GNPs to construct a first order model that can predict the dependent variable (SPR band intensity of GNPs) as a function of independent variable (media conditions) as shown in Table 3. The analysis of variance (ANOVA) consists of classifying and cross classifying statistical results and testing if the means of a certain classification significantly differ. This was carried by Fischer's statistical test for the analysis of variance. The Fischer's F- test showed a value of 42.303 which is much greater than that of the F tabulated 0.0001 and that demonstrates that the model terms are significant. The model equation fitted by regression analysis is given by:

$$\text{Actual} = 1.12+0.33X_1-0.05X_2+0.02X_3-0.10X_4+0.10X_5-0.13X_1X_2-0.01X_1X_3+0.05X_1X_4-0.15X_1X_5-0.04X_2X_3-0.06X_2X_4-0.10X_2X_5+0.09X_3X_4+0.17X_3X_5-0.19X_4X_5$$

Where X_1 = Yeast extract, X_2 = KNO_3, X_3 = Glucose, X_4 = Malt extract, X_5 = Incubation Period.

The model determination coefficient R^2 (0.8953) suggested that the fitted model could explain 89.53 % of the total variation. This implies a satisfactory representation of the process by the model (Fig. 3). To assure the significance of factors studied, both the statistical parameters P-value and t-value were used. The t-value that measured how large the coefficient is in relationship to its standard error (SE) was obtained by dividing each coefficient by its SE. The p-value is the possibility of getting a larger t-value (in absolute value) by chance alone. The smaller the p-value and larger the magnitude of the t-value, more significant is the corresponding coefficient [19].

The intensity of the Surface Plasmon Resonance (SPR) band of the formed GNPs was significantly affected with malt, KNO3, yeast and incubation period (Table 4). The glucose factor had no influence (Fig. 4). This may be due to a complex of organic nitrogen sources which resulted in high biomass production [12], high extracellular enzymes secreted by *P. ostreatus* and resulted in more reduction of gold ions to

GNPs. Basidiomycetes prefer complex organic nitrogen sources. This may be due to some essential amino acid(s) may not be synthesized from inorganic nitrogen sources in the submerged culture of higher fungi [20,21]. Most researches have used organic nitrogen sources for the production of bioactive compounds from basidiomycetes [22].

3.3 GNPs Characterization

The DLS measurement showed that the particle size average obtained was found to be 57 nm (Fig. 5). FTIR absorption spectra of biosynthesized vacuum dried gold nanoparticles (Fig. 6) showed the presence of bonds due to O–H stretching (around~3262 cm_1) and aldehydic C–H stretching (around ~2348 cm_1). These peaks proved the presence of organic residues and proteins, which might have been extracellulary secreted by P. ostreatus and might cause the reduction and synthesis of GNPs [5].

TEM image (Fig. 7) revealed different sizes of GNPs which arose from the bio- reduction of $HAuCl_4$ by FCF at room temperature (37°C). These observations showed that the GNPs formed were spherical in structure and the average diameter was of 57 nm.

The effect of temperature was tested by carrying out the reaction using $HAuCl_4$ solution (2.5 mM) and FCF by ratio 1:10 v/v at different temperature (40-100°C). The increase in the temperature resulted in an increase in the SPR of synthesized GNPs (Fig. 8). The effect of FCF quantity indicates that only 1:10 ratio of $HAuCl_4$: FCF v/v ratio was enough to accomplish the reaction at a concentration of 2.5 mM stock solution of $HAuCl_4$ (Fig. 9).

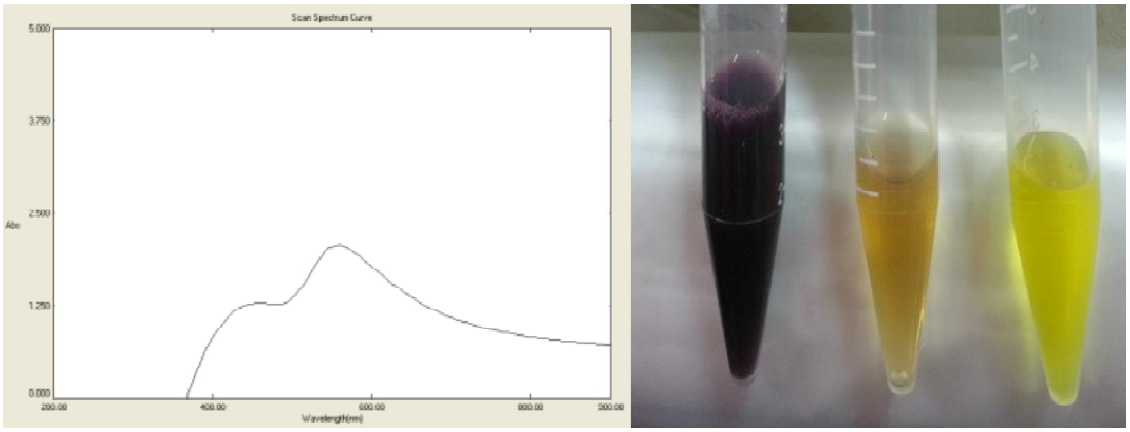


Fig. 1. (a) UV/Vis spectra recorded as a function of reaction time of an aqueous solution of 2.5 mM $HAuCl_4$ with the fungal culture filtrate (FCF) (b) Three tubes containing reaction mixture of the FCF with $HAuCl_4$ solution (1), (2) only $HAuCl_4$ solution (3) only the FCF

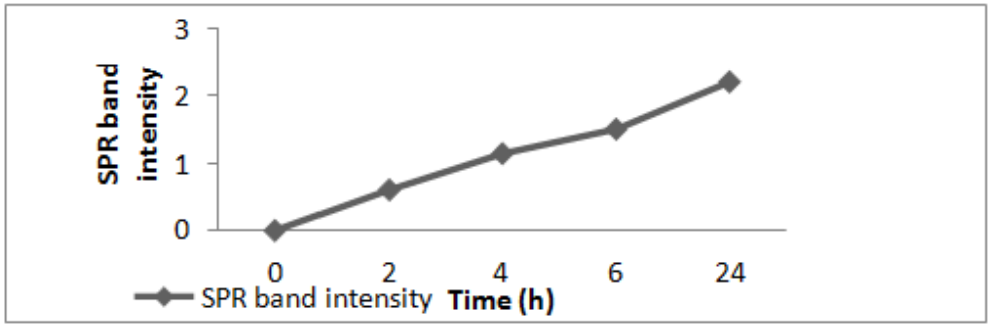


Fig. 2. SPR band intensity was recorded at different reaction times using HAuCL4 to cell culture filtrate concentration 1:10 v/v showing increase in the intensity with time

Table 2. The experimental conditions and the results of the mean SPR band intensity of the synthesized GNPs (combinations of variables and full factorial design)

Run no.	YE	KNO_3	Glucose	ME	IP	Mean SPR	S.D
1					5	0.40	0.06
2				0.5	8	1.70	0.16
3					5	1.00	0.36
4			0.5	1	8	1.70	0.17
5					5	0.30	0.10
6				0.5	8	0.70	0.10
7		0.2			5	0.06	0.01
8			1	1	8	1.60	0.33
9					5	0.40	0.13
10				0.5	8	1.80	0.09
11					5	0.90	0.10
12			0.5	1	8	1.50	0.13
13	0.2				5	0.50	0.10
14				0.5	8	2.00	0.21
15		0.3			5	0.70	0.11
16			1	1	8	1.10	0.16
17					5	1.25	0.13
18				0.5	8	1.60	0.09
19					5	1.20	0.15
20			0.5	1	8	1.40	0.06
21	0.5	0.2			5	1.40	0.26
22			1	0.5	8	2.28	0.08
23					5	1.70	0.13
24				1	8	1.10	0.12
25					5	0.60	0.09
26				0.5	8	1.40	0.24
27					5	0.30	0.04
28			0.5	1	8	0.60	0.10
29		0.3			5	0.70	0.11
30				0.5	8	1.70	0.12
31					5	1.00	0.10
32			1	1	8	1.19	0.01

IP= Incubation period/day, ME = Malt extract, YE = Yeast extract, KNO_3 = Potassium nitrate, SPR = Surface Plasmon Resonance, SD= Standard Deviation

Table 3. Regression analysis of the full factorial design

Source	Df	Sum of squares	Mean square	F ratio
Model	15	30.142	0.972	42.303
Error	16	1.47	0.02	Prob> F
C. total	31	153.04	-------	<0.0001*

**: Significant; df: Degree of freedom; F: Fischer's F-test*

3.4 Bacterial Susceptibility to GNPs

Table 5 shows the zone of inhibition of GNPs and FCF of *P. ostreatus* as a control against Gram negative, Gram positive and yeast. The present study clearly indicates that GNPs have good antibacterial action against Gram negative *Pseudomonas aeruginosa* and *Escherichia coli* when compared with the Gram positive organism *Staphylococcus aureus*, similar results was obtained by [23,24]. It can be suggested that the outer membrane proteins (OMP) in gram negative *Pseudomonas aeruginosa* and *Escherichia coli* were deteriorated by GNPs, while compared to gram positive *Staphylococcus aureus* might be inhibited through enhanced penetration and interaction with inner membrane proteins. No antifungal activity detected against *Candida albicans*. No antimicrobial activity for tetrachloro-auric acid alone was detected.

Table 4. Regression coefficients for the SPR band intensity under different physical conditions

Term	Coefficient	SE coefficient	T- value	P- value
Constant	1.12	0.02	72.69	0.000*
YE	0.33	0.023	21.56	0.000*
KNO_3	-0.05	0.025	3.17	0.002*
Glucose	0.02	0.026	1.45	0.153
ME	-0.10	0.024	6.70	0.000*
Ip	0.10	0.022	6.75	0.000*
YE*KNO_3	-0.13	0.025	8.65	0.000*
YE*Glucose	-0.01	0.024	0.40	0.688
YE*ME	0.05	0.023	3.34	0.001*
YE*Ip	-0.15	0.024	9.50	0.000*
KNO_3*Glucose	-0.04	0.022	2.29	0.025*
KNO_3*ME	-0.06	0.022	3.72	0.000*
KNO_3*Ip	-0.10	0.022	6.63	0.000*
Glucose*ME	0.09	0.024	5.89	0.000*
Glucose*Ip	0.17	0.023	10.99	0.000*
ME*Ip	-0.19	0.023	12.55	0.000*

**: Significant values at level of P< 0.05*

Table 5. Diameter of inhibition zone (mm) of GNPs and FCF of *Pleurotus ostreatus* as a control against bacteria and yeast tested

Microorganism	Diameter of inhibition zone (mm) produced by GNPs	Diameter of inhibition zone (mm) produced by FCF
Escherichia coli	17 ± 0.2	14± 0.1
Staphylococcus aureus (MRSA)	15 ± 0.1	12 ± 0.1
Pseudomonas aeruginosa	19 ± 0.1	14±0.2
Candida albicans		

3.5 *In vitro* Cytotoxicity Assays of GNPs

3.5.1 Trypan blue exclusion method

In vitro cytotoxicity of gold nanoparticles was evaluated on human Breast carcinoma (T47D) cells, Prostate carcinoma (PC3) cells and human Hepatocellular carcinoma (HEPG2) cells using Trypan blue exclusion method. The resulted percentage of viable cells expressed as the % cell viability, using formula CSF= T/C × 100. The tested GNPs showed good anticancer activity against all the tested carcinoma cells. The cells reduced by 85%, 70% and 67% for T47D, PC3 and HEPG2 cell lines respectively. Also, the FCF showed less anticancer activity. It reduced the cell viability by 98%, 96% and 96% for T47D, PC3 and HEPG2 respectively. It may be attributed to B-glucans polysaccharides produced by *P. ostreatus* which is an important constituent of the cellular wall of the mycelium [22].

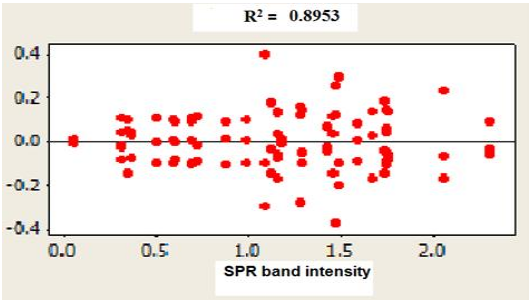


Fig. 3. SPR band intensity of the synthesized GNPs at wavelength 550 nm actual by predicted plot

3.5.2 SRB assay

The effect of GNPs on cell viability of (HEPG-2) cells and (WISH) cells were assessed by SRB assay. The cells were treated with varying concentrations of GNPs (0.01, 0.1, 1, 10 and 100 µg ml^{-1}) for 48 h. There is significant difference on cell viability at all concentrations of GNPs on HEPG2 cells, while there was no significant difference at concentrations (0.01, 0.1 and 1 µg ml^{-1}) on wish cells. The cell viability was reduced in a dose dependent manner in both the cell lines (Fig. 10).

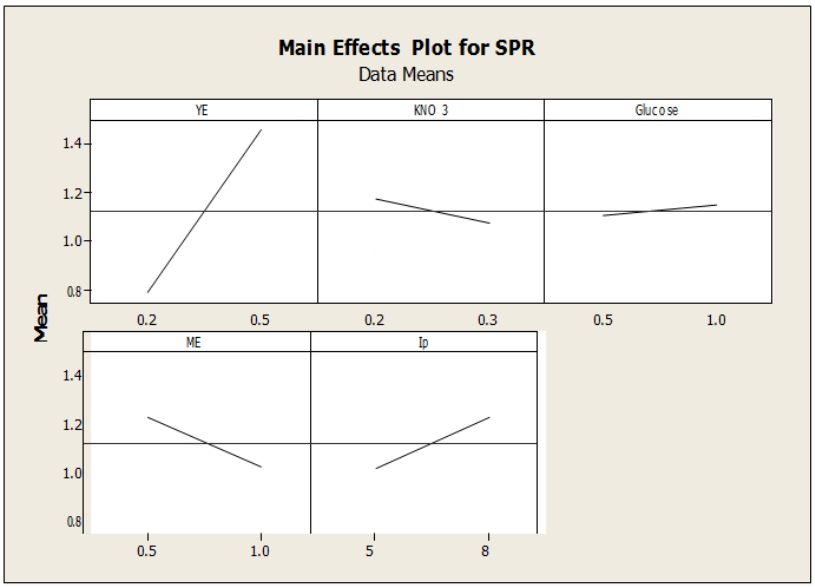


Fig. 4. Effect of superior and inferior level of each factor (independent variable) on the dependent variable (SPR band intensity of GNPs)

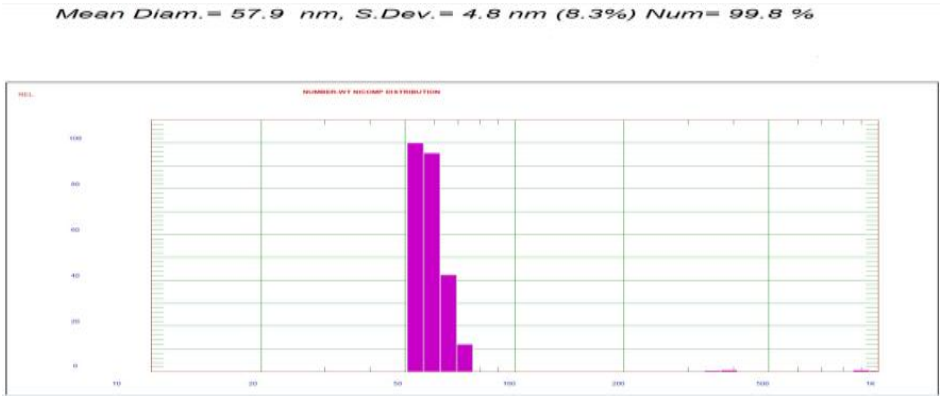
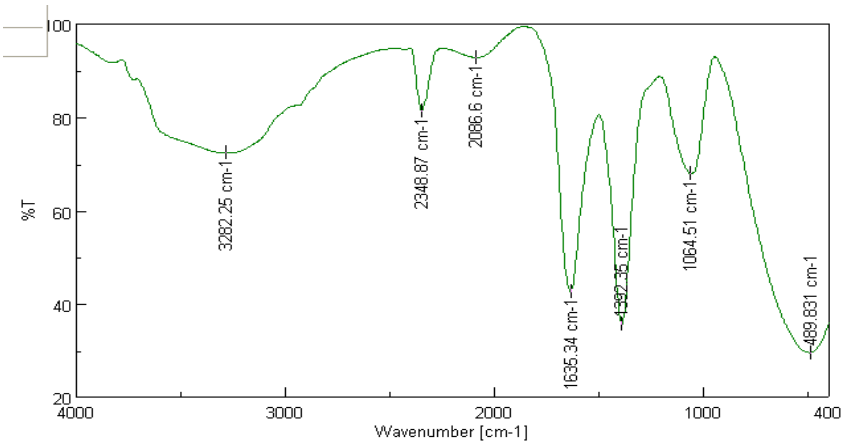


Fig. 5. Particle size distribution by dynamic light scattering (DLS) showing mean diameter 57.9 nm



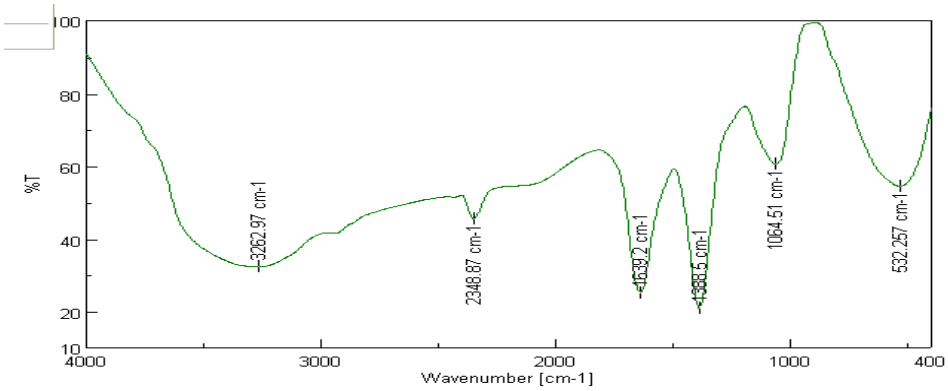


Fig. 6. FTIR spectra of (a) Free cell filterate (b) Formed GNPs after 24 hours incubation of the free cell filterate treated with $HAuCL_4$ (2.5 mM) solution

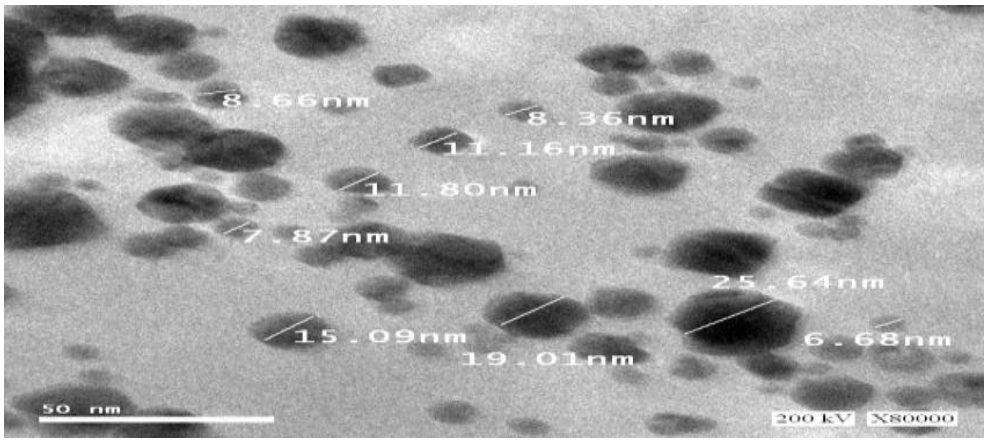


Fig. 7. TEM recorded from a region drop-coated film of HAuCl4 solution treated with the cell free filtrate of for 24 hrs showing spherical nanoparticles with average size 5-35 nm with scale bars correspond to 50 nm

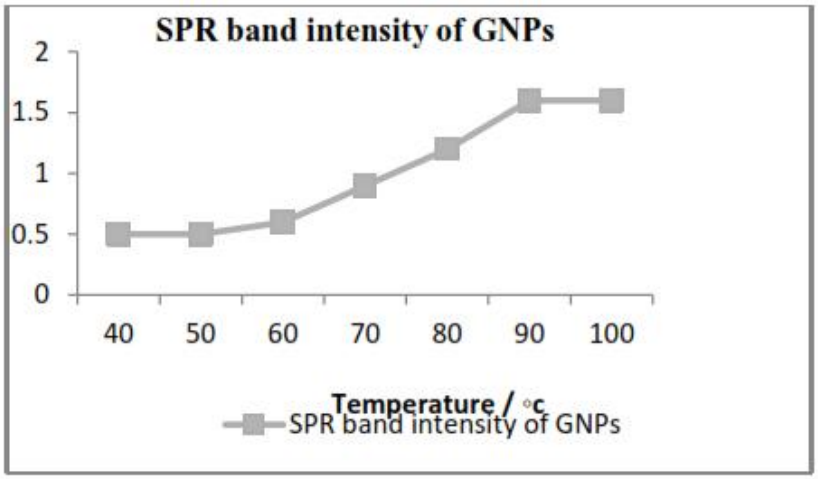


Fig. 8. Effect of temperature on SPR band intensity of GNPs

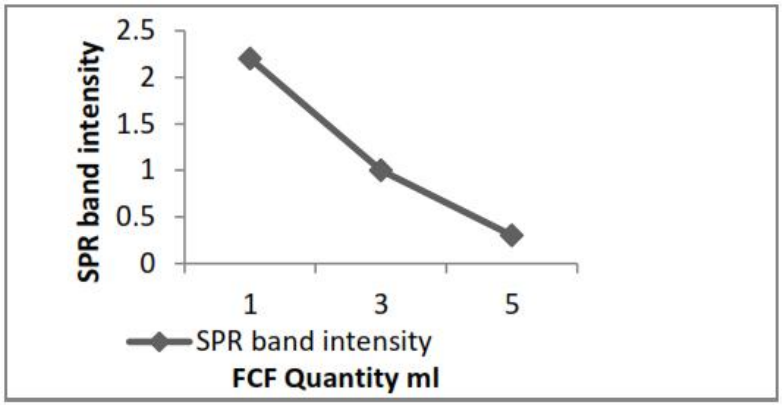


Fig. 9. SPR band intensity recorded for the formed GNPS using different volumes of cell culture filtrate to 0.1 ml HAuCl4 (2.5 mM), it shows decrease of the SPR band intensity by increasing cell culture filtrate volume

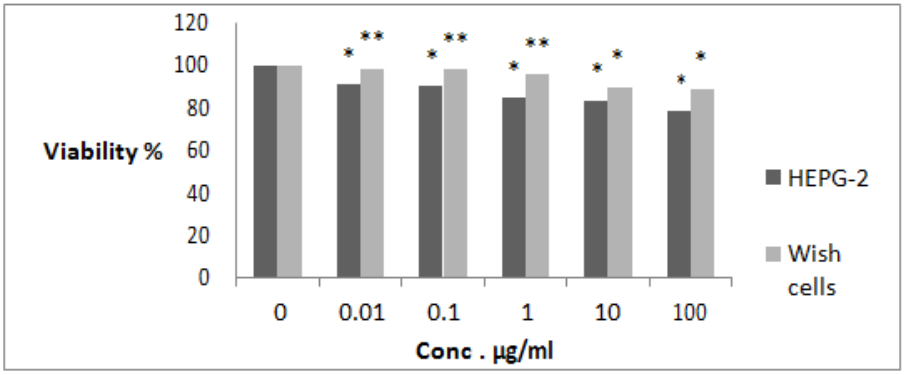


Fig. 10. SRB assay of GNPs against Human hepatocellular carcinoma (HEPG-2) cells and Human normal amnion (Wish) cells. Data are reported as means ± standard error (SE) of triplicate experiment. The values were analyzed by student's t-test ($p<0.05$) statistical analysis. P values for significantly different means

**P<0.05 significant value; ** P> 0.05 non-significant value*
** p< 0.05 and ** P>0.05 vs control*

3.6 Exposure of Microorganism and FCF to Gamma Irradiation

Treatment of fungal strain with different doses of gamma irradiation showed the maximum SPR intensity (Optical density 3.2) at low dose 0.25 kGy (Fig. 11). This may suggest that gamma irradiation resulted in viable and stable enhanced isolate with increased production of enzymes [25]. Similarly, several studies showed that gamma irradiation could change the genomic structure [26]. Further increases in the radiation dose decreases the SPR band intensity which could be attributed to the deterioration in the vitals of the microorganism and decrease in the enzyme synthetic activity due to radiation which resulted in decrease in the reduction of Au+ ions to GNPs.

Treatment of fungal FCF with different doses of gamma irradiation before and after mixing with gold chloride solution showed that the FCF irradiated after mixing with solution HAuCl4 was more rapid and effective (immediate reduction and change in color was observed) than that of the FCF irradiated before mixing with HAuCl4 solution in the formation of GNPs (Fig. 12). This could be attributed to that, in case of samples irradiated before mixing the only one mechanism is due to different enzymes activity and components of FCF secreted by the fungal strain *P. ostreatus* e.g. Nitrate reductase. In case of samples irradiated after mixing,

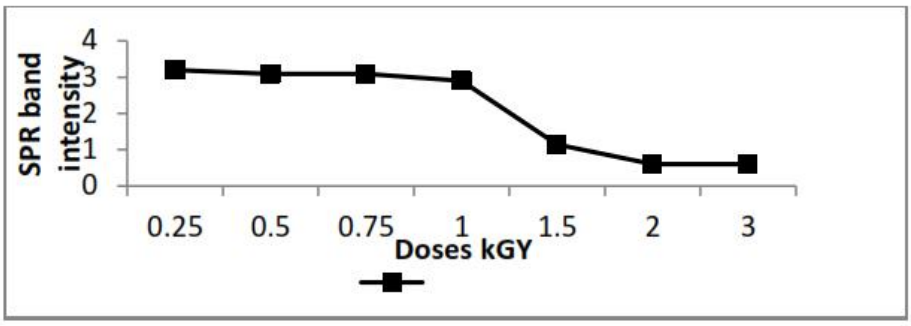


Fig. 11. Effect of gamma irradiation on *Pleurotus ostreatus* irradiated with different doses of gamma radiation (kGy) before inoculation, fermentation and using FCF for synthesis of GNPs

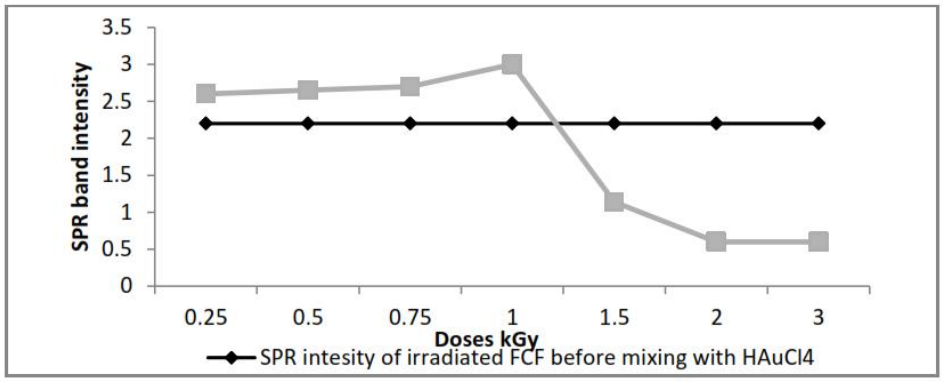


Fig. 12. The recorded effect of gamma irradiation on FCF of *Pleurotus ostreatus* irradiated before and after mixing with $HAuCl_4$ using different doses of gamma radiation (kGy)

the nanoparticles synthesis is due to two mechanisms; the first is the extracellular enzymes and components of FCF and the second is radiolytic mechanism of gamma irradiation which increased the synthesis of GNPs.

The formation of GNPs in case of radiation could be related to the radiolytic reduction which requires radiolysis of aqueous solutions and renders an efficient method to reduce metal ions. In the radiolytic method, when aqueous solutions are subjected to gamma rays, they create solvated electrons, which reduce the metal ions and the metal atoms finally coalesce to form aggregates. The effect of radiolytic reduction resulted in GNPs synthesis by radiolytic reactions and stabilization by prevention of aggregates formation by "capping". The maximum SPR band intensity was at (3.1), after which the SPR band intensity decreased by increasing the irradiation dose more than 1 kGy. This may be due to the destructive impact of free radicals produced by gamma irradiation on the bioactive compounds present in the extract [18].

4. CONCLUSION

In this study, we present a low cost and rapid approach for the bio-reduction and synthesis of gold nanoparticles utilizing an edible mushroom, *P. ostreatus* for the bio- reduction of $HAuCl_4$. The free cell filtrate enriched with extracellular enzymes and proteins can be used for efficient green synthesis of stabilized GNPs. The combined effect of both ɤ-radiation and proteins in synthesis and stabilization of GNPs offers a highly efficient and inexpensive method which can be used in large scale production of GNPs.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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