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Mycosynthesis of gold nanoparticles using the extract of *Flammulina velutipes*, Physalacriaceae, and their efficacy for decolorization of methylene blue

Muwafaq Ayesh Rabeea[a], Mustafa Nadhim Owaid[b,c,*], Azlan Abdul Aziz[d], Mahmood S. Jameel[d], Mohammed Ali Dheyab[d]

[a] Department of Applied Chemistry, College of Applied Sciences-Hit, University Of Anbar, Hit, 31007, Anbar, Iraq
[b] Department of Heet Education, General Directorate of Education in Anbar, Ministry of Education, Hit, 31007, Anbar, Iraq
[c] Department of Environmental Sciences, College of Applied Sciences-Hit, University Of Anbar, Hit, 31007, Anbar, Iraq
[d] Nano-Optoelectronics Research and Technology Lab (NORLab), School of Physics, Universiti Sains Malaysia, 11800, Pulau Pinang, Malaysia

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ABSTRACT

This study is the first report on the usage of fresh fruiting bodies of Enoki mushroom (*Flammulina velutipes*) as a new green reducer agent incorporated in the mycosynthesis of gold nanoparticles. The mycosynthesized AuNP was characterized for its physical and chemical properties using optical observation, UV–vis spectrometer, FESEM, AFM, DLS, XRD, Zeta Potential, and FTIR analyses. The optimized AuNPs were then tested for its efficacy in the decolorization of Methylene Blue (MB). The purple color of AuNPs indicates that the solution contains a mixture of triangular, spherical, and irregular shapes with an average of size 74.32 nm. The pattern of XRD clearly showed that the formed AuNPs were crystalline while DLS and Zeta Potential data showed that myco-synthesized AuNPs exhibited good stability and aggregation properties in colloidal form. FTIR showed the attachments of polysaccharides, amino acids and phenol as reducer agents on AuNPs. The efficacy test exhibited that the degradation of MB increased with the increase of the incubation time in presence of AuNPs. The proof-of-concept that mycosynthesized AuNPs by fungal natural organic materials of *Flammulina velutipes* can act as catalysts in reducing the MB dye is proven by the best decolorization of MB at 75.35 % after 4 h soak.

1. Introduction

The term Myco-nanotechnology is called for the green formation of nanoparticles from myco-materials, which present in fungi like yeasts, molds, and mushrooms [1,2]. In many studies, the nanoparticles have been synthesized by chemical approaches using different reducing agents, but the chemical methods might be intimidating health of human when they are used in removing many pollutants of nature, and in some medical applications [3,4]. Recently, green synthesis of NPs, as a green approach, is utilized to eliminate the resulted cytotoxicity from chemical agents by using biomaterials [5] from plant [6–8], algae [9], mushrooms [10,11] and truffles [12].

This synthesis is called the green chemistry method to biosynthesize metallic nanoparticles, which are considered ecofriendly. Using the macrofungus in the biosynthesis of different metallic NPs is very useful due to the produced massive amounts of enzymes, mycelia, fruiting bodies globally [1]. The synthesis of a triangular nanoparticle is more complicated than other shapes [13]. Nevertheless, extracts of the edible mushroom were used coupled with microwave irradiation method, to biosynthesize spherical AuNPs with 50 nm [14] and 20 nm [15]. On another hand, few researches have succeeded to mycosynthesize triangular and spherical AuNPs [10] from mushrooms, including *Volvariella volvacea* (20 − 150 nm) [16], *Pleurotus florida* (10 − 50 nm) [17], *Pleurotus sapidus* (15 − 100 nm) [18], and *Lentinula edodes* [2].

Recently, several studies have emerged about the synthesized AuNPs using plant [19] and mold extracts [20] to evaluate the catalytic efficacy in the degradation of organic dyes employed in the industry. Very few researches have also reported that the mycosynthesized silver nanoparticles (AgNPs) from mushrooms such as the oyster mushroom *Pleurotus sajor caju* [21], *Pleurotus ostreatus* [22] *Agaricus bisporus* and *Ganoderma lucidum* [23] showed remarkable catalytic activities toward Azo dyes such as orange dye, congo red and Direct blue 71. Nonetheless, no work has been reported on the applicability of myco-synthesized AuNPs for the degradation of Azo dyes. However, just for

* Corresponding author.
E-mail address: mustafanowaid@uoanbar.edu.iq (M.N. Owaid).

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the first time, *Flammulina velutipes* was used to mycosynthesize triangular AuNPs within hyphae (intracellularly) with size ranged from 5 − 150 nm [24], but no published study was ever achieved in the formation of AuNPs (extracellular) from the fruiting body extract of the current mushroom.

Thus, this study aims to produce the extracellular mycosynthesized spherical and triangular AuNPs using the extract of *Flammulina velutipes* fruitbodies for the first time and to describe their chratcteristics using the change in color, FTIR, UV–vis spectrum, FE-SEM, AFM, DLS, Zeta Potential, and XRD analyses. Moreover, the mycosynthesized AuNPs were tested for decolorization of methylene blue (MB) dye for the first time by using gold NPs synthesized from mushrooms.

2. Materials and methods

2.1. Chemicals

Gold (III) chloride hydrate or Chloroauric acid ($HAuCl_4.4H_2O$) was purchased from (Sigma Aldrich, Germany) with a purity of 99.995 %.

2.2. Samples of enoki mushroom

150 g of fresh fruitbodies of *Flammulina velutipes* (Enoki mushroom) were purchased from the local market on Penang Island, Malaysia.

2.3. Enoki mushroom preparation and extraction

The Enoki mushroom watery extract was prepared using extraction of 20 *g* of cutted mushrooms in a 200-ml flask with 150 ml of distilled water (D.W) and then boiled for 10 min at 400 rpm using magnetic strierr hotplate (Fisher Scientific, USA). After that, the solution was removed from the heating source for cooling down at room temperature. However, this extract was filtered and centrifuged for 10 min at 25 °C and 6000 rpm. The final aqueous was storage at − 18 °C for later use.

2.4. The mycosynthesis of gold nanoparticles

Mycological synthesis of Au nanoparticles was achieved using the following approach. Briefly, 135 ml of the prepared 0.001 M $HAuCl_4.4H_2O$ (Chloroauric acid) solution (Sigma Aldrich, Germany) was stirred and heated to 80 °C in a 200 ml flask. Then, 15 ml of the watery extract of Enoki mushroom was mixed with the gold salt solution and the temperature was increased to 100 °C. After 30 min, the color of the solution changed from bright yellow to light purple and during this time, a sample of the mixture was taken for UV–vis analysis and repeated after each 15 min, each with a 3 ml of the watery extract (at 30, 45, 60, 75 and 90 min) until constancy the mixture color at deep violet which indicates to the nucleation process had taken place. The final colloidal solution was kept at 4 °C for later usage. The schematic illustration of the synthetic steps for gold nanoparticles from *Flammulina velutipes* mushroom extract was exhibited in Fig. 1.

2.5. Characterization of AuNPs

The synthesized Au nanoparticles from the extract of *F. velutipes* were described by visual inspection on changes in color, the UV–vis spectrum (Agilent Technologies, Cary Series UV–vis-NIR Spectrophotometer), FTIR (PerkinElmer FT-NIR Spectrometer), FE-SEM (FESEM-FEI/Nova NanoSEM 450), AFM, Dynamic Light Scattering method (Zeta Potential, and Zetasizer (Nanoseries Model ZEN 3600, Malvern Instruments)), and XRD (PANalytical X'pert PRO MRD PW 3040) analyses. All analyses and tests were achieved at USM (Universiti Sains Malaysia), Malaysia.

2.6. Decolorization of methylene blue dye

The mycosynthesized AuNPs were investigated to degrade methylene blue (MB) dye at a concentration of 20 ppm. About 5 mg of AuNPs was mixed with 25 ml solution of MB dye with continuous shaking on the magnetic stirrer at 400 rpm for 1, 2, 3, and 4 h at room temperature. The absorption of the final solution is determined using UV–vis at ƛ. max664 nm. The decolorization of MB was estimated by the following equation [25]:

$$\text{Decolorization of methylene blue, } \% = \frac{M_0 - M}{M} * 100$$

Where M is the concentration of MB after decolorization using AuNPs, M_0 is the initial concentration of MB.

3. Results and discussion

The extracellular recovery of Au (gold) using the mycoreduction of ions of Au^+ by Enoki mushroom *Flammulina velutipes* fruiting bodies haven't been tested previously. Fig. 2 shows that the color of the Enoki mushroom (*F. velutipes*) extract mixed with the 10^{-3} M Chloroauric acid ($HAuCl_4.4H_2O$) has altered from bright yellow to purple color. This color is an indicator for successful biosynthesis of gold nanoparticles (AuNPs) in the present work that agrees with the visual photograph of gold nanoparticles from other work [26] that was myco-synthesized using the yellow oyster mushroom *Pleurotus cornucopiae*. In this research, five periods were applied to mycosynthesize AuNPs from *F. velutipes* fruiting bodies, i.e. 30, 45, 60, 75, and 90 min using the magnetic stirrer hot plate at 100 °C and 700 rpm. However, the interaction solution altered to bright purple after 30 min, referring to the initial mycosynthesis of gold nanoparticles. Then the intensity of color increased with increased interaction time, which indicates that the formation of more AuNPs, see Fig. 2. Optical analysis increased when the time of reaction increased, and the fungal compounds reduced more ions of gold in the interaction solution [26]. In other words, the intensity of gold nanoparticles associated with concentrations of polysaccharides, and proteins, which capped and reduced these NPs, as reported in many previous investigations [16]. Finally, the optimum condition for the synthesis of AuNPs in this study was as the following: the temperature (100 °C), the salt concentration (10^{-3} M), ratio of the watery extract volume to the salt solution volume (1:9), and stirring (700 rpm).

The UV–vis spectra of AuNPs were recorded by the spectrophotometer operated from a range of 400 − 700 nm and the peak of absorption at 563 nm was observed because of the incitation of SPR in the solution of Au nanoparticles. UV–vis spectra exhibit variation in the top of peak depending on increasing of the time of interaction. As shown in Fig. 2, peaks of colloidal AuNPs absorbance redshifted from 551 − 563 nm at 30 − 90 min. At 30 min, the highest peak was 0.705 cm^{-1}; afterwards the absorption raised to 0.962 cm^{-1}, 1.151 cm^{-1}, and 1.333 cm^{-1} after 45, 60, and 75 min from the time of interaction for next wavelengths 557 nm, 563 nm, and 563 nm. Nevertheless, at 90 min, Au nanoparticles showed the higher absorbance (1.448 cm^{-1}) at the higher peak of 563 nm in the current research. Hence, the venatic relation between the increasing color intensity and the absorbance wavelength was remarkable. The UV–vis spectrum supplied Surface Plasmon Resonance phenomenon discovers for Au [27]. It is noticed from UV–vis spectra that the peak of AuNPs occurs at 563 nm, and the last absorbance stably increased with increasing reaction time. The redshift, splitting and broadening of SPR is possible because of the dampening of Surface Plasmon Resonance caused by the change in the refractive index of the surrounding medium, and increase in size of gold nanoparticles in the solution [28].

Fig. 3 showed the size and shape of the mycosynthesized gold NPs from *Flammulina velutipes* (Enoki mushroom). Most of the biosynthesized gold nanoparticles have spherical, triangular, and irregular

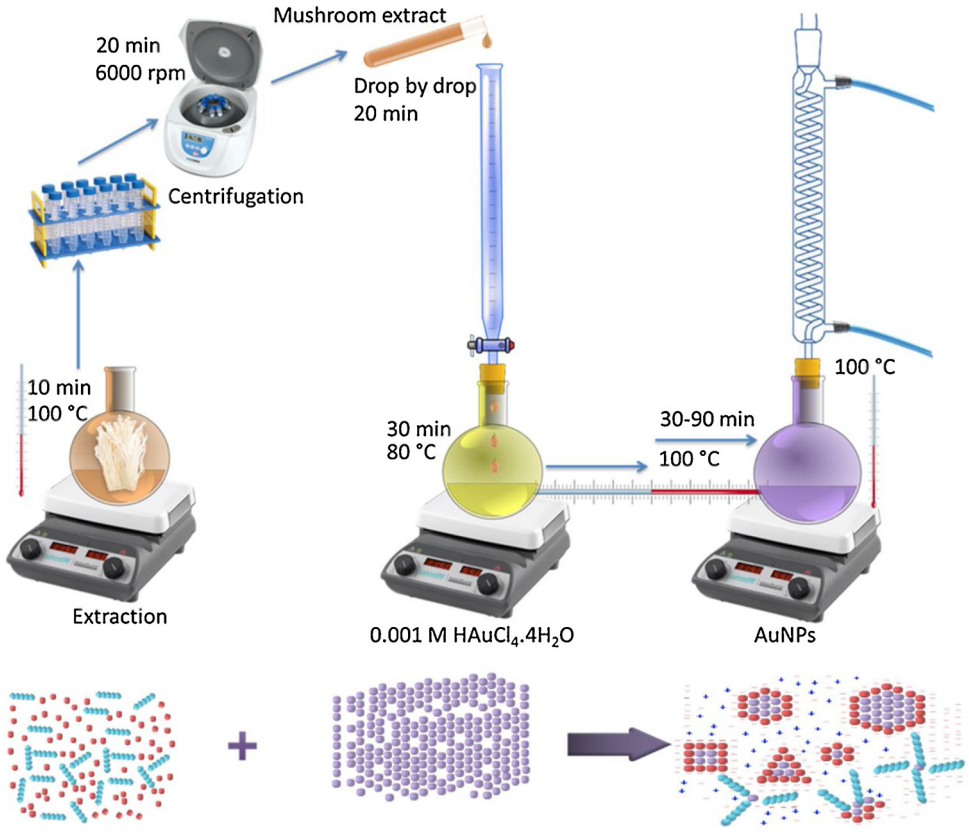

Fig. 1. Schematic illustration of the synthetic steps for gold nanoparticles from mushroom extract.

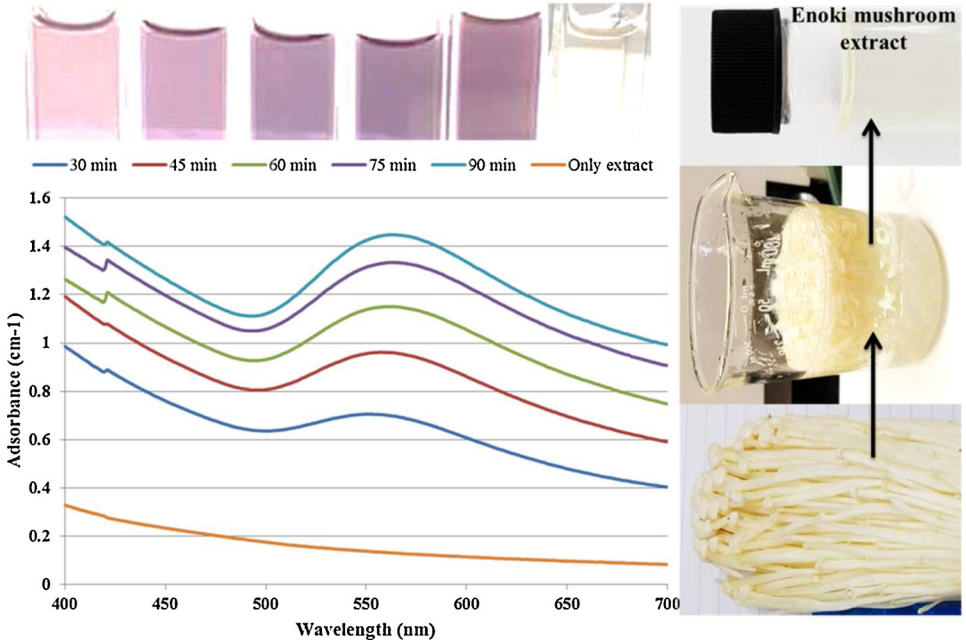

Fig. 2. Optical and UV–vis analyses of the mycosynthesized gold nanoparticles.

shapes, as shown in images of FESEM (Fig. 3). Nevertheless, the average size of AuNPs was 74.32 nm, as seen in Fig. 3. Otherwise, the histogram of granularity showed the size distribution of myco-synthesized AuNPs average 63.0 nm, as shown in Fig. 4. The difference in the variety of shapes and sizes of the nanoparticles is due to the difference and diversity of biological materials in the mushroom extract such as polysaccharides, phenols, amino acids and proteins that are reducing agents for gold ions, therefore it results in a large variety of shapes and sizes in AuNPs biosyhthseized from myco-materials, contrary to the consistency of the sizes and shapes of nanoparticles synthesized by chemical approaches [29] (Fig. 5).

XRD peaks in Fig. 6 located at 2θ of 38.14°, 44.41°, 64.61° and 77.52° could be attributed to the 111, 200, 220 and 311 crystallographic planes of face-centered cubic gold crystals, respectively, which corresponds with Reference Code 01-089-3697 values [30,31]. The averages of particle size of the grains are 43.21 nm, 21.57 nm, 89.21 nm, and

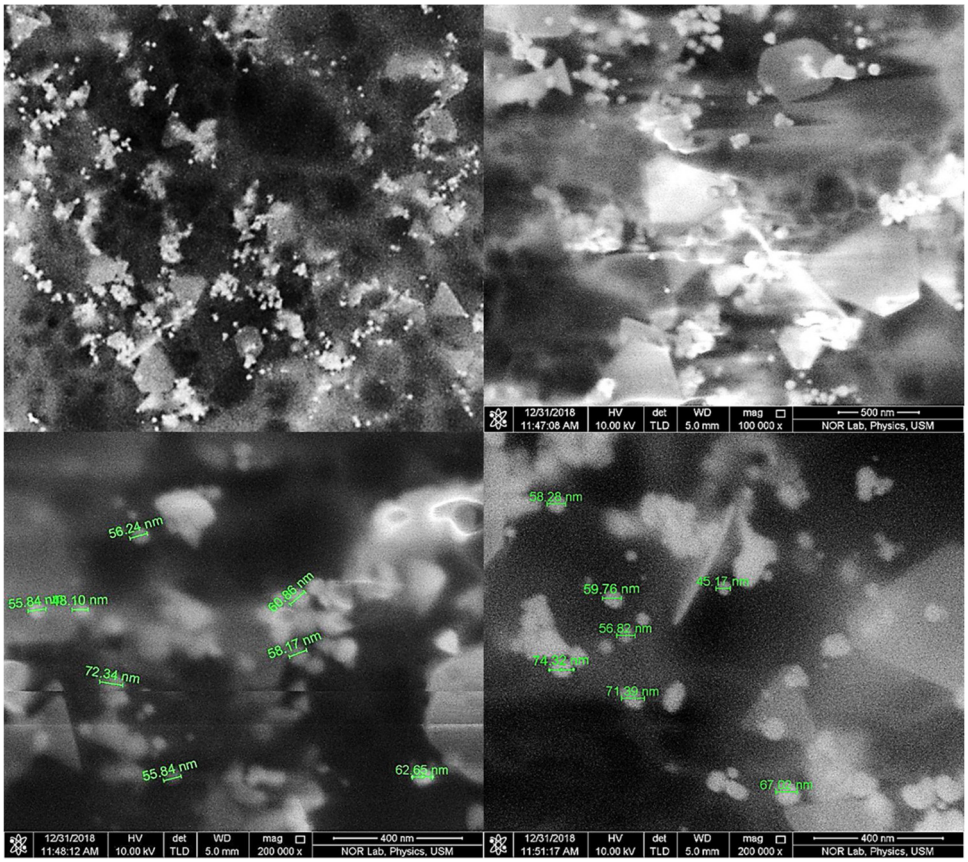

Fig. 3. FE-SEM images of the synthesized gold nanoparticles.

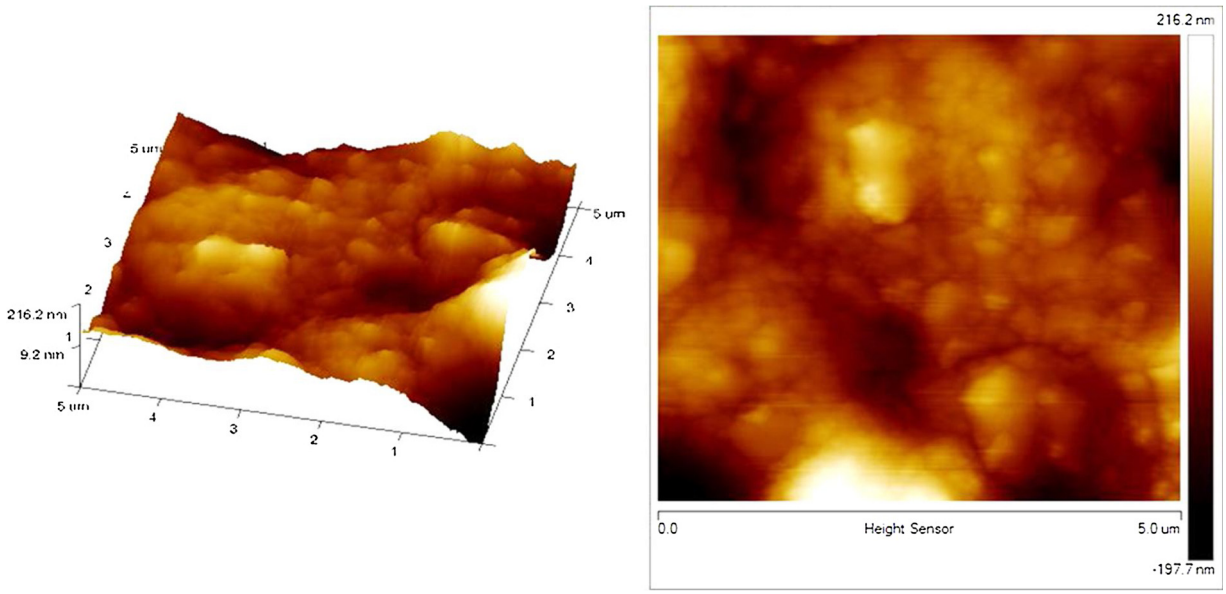

Fig. 4. AFM image of the synthesized gold nanoparticles.

35.42 nm, respectively. The peak of 111 has the highest intensity close of 84 %, pointing out that this peak is a remarkable orientation, which shows the mean crystallite size of gold nanoparticles is 43.21 nm. The pattern of XRD evidently illustrated that the mycosynthesized Au nanoparticles had a crystalline nature. The average of grains size of Au nanoparticles can be measured by the peak of X-ray diffraction using the equation of Debye–Scherrer [32]:

$$D = k\lambda / \beta \cos 2\theta$$

Where D is the average thickness of crystalline grains vertically at the plane of a crystal (nanometers), K is the Scherrer constant (0.89), β is the Full-Width Half Maximum (FWHM), θ is the angle of diffraction, and λ is the wavelength of X-ray (CuK_{α} source) of 0.15418 nm. Significant peaks with intensity percentage reached to 83.55 %, 14.74 %, 10.61 %, and 8.77 % were recorded at 2θ = 38.14°, 44.41°, 64.61° and 77.52°, respectively as shown in Fig. 6.

Fig. 7A exhibited a typical FTIR profile of the Enoki mushroom

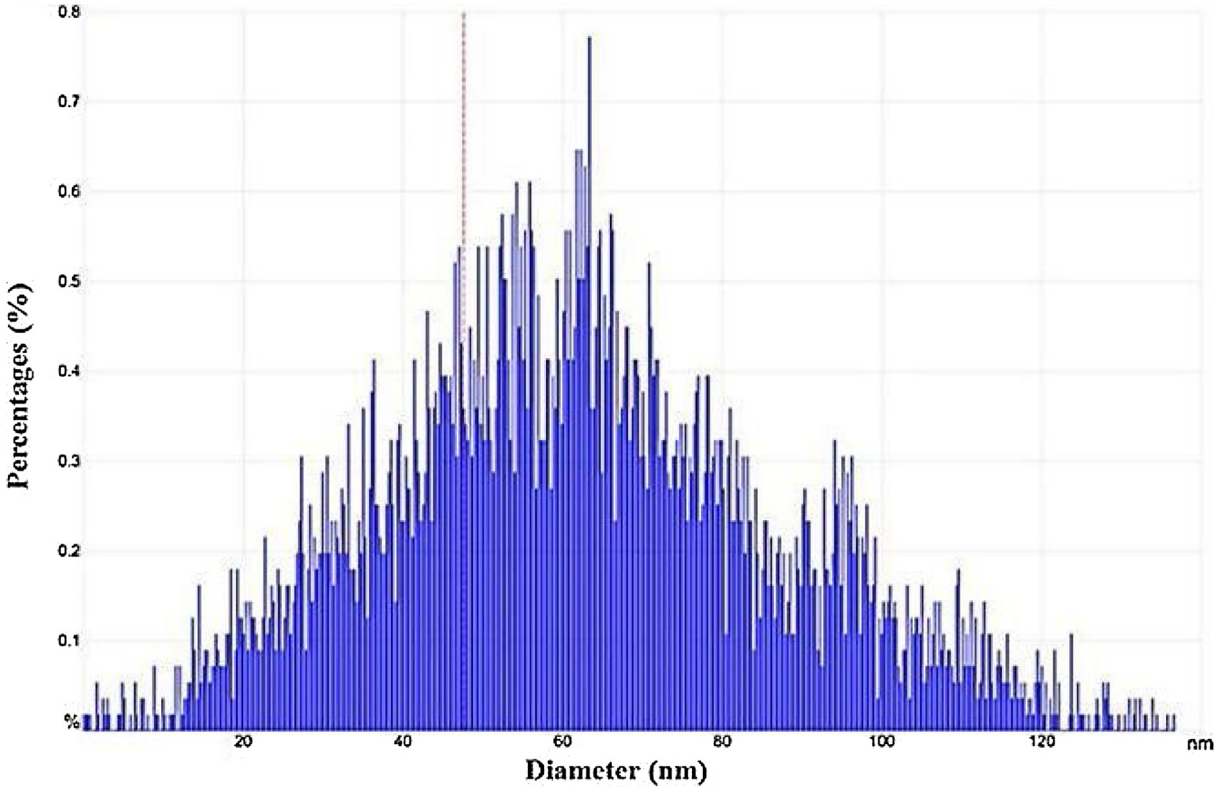

Fig. 5. Histogram of granularity of the biosynthesized gold nanoparticles.

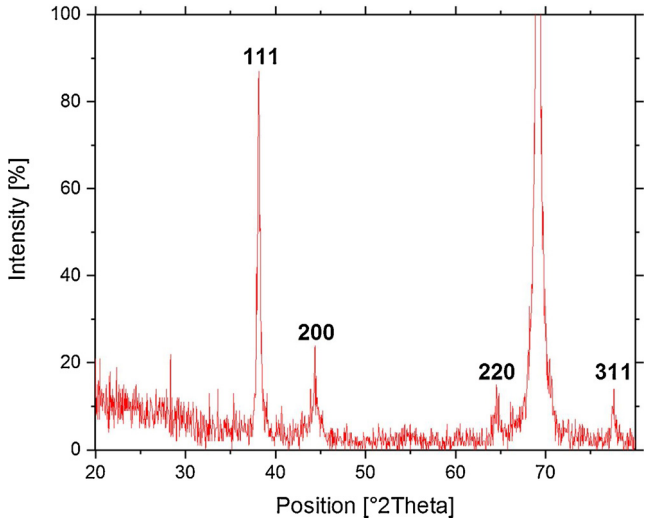

Fig. 6. XRD pattern of the produced AuNPs using extract of Enoki mushroom.

extract to be used as a reference profile. The band 3357 cm^{-1} belongs to O—H stretching vibrations in sugar while the aliphatic C–H stretching is registered to band 2950 cm^{-1} [33]. The band 2896 cm^{-1} referred to the O—H stretch in carboxylic acids, whereas the band 1634 cm^{-1} associated with the C—N stretch of amide-I in proteins. The band at 1389 cm^{-1} belongs to the stretch R—COOH group in the carboxylic acids while the band 1201 cm^{-1} may be related to the group of C—H in the polysaccharide or stretching of the vibration of phenol (C—O) and has the remarkable absorbance peak of lipids. Finally, the band 1036 cm^{-1} maybe belonged to β-D-linked glucopyranosides [34] or the stretching of the group of C—O—C in glucopyranose in carbohydrates and the group of C—O in the polysaccharide [35].

Fig. 7B exhibited the bands of the mycosynthesized AuNPs from Enoki mushroom. The band 3368 cm^{-1} exhibited the finding of the group of hydroxyl (O—H) stretching vibrations in sugar. Also, the hydroxyl group (—OH) existed in the band of 1200 cm^{-1} [36]. The band 2897 cm^{-1} reverts to —CH stretching. Moreover, the band 1634 cm^{-1} associated with the C—N stretch of amide I in proteins as in *F. velutipes* extract (Fig. 6A). The band 898 cm^{-1} belonged to the aromatic structures (—CH), whereas the band 1036 cm^{-1} related to the C—O—C group in glucopyranose in the carbohydrate. There is a slight shift in the infrared values in Fig. 7B compared with Fig. 7A of some functional groups, which were the reason for reducing the gold ions, whereas it didn't appear with other chemical groups, which have no role in the reducing.

The stability of biosynthesized AuNPs was tested by calculation of zeta potential (Fig. 8). The surface zeta potential exhibited the surface charge on AuNPs. The value of zeta potential showed the constancy of mycosynthesized nanoparticles [37]. Thus, the synthesized AuNPs exhibited a good zeta potential value of -7.47 ± 6.3 mV produced from the extract of Enoki mushroom. However, the value of zeta potential indicated good quality with an aggregation of some Au nanoparticles, because of some biological materials which led to precipitation especially which reduced gold ions and that was clear in FTIR (Fig. 7).

Many reported work has described that the surface-active bio-molecules (stabilizers) in the interaction solution created electrostatic interactions are giving more stable gold nanoparticles [38,39]. It is proposed that fungal organic molecules such as amino acids, proteins, phenol, alkaloids, glycosides, and flavonoids can act as stabilizers responsible for the mycosynthesis and constancy of AuNPs. The nanoparticles are considered to be stable if their potential surface value is between −30 mV and +30 mV [38]. Finally, the low negative value of zeta potential in this study indicated that metallic nanoparticles are surrounded by negatively charged organic compounds, which reduce repulsion among gold NPs and thus increase their stability [40].

The chemical interaction of biomolecules of mushrooms with ions of gold leads to reduce the last (Au^+) by these natural mycomaterials and form the Au atom (Au^0) [1]. The organic compounds in Enoki

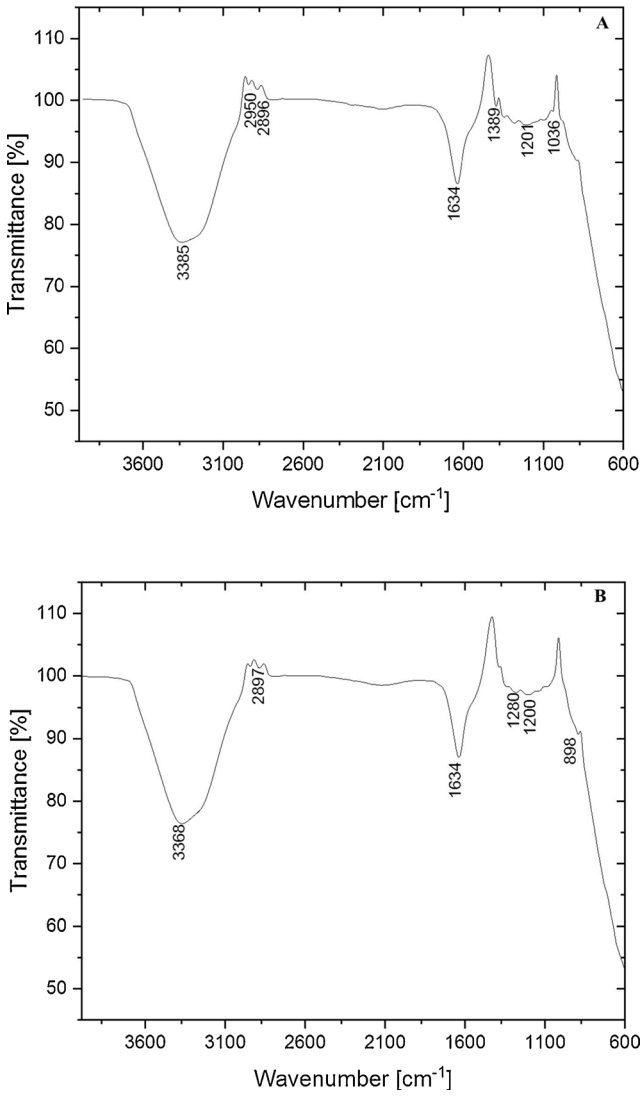

Fig. 7. FTIR of the extract of mushroom (A) and mycosynthesized gold NPs (B).

mushroom may interact with gold ions to form atoms of Au coated by polysaccharides, amino acids, and phenol is evident in the spectra of FTIR (Fig. 7A & 7B) of AuNPs in this study.

The catalytic efficacy of the mycosynthesized AuNPs using *Flammulina velutipes* was tested by observing the decolorization of the dye of MB (methylene blue). Fig. 9 exhibited the percentage of MB degradation using Bio-AuNPs at different times. The catalytic degradation of methylene blue was determined by the decreasing intensity of the absorption band at 664 nm.

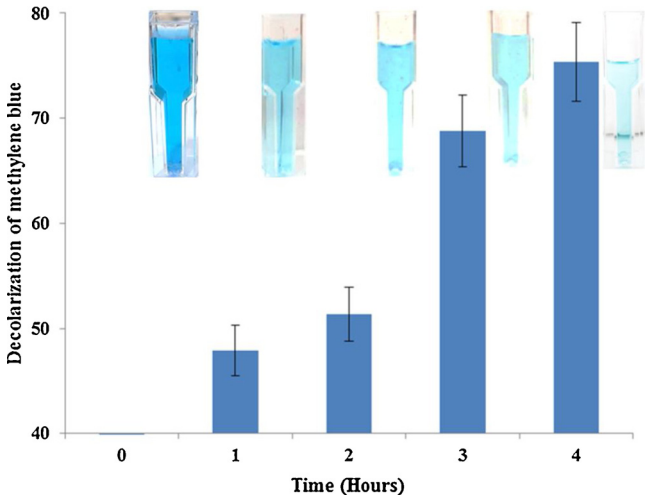

Fig. 9. Decolorization of methylene blue dye by the mycosynthesized AuNPs (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

Moreover, the band of absorption at 664 nm for MB dye that decreased gradually with the increase in the reaction time indicates that the MB dye was degrading slowly, in agreement to findings by Reddy et al. [41]. The colors of the mixed dye with colloidal AuNPs gradually changed from deep blue to light blue with time compared to the dye of MB alone which stayed deep blue as control. In other words, the catalytic activity of the mycosynthesized AuNPs from *F. velutipes* mushroom showed degrading MB to Leucomethylene Blue, as in Fig. 9. Increasing the time of incubation led to increasing the decolorization percentage of MB dye from 47.8 %, 51.3 %, and 68.8 % after 1 h, 2 h, and 3 h, respectively, to 75.3 % after 4 has observed in Fig. 9. The high surface energy of the mycosynthesized AuNPs gives an active role in reducing dyes where they act effectively as catalysts in the decolorization of methylene blue dye [42]. This also in agreement with Qu et al. [20] which proposed that AuNPs can greatly enhance rates of decolorization of organic dye with high catalytic efficacy. However, Table 1 exhibited differences among this study and others which synthesized nanoparticles from mushrooms and applied to decolorize Azo dyes.

The mechanism of MB decolorization is related to the organic materials on the surface of the AuNP. The degradation of the blue dye didn't occur to proceed in the absence of gold nanoparticles catalysts. Azo dyes bearing the functional group –N = N– are widely employed in textiles and reduced to colorless amines (–NH–NH–) in the presence of gold NPs as a catalyst [20]. Also, the mycosynthesized AuNPs are playing as catalysts in reducing MB by natural organic matters of *Flammulina velutipes,* which found on the surface of Au nanoparticles. FTIR analysis (Fig. 6) exhibited that functional groups like – OH, –NH,

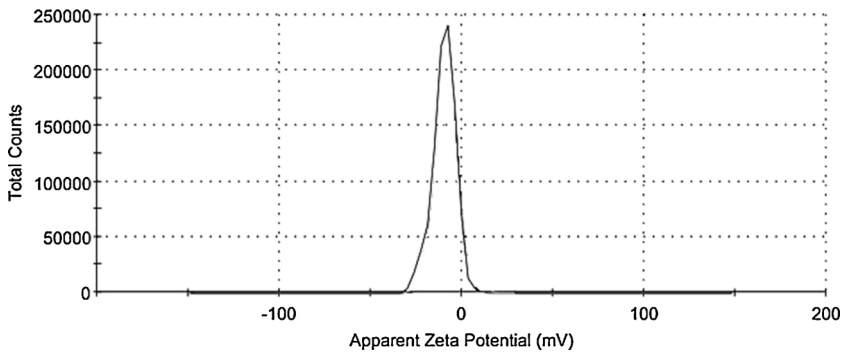

Fig. 8. Distribution of Zeta potential (mV) of the mycosynthesized AuNPs.

Table 1
Rsults of this study compared with the previously published related literature.

Species of mushroom	The used parts	Type of mttalic NPs	Shapes and sizes of of NPs	Type od dye and its concentration	Conditions and time	Activity percentages to decolour MB	References
Pleurotus ostreatus	Fruiting bodies and cell free filtrate	SeNPs	Cubic, Spherical (5 – 40 nm)	Orange dye (20 ppm)	3 hrs	39 %	[22]
Pleurotus sajor caju	mycelium culture	AgNPs	Spherical (40 nm)	Congo red (50 μM)	30 °C with shaking (24 hrs)	78 %	[21]
Agaricus bisporus	Fruiting bodies	AgNPs	Sponge-like (10 – 80 nm)	Direct blue 71 (10 ppm)	UV 265 nm (2.5 hrs)	93-96 %	[23]
Ganoderma lucidum	Fruiting bodies	AgNPs	needle-like, rod (10 – 80 nm)	Direct blue 71 (10 ppm)	UV 265 nm (2.5 hrs)	78-97 %	[23]
Flammulina velutipes	Fruiting bodies	AuNPs	triangular, spherical, irregular (74 nm)	Methylene Blue (20 ppm)	25 °C with shaking (4 hrs)	75 %	This study

and −COOH may be reduced the basic dye MB, which indicated that the adsorption process generally depended on the chemical adsorption onto the biomaterials surfaces of *F. velutipes* in Myco-AuNPs through electrostatic attraction, complexation, and coordination reaction and that agrees with [43].

4. Conclusion

The current study aims to apply fresh fruitbodies of *Flammulina velutipes* (Enoki mushroom) to myco-synthesize AuNPs (gold nanoparticles) for the first time. The UV–vis spectra exhibited a peak of absorbance at 568 nm. FTIR showed the attachments of polysaccharides, amino acids and phenol as reducer agents on AuNPs. The purple color of AuNPs indicates that the solution contains a mixture of triangular, spherical, and irregular shapes with an average of size 74.32 nm. The XRD pattern indicates that the synthesized gold nanoparticles were crystalline. The AuNPs showed the best decolorization of MB reached 75.35 % after 4 h. Finally, the mycosynthesized AuNPs by fungal natural organic materials of *Flammulina velutipes* are acting as catalysts in reducing the MB dye.

CRediT authorship contribution statement

Muwafaq Ayesh Rabeea: Conceptualization, Software, Validation, Investigation. **Mustafa Nadhim Owaid:** Conceptualization, Methodology, Formal analysis, Data curation, Writing - original draft, Project administration. **Azlan Abdul Aziz:** Writing - review & editing, Supervision, Project administration, Funding acquisition. **Mahmood S. Jameel:** Validation, Investigation, Resources, Visualization. **Mohammed Ali Dheyab:** Validation, Investigation, Resources, Visualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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