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# Mushroom-assisted synthesis of triangle gold nanoparticles using the aqueous extract of fresh *Lentinula edodes* (shiitake), Omphalotaceae

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ABSTRACT

This work aims to find a new green reducer agent for synthesis gold nanoparticles (AuNPs). Fresh fruiting bodies of shiitake mushroom (*Lentinula edodes*) were used to mycosynthesize AuNPs, which were characterized using change in color, UV–vis, FE-SEM, AFM, Zetasizer, Zeta Potential, XRD, EDX, and FTIR analyses. The purple color is a sign of biosynthesizing AuNPs. The UV–vis spectrum showed an absorption peak at 568 nm due to incitation of Surface Plasmon Resonance in AuNPs colloidal solution. FE-SEM images exhibited that most of the AuNPs have triangular, hexagonal, spherical and irregular shapes with an average of sizes reached 72 nm. The XRD pattern clearly explained that AuNPs formed in this work were crystalline. The EDX proved that the nanoparticles are AuNPs. FTIR exhibited the role of amino acids, polysaccharides and phenols as reducer agents. The value of zeta potential indicates good quality with the aggregation of particles.

## 1. Introduction

Myconanotechnology is a recent technique for the green synthesis of nanoparticles from fungi, which include molds, yeasts, and mushrooms (Owaid and Ibraheem, 2017). In past decades, the nanoparticles were synthesized using chemical methods using various reducing agents, and this approach might be threating human health when it is used in medical applications, and in removing pollutants in nature (Noruzi et al., 2011; Shang et al., 2013). In recent years, biosynthesis of nanoparticles, as a green method, is utilized for eliminating the resulting toxicity from chemical reducing agents (Virkutyte and Varma, 2011).

The green chemical synthesis of nanoparticles base on the usage of extracts of plant (Al-Bahrani et al., 2018; Noruzi, 2015; Owaid et al., 2019), algae (Kathiraven et al., 2015), mushrooms (Owaid, 2019; Owaid et al., 2015) and truffles (Owaid et al., 2018) are eco-friendly. Using mushroom in the formation of metallic nanoparticles returns to massive amounts of fruiting bodies, mycelia, enzymes that produced both within farms and in labs (Owaid and Ibraheem, 2017). Sun et al. have confirmed the manufactured of triangular nanoparticle more difficult than other shapes (Sun et al., 2003). Shankar et al. have synthesized a triangular gold nanoparticle with 50–500 nm by using the extract of lemongrass plant and chloroaurate ions (Shankar et al., 2004). However, Maryam et al. have used edible mushroom extract, that extraction by microwave irradiation, to synthesize spherical gold nanoparticles with 50 nm (Eskandari-Nojehdehi et al., 2016) and 20 nm (Eskandari-Nojedehi et al., 2018).

However, few studies have successfully achieved to synthesize triangular gold nanoparticles (Owaid, 2019) from the mushroom *Pleurotus florida* (10–50 nm) (Bhat et al., 2013), and triangular and hexagonal AuNPs from *Volvariella volvacea* (20–150 nm) (Philip, 2009), *Pleurotus sapidus* (15–100 nm) (Sarkar et al., 2013), and *Flammulina velutipes* (5–150 nm) (Narayanan et al., 2015). However, only two studies *Lentinus sajor caju* (Chan and Don, 2013) and *Lentinula edodes* (Sujatha et al., 2015) have referred to form silver nanoparticles from their mycelia extracts without mention the size of the synthesized AgNPs.

*Lentinus edodes* was used for the only first time to synthesize spherical AuNPs by Laccase and Tyrosinase inside hyphae with size ranging from 5 nm to 50 nm (Vetchinkina et al., 2013) and no published work was ever produced in the synthesis of gold nanoparticles from extracts of fruiting bodies of this macrofungus. Hence, this study is considering the first attempt the extracellular mycosynthesizing triangular and hexagonal AuNPs from the extract of *Lentinula edodes* fruitbodies.

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## 2. Materials and methods

### 2.1. Mushroom samples

One hundred fifty grams of fresh fruiting bodies of shiitake mushroom (*Lentinula edodes*) were obtained from the market in the local surroundings of Penang Island, Malaysia. This mushroom has cultivated in Champ Fungi, Sdn. Bhd., Klang, Malaysia.

### 2.2. Extraction of shiitake mushroom

#### 2.2.1. Preparation of mushroom extracts

The shiitake mushroom aqueous extract was achieved by extraction of 20 *g* of finely cut mushroom in a 200 ml flask with 150 ml D.W and then boiled for 10 min and removed the heating source to cool in the room temperature. The combined extract was filtered and centrifugation at 25 °C for 10 min at 6000 rpm, Next, the final solution obtained was storage at −18 °C to use later.

#### 2.2.2. Green synthesis of Au nanoparticles

Biological synthesis of AuNPs was performed by using the following procedure. Briefly, 0.001 M Chloroauric acid ($HAuCl_4.4H_2O$) solution (was obtained from Sigma Aldrich, Germany) was added in a 200 ml Erlenmeyer flask with 135 ml of distilled water and vigorously stirred with heated to 80 °C then, 15 ml of shiitake mushroom extract solution was added to the flask and increase the temperature to 100 °C after 25 min the mixture color change from yellow to colorless, after 30 min, the solution color changed to light purple in this time 3 ml of the solution took for UV–vis test, then after every 15 min extra, 3 ml of the solution was taken (at 30, 45, 60, 75 and 90 min) until the color of the mixture became fixed at the dark violet which pointing to the nucleation process had occurred. Au nanoparticles solution was stirred continuously for an extra 10 minutes with removing the heating source to cool at room temperature. The final product was stored at 4 °C.

#### 2.2.3. Characterization of Au nanoparticles

The gold nanoparticles synthesized from *L. edodes* extract were characterized using change in color, UV–vis spectrum, FE-SEM, AFM, Size Distribution by intensity using Zetasizer (Zetasizer Ver. 7.11, Malvern Instruments Ltd), Zeta Potential (Malvern Ver. 2.3, Malvern Instruments Ltd, 2008), XRD, EDX and FTIR analyses. All these tests and analyses were conducted at USM (Universiti Sains Malaysia), Malaysia.

## 3. Results and discussion

The extracellular recovery of gold (Au) by fungal reduction of $Au^+$ ion using the shiitake mushroom *Lentinula edodes* fruitbody has not been investigated previously. Fig. 1 exhibits that the color of the $10^{-3}$ M gold salt (Chloroauric acid: $HAuCl_4.4H_2O$) mixed with the shiitake mushroom (*L. edodes*) extract has changed from colorless to purple. The purple color is a sign for biosynthesizing the gold nanoparticle (AuNP) in this study that agrees with optical photographs of AuNPs, which mycosynthesized using the mushroom *Pleurotus cornucopiae* var. *citrinopileatus* (Owaid et al., 2017). In this study, there are five periods have applied to achieve the formation of AuNPs from fruiting bodies of *L. edodes,* which including 30, 45, 60, 75, and 90 min at 80 °C on the magnetic stirrer hotplate. The solution turned light purple after 30 min, indicating the initial synthesis of AuNPs. The color intensity has increased with the increase in reaction time, indicating that the AuNPs are more intensity like at 90 min compared with 30 min, as in Fig. 1. Visual analysis increased with increasing the interaction time, which may be allowed to other organic compounds to reduce more gold ions in the mixture (Owaid et al., 2017). On the other hand, the intensity of AuNPs relates to proteins and polysaccharides which reduced and capped those nanoparticles as mentioned by some previous studies (Philip, 2009). At an earlier work, AuNPs has formed at light conditions after 12 h (Bhat et al., 2013).

The UV–vis spectrum of gold nanoparticles (AuNPs) was recorded using the spectrophotometer operated at a resolution of 1 nm from a range of 400–700 nm and observed absorption peak at 568 nm due to incitation of Surface Plasmon Resonance (SPR) in AuNPs colloidal solution. UV–vis spectra show differences in the top of peaks according to interaction time. As in Fig. 1, absorbance peaks of AuNPs colloidal transferred from 558 nm to 568 nm at 30–90 min. At 30 min, the higher peak was 0.581 $cm^{-1}$ then the absorbance increased to 0.840 $cm^{-1}$, 0.890 $cm^{-1}$, and 1.262 $cm^{-1}$ after 45, 60, and 75 min from interaction time for the following wavelengths 560 nm, 562 nm, and 566 nm. However, at 90 min, AuNPs recorded the highest absorbance 1.411 $cm^{-1}$ at highest peak 568 nm in this study. The extrusive relation between increasing color intensity with wavelength of absorbance was evident. UV–vis spectra provided SPR phenomenon exists for gold (Singh et al., 2016). It is observed from UV–vis spectrum that the gold SPR peak takes place at 568 nm and this absorbance steadily increases with increasing time of reaction. The redshift, broadening and splitting of the surface plasmon resonance is probably due to the dampening of the SPR caused by the change in the refractive index of the surrounding medium and increase in sizes of metallic nanoparticle in colloidal solution (Basavaraja et al., 2008).

Fig. 2 exhibited the shape and size of the biosynthesized AuNPs from *Lentinula edodes* (shiitake mushroom). Most of the mycosynthesized AuNPs have triangular, hexagonal, spherical, and irregular shapes, as in FE-SEM images (Fig. 2). The average of the size of gold NPs was 72 nm, as in Fig. 2. Otherwise, Fig. 3 showed topographical pictures 2D, and 3D of the surface of AuNPs with thickness reached 77.7 nm. Granularity Cumulation Distribution chart as seen in Fig. 3 exhibited the size distribution of the mycosynthesized gold nanoparticles average 72 nm, which started from 1 nm to 145 nm in different percentages (Fig. 4).

XRD peaks in Fig. 5 located at 2θ of 38.13°, 44.47°, 64.68° and 77.66° could be ascribed to the 111, 200, 220 and 311 crystallographic planes of the face-centered cubic gold crystals respectively which agrees with the values (Ref. Code 01-089-3697) (Abod et al., 2017; Ghareib et al., 2016). The (111) peak has the highest intensity, indicating that (111) is the preferred orientation. The result showed that the mean crystallite size of AuNPs is 34.54 nm. The XRD pattern clearly explained that AuNPs formed in this work were crystalline. The average grain size of AuNPs can be calculated using the X-ray diffraction peak by Debye–Scherrer equation (Abdelrahim et al., 2017):

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

Where; D is the average thickness of the crystal grains perpendicular to the crystal plane (nm). K is the Scherrer constant equal (0.89). β is the Full-Width Half Maximum (FWHM). λ is an X-ray wavelength ($CuK_\alpha$ source) of 0.15418 nm, θ is the diffraction angle, reflected at 2θ = 38.13°, 44.47°, 64.68° and 77.66°. The average particle size of the grains is 34.54 nm, which is 43.62 nm, 70.04 nm, and 49.68 nm, respectively.

Fig. 6A exhibited FTIR of the shiitake mushroom extract. The band 3357 $cm^{-1}$ belongs to OH− stretching vibrations in sugar. The band 2941 $cm^{-1}$ belongs to aliphatic C−H stretching (Xiang et al., 2016). However, the band 2890 $cm^{-1}$ referred to OH− stretch in carboxylic acid. While the band 1633 $cm^{-1}$ related to CN− stretch of amide-I in protein. The following bands at 1398 $cm^{-1}$ and 1350 $cm^{-1}$ belong to the group of R−COOH stretch in a carboxylic acid. The band 1280 $cm^{-1}$ maybe belonged to the CH− group in polysaccharides or to stretching vibration of phenols (C−O) and has characteristic absorption peak of the lipids. The band 1092 $cm^{-1}$ may be related to β-D-linked glucopyranoside (Li et al., 2012) or the stretching of the C−O group in polysaccharides and the group of C−OC− in glucopyranose in carbohydrates (Radzki and Kalbarczyk, 2010). Finally, the band 896 $cm^{-1}$ indicates the presence of the −CH aromatic structures or Out-Plane bending of $CO_3^{-2}$.

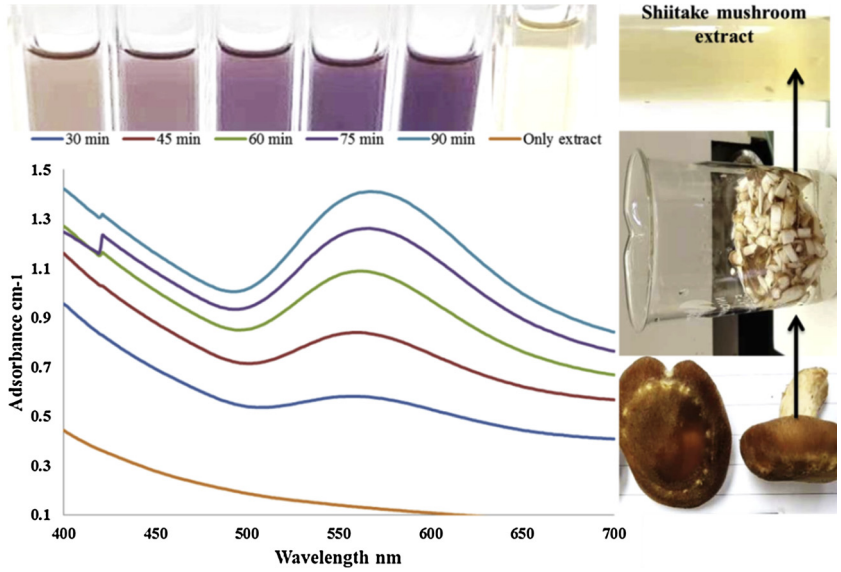



**Fig. 1.** Visual and UV–vis analysis of the biosynthesized AuNPs.

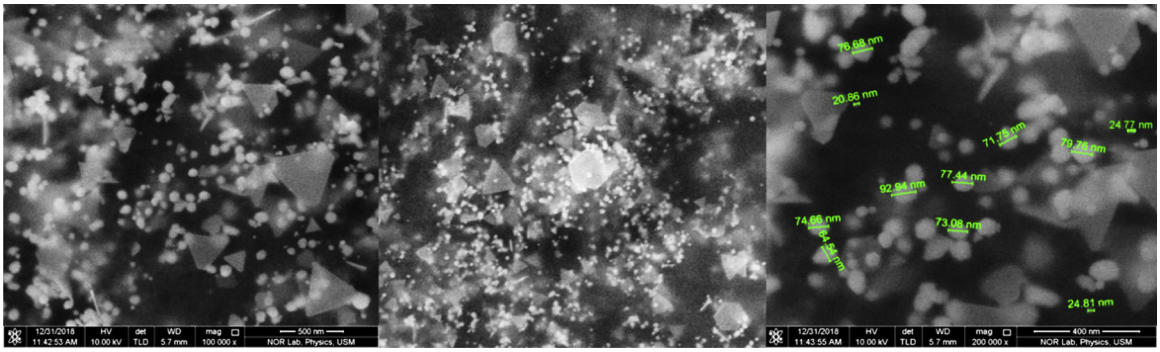


**Fig. 2.** FE-SEM images of the mycosynthesized AuNPs.

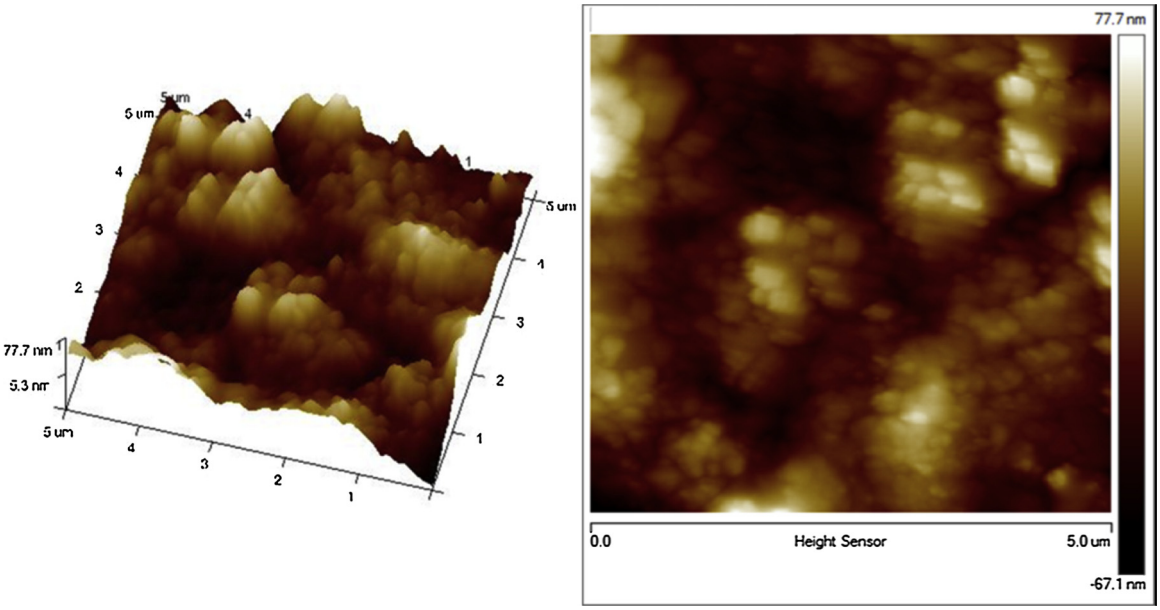



**Fig. 3.** AFM image of the mycosynthesized AuNPs.

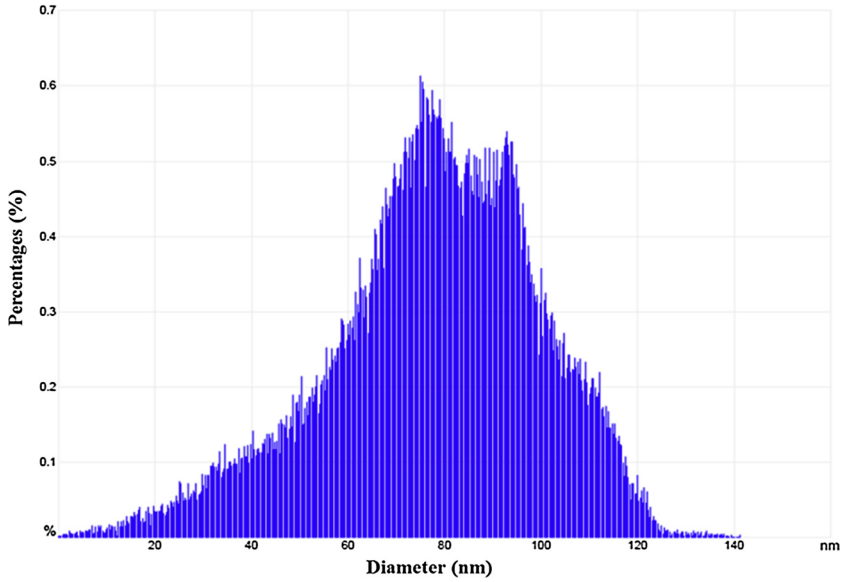



**Fig. 4.** Histogram of granularity (distribution of sizes) of the mycosynthesized AuNPs.

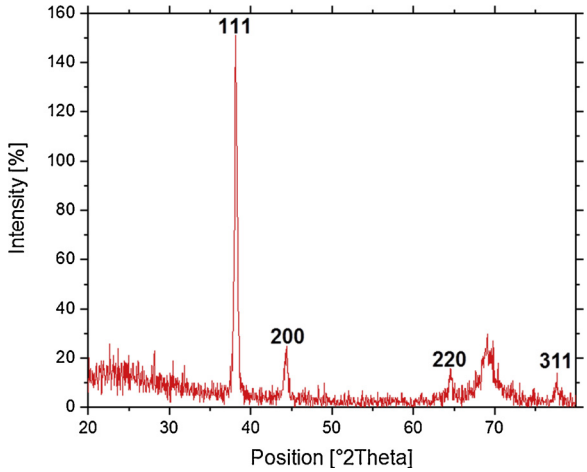
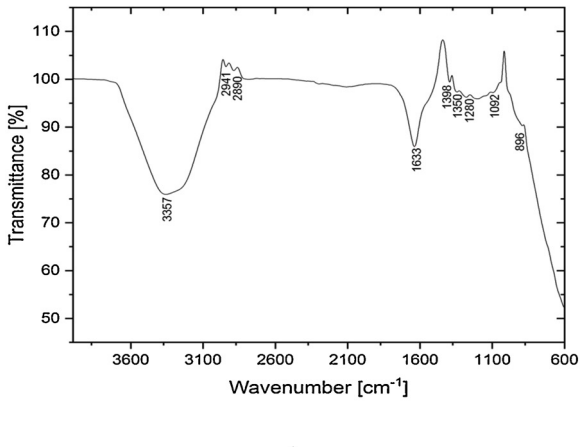
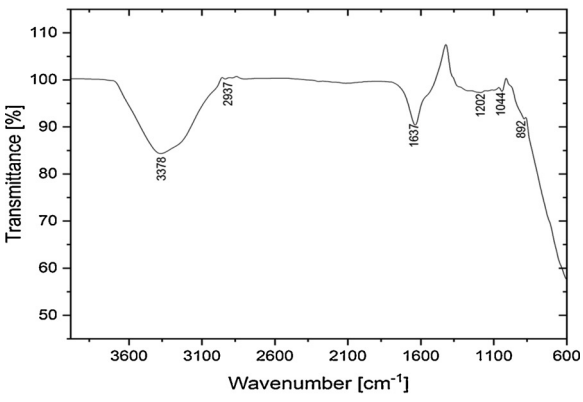



**Fig. 5.** XRD of Au nanoparticles produced from shiitake mushroom extract.




A




B

**Fig. 6.** FTIR of mushroom extract (A) and the biosynthesized AuNPs (B).

Fig. 6B showed the bands of the nanogold, which synthesized from shiitake mushroom. The band 3378 $cm^{-1}$ showed the presence of the hydroxyl group (O—H) stretching vibrations in sugar. The hydroxyl group (—OH) also found at the band of 1202 $cm^{-1}$ (Socrates, 2004). The band 2937 $cm^{-1}$ belongs to C—H stretching. Also, the band 1637 $cm^{-1}$ belongs to the CN— stretch of amide-I in protein as in the sample of mushroom extract alone. The band 1044 $cm^{-1}$ belongs to the group of C—OC— in glucopyranose in carbohydrates, while the band 892 $cm^{-1}$ belongs to the C—H aromatic structures.

The stability of mycosynthesized gold nanoparticles was investigated by measurement of zeta potential. Fig. 7 exhibited surface zeta potential which was achieved for recording the surface charge on the gold nanoparticle. The value of zeta potential indicates the stability of the biosynthesized particles (Srikar et al., 2016). The synthesized AuNPs showed good zeta potential value of -2.26 ± 8.3 mV prepared from shiitake mushroom extract. This value of zeta potential indicates good quality with aggregation of particles. The negative zeta potential value refers to that the metallic NP is surrounded by negative organic molecules, which reduces repulsion among the AuNPs, prevents the aggregation, and then increases its stability (Suresh et al., 2011).

Many kinds of research have referred to that the surface-active biomolecule (stabilizer) in the mixture of reaction creates electrostatic interactions giving more stable AuNPs (Ahmad et al., 2015; Anand et al., 2015). It is suggested that organic biomolecules like proteins, amino acids, phenols, flavonoids, alkaloids, and glycosides can act as surface-active molecules responsible for the synthesis and stability of gold NPs. The nanoparticle is considered to be stable if its potential surface value is between −30 mV and +30 mV (Anand et al., 2015). However, the stable dispersion of AuNPs is evident, as in Fig. 7 (the zeta

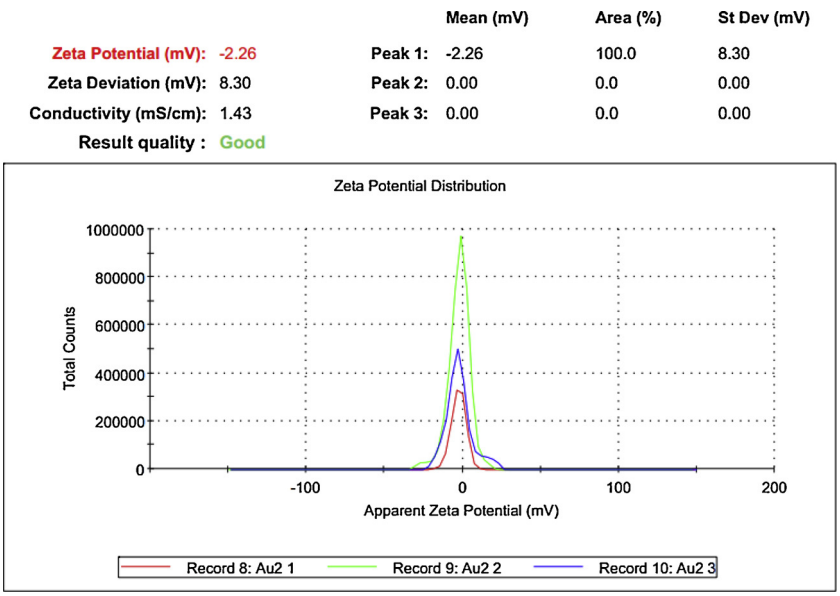



**Fig. 7.** Zeta potential distribution (mV) of mycologically synthesized gold nanoparticles.

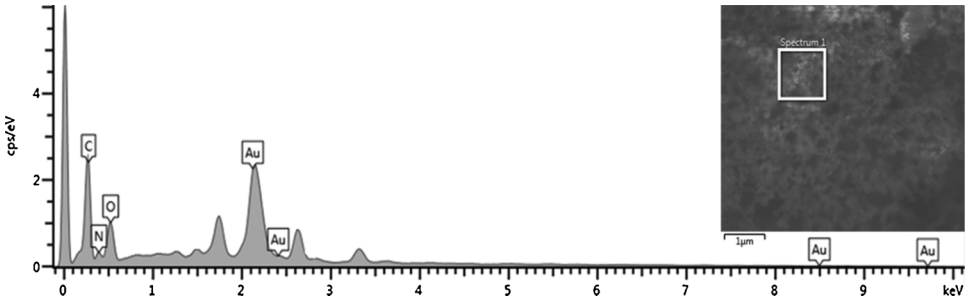



**Fig. 8.** EDX of mycologically synthesized gold nanoparticles.

potential of −2.26 mV).

The spectrum of EDX of the mycosynthesized AuNPs, as in Fig. 8, exhibits finding the gold element (42.76 %) as an indicator for producing Au nanoparticles from L. *edodes* fruiting bodies extract. Also, it shows the C (33.46 %), O (9 %), N (4.74 %), and Cl elements have been presented in the employed specimen. Carbon, nitrogen, and oxygen are shown in this specimen because of the organic molecules found in the mushroom extract. These biomolecules may be amino acids, polysaccharides, or phenols which capped the gold NPs as capping and stabilizing agents. The presence of the elemental Au can be seen in the EDX spectrum (Fig. 8) that proves the reduction of gold ions to the element Au (Owaid et al., 2017).

The chemical interaction between biomolecules of mushrooms such as polysaccharides, proteins, amino acids, and phenols and gold ions relates to reducing Au + by these natural organic myco-materials and form $Au^0$ (gold atom) (Owaid and Ibraheem, 2017). The EDX image (Fig. 8) illustrates the presence of carbon, nitrogen, and oxygen. These organic matters are considered essential of organic compounds in the shiitake mushroom, and these molecules interact with ions of gold and form gold atoms covered by amino acids, polysaccharides, and phenols, as shown in FTIR spectra (Fig. 6A & B) of AuNPs in this study.

## 4. Conclusion

This paper aims to use fresh fruiting bodies of shiitake mushroom (*Lentinula edodes*) for the first time to mycosynthesize gold nanoparticles (AuNPs). These AuNPs were characterized using change in color, UV–vis, FE-SEM, AFM, Zetasizer, Zeta Potential, XRD, EDX, and FTIR analyses. The UV–vis spectrum showed an absorption peak at 568 nm due to the incitation of Surface Plasmon Resonance in AuNPs colloidal solution. FTIR exhibited the role of amino acids, polysaccharides and phenols as reducer agents. FE-SEM images showed that most of the AuNPs have triangular, hexagonal, spherical, and irregular shapes with average of sizes of were 72 nm. The EDX proved that the nanoparticles are AuNPs. The XRD pattern clearly explained that AuNPs formed in this work were crystalline.

## Declaration of Competing Interest

None.

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