

## Green Synthesis of Gold Nanoparticles from *Coprinus comatus*, Agaricaceae, and the Effect of Ultraviolet Irradiation on Their Characteristics

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### Abstract

The present research aims to produce gold nanoparticles (AuNPs) from the aqueous extract of locally isolated mushroom *Coprinus comatus* from Hit city, Iraq. Its properties were studied using the optical vision, UV-Vis, EDX, XRD, FTIR, AFM, and Zetasizer analyses. The exposure of the colloidal solution of AuNPs to UV radiation was investigated for 1, 2, and 3 h. The results showed the color change of the interaction mixture from light yellow to purple after 25 min. The lambda max of the absorbance reached 530 nm using UV-Visible spectrum as evident in the formation of AuNPs. FTIR spectra revealed the presence of functional groups related to peptides, proteins, flavonoids, monosaccharides, and phenolic compounds, which reduced gold ions. The EDX technique showed that the formed nanoparticles were AuNPs. XRD results showed that AuNPs have a face-centered cubic (fcc) crystal. The UV irradiation at different times led to an increase in the intensity of absorbance and sizes of AuNPs from 17.39 nm before the irradiation and switched to 58.16, 59.13, and 47.35 nm after 1, 2, and 3 h, respectively, but their sizes remained within the nanoscale range (less than 100 nm). In conclusion, the best result was observed after about an hour on the effects of UV irradiation on sizes of AuNPs, which reached smaller nanoparticles compared with times 2 and 3 h.

**Keywords:** AuNPs, Mycosynthesis, Shaggy mane, Ultraviolet irradiation, XRD

### Introduction

Green synthesis of metallic nanoparticles is considered an ecofriendly approach. This method has been used worldwide in recent studies as nano drugs against cancers, bacterial and fungal infections, and other inflammations [1]. Mycological materials were utilized as myco-reducer to synthesize green nanoparticles, which is called myconanotechnology. Mycomaterials include purified materials such as enzymes/proteins, polysaccharides, polysaccharides protein complexes, or crude extracts such as fruiting bodies extracts, fungal mycelia extracts, and free cell filtrate. Green chemistry methods have attempted to myco-synthesize silver, gold, iron, NPs, and others using mycomaterials in different ways. The green mushroom nanoparticles were investigated as antimicrobial, antioxidant, antitumor agents [2].

The production of metallic nanoparticles from mushrooms is one of the recent studies that tend to use fruiting bodies or mycelia as a reducing agent in the formation of nanoparticles in a clean and environmentally friendly approach. This specialty lies within the biosynthesis of nanoparticles, also called

Mycosynthesis of NPs [3]. The volume of the produced mushrooms is significant and economical in introducing this fresh food into the formation of metallic nanoparticles used for biomedical treatments [4]. The mycosynthesized gold nanoparticles from mushrooms were used as anticancer agents [5,6]. These nanoparticles also were applied in the decolorization of dyes [7]. The mycosynthesized AuNPs from mushrooms were succeeded by using few edible mushrooms, including *Pleurotus sapidus* [8], *Pleurotus florida* [5,9], *Pleurotus ostreatus* [7,10], *Pleurotus cornucopiae* [11], *Lentinula edodes* [12], and *Flammulina velutipes* [13].

*Coprinus comatus* (Shaggy mane) is one of the medical and edible mushrooms belonging to the family of Agaricaceae. Its extracts were used in treating Alzheimer's disease [14]. It has many medical benefits such as antibacterial [15], anticancer, anti-oxidant [16,17], hepatoprotective, anti-inflammatory [18], anti-androgenic [19], anti-diabetic [20], anti-obesity [21], and acetylcholinesterase inhibitory [22] activity. From the literature, no research used *Coprinus comatus* for synthesizing any metallic nanoparticles.

Hence, this study is considered the first to attempt to mycosynthesize AuNPs from the extract of *Coprinus comatus* fruiting bodies, and it aims to screen the influence of ultraviolet irradiation at different times on characteristics of AuNPs, and their crystalline nature.

## Materials and methods

### Mushroom samples

The fruiting bodies of the fungus *Coprinus comatus* (Shaggy mane), Agaricaceae, were collected from one of the groves of the city of Hit, Iraq. Morphological characteristics authenticated the species level as per the Atlas of mushrooms for Hall *et al.* [23]. The common name is which belongs to. The fungal samples were cleaned, chopped, and used in future tests to mycosynthesize gold nanoparticles (AuNPs).

### Extraction of *Coprinus comatus* fruiting bodies

The aqueous extract was prepared by taking 30 g of fresh fruiting bodies of *Coprinus comatus* boiled (100 °C) in 200 mL distilled water (DW) on the hotplate magnetic stirrer for 10 min. Then it was filtered with filter paper (Whatman No.1) with a centrifuge at 4,000 rpm. The filtrate was used in the mycosynthesis of AuNPs. The watery extract was stored at four °C until using it to synthesize AuNPs.

### Mycosynthesis of gold nanoparticles

Ten millimeters of the mushroom extract was mixed with 90 mL of 1 mM Chloroauric acid ( $\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$ ) solution (purchased from Direvo Industrial Biotechnology, Germany). The extract was left on the hotplate magnetic stirrer for 25 min at 80 °C until the color changed from bright yellow to purple [12].

### Characterization of mycosynthesized AuNPs

The properties of the mycosynthesized AuNPs were studied using the change in color, UV-Visible spectrum, FTIR (Bruker, Germany, Tensor 27 Model), Zetasizer, AFM (AFM model AA 3000 SPM 220 V-Angstrom Advanced INC.), XRD (Model 6000 X-Ray, SHIMADZU in Central Organization for Standardization and Quality Control, Baghdad), and EDX (Energy Dispersive X-Ray Spectroscopy, Bruker Company, Germany, X Flash 6L10 Model in Al-Nahrain University, Baghdad).

### The exposure to UV radiation

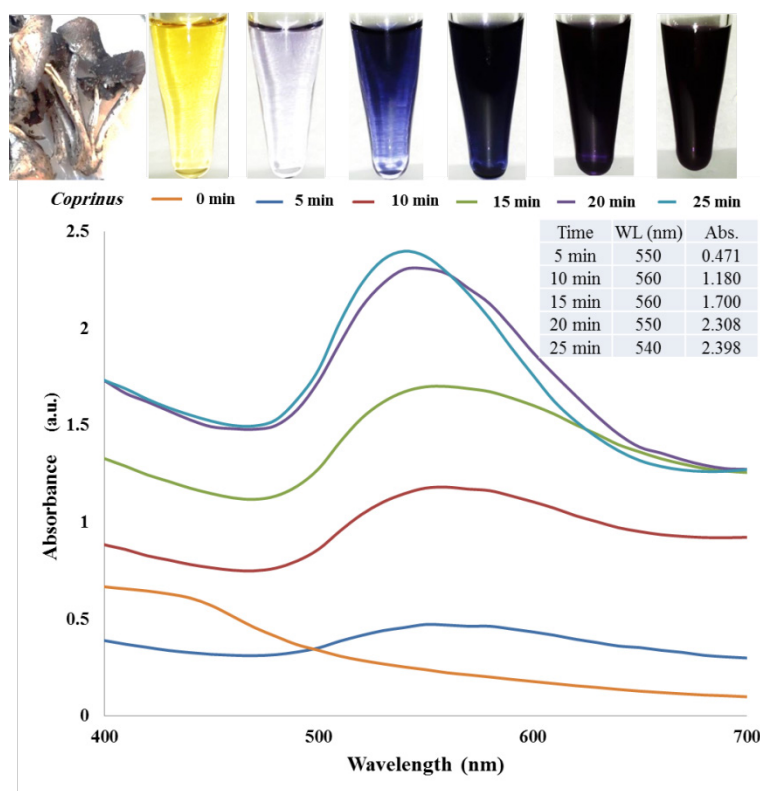
Samples of the colloidal AuNPs were exposed for 1, 2, 3 h with the UV lamp (wavelength 256 nm), at a temperature of 15 °C [24]. The characteristics of the various samples were measured before and after they were exposed to UV radiation. FTIR and UV-Vis were achieved to see the changes in these samples.

## Results and discussion

### Optical vision and UV-Visible analyses

The optical vision of the mycosynthesized AuNPs is presented in **Figure 1**. The mixture color of the aqueous extract of *Coprinus comatus* with the HAuCl<sub>4</sub>·4H<sub>2</sub>O solution (1 mM) changed from yellow to purple after 25 min. This change is a piece of clear evidence for the synthesis of AuNPs from the mushroom due to the behavior of these NPs in the absorption of the UV-Visible [25]. This agrees with the results of Owaid *et al.* [11], who mycosynthesized AuNPs from the oyster mushroom *Pleurotus cornucopiae*.

Moreover, it was found that the highest absorption value of colloidal gold nanoparticles occurred at the wavelength of 530 nm as in UV-visible spectra (**Figure 1**). The UV-Visible spectrum supplied Surface Plasmon Resonance phenomenon discovers for Au [26]. This experiment showed that time was an affirmative act to increase the intensity of the purple color. This increase is evidence that the rise in the heating leads to an increase in the activation energy [12] and allowed to active substances of the extract to play an essential role as a reducing agent for gold ions in the form of atoms, as mentioned by Owaid *et al.* [11].

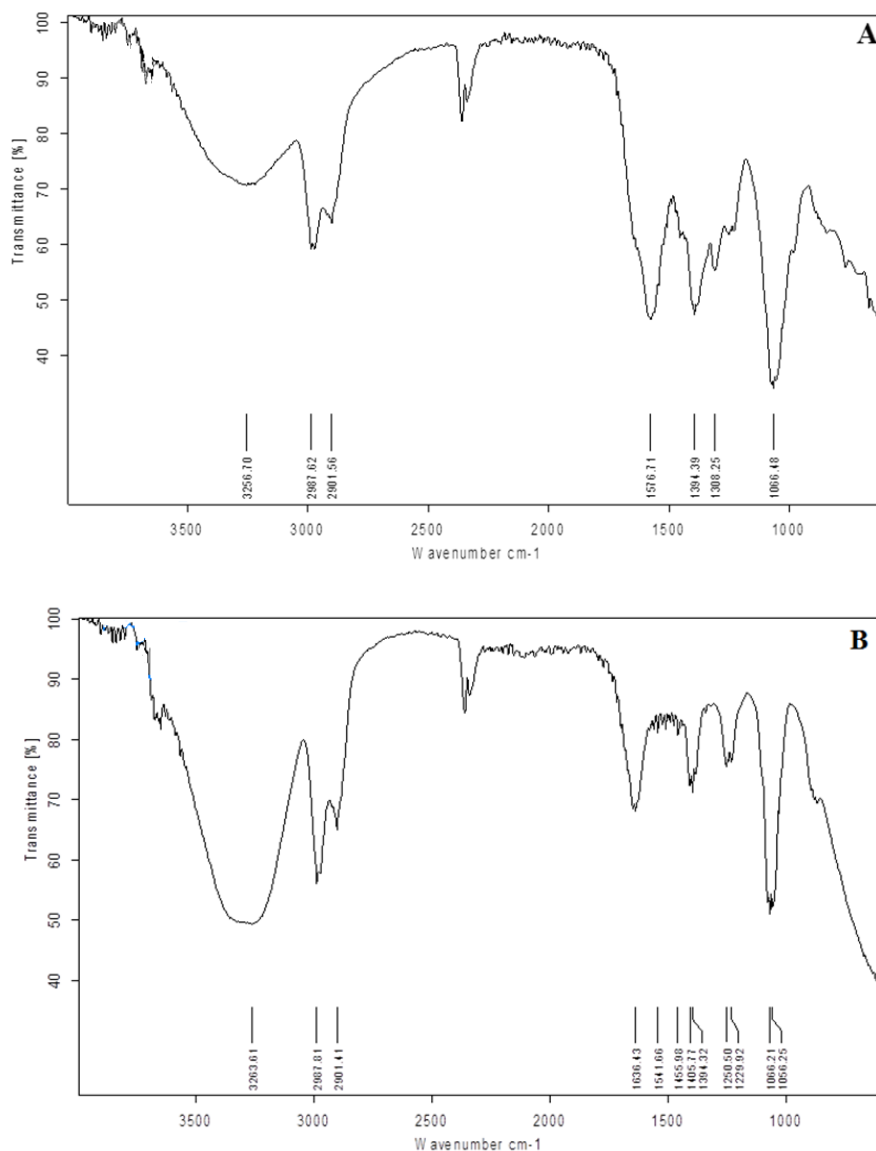


**Figure 1** UV-Visible spectra of the mycosynthesized AuNPs from *Coprinus comatus*.

**Figure 2A** showed the absorption band at 1,394 cm<sup>-1</sup>. This band is due to the homogeneous bending vibration of the -CH group and the absorption band at 2,901 and 2,987 cm<sup>-1</sup> are due to the homogeneous stretch vibration of the -CH group. In contrast, the absorption band at 1,066 cm<sup>-1</sup> belongs to the single bond (C-C) in methylene groups (CH<sub>2</sub>) and methyl groups (CH<sub>3</sub>). However, all the mentioned peaks indicate the presence of Alkane compounds such as methyl (-CH<sub>3</sub>) and methylene (-CH<sub>2</sub>) present in the composition of monosaccharides, polysaccharides, amino acids, and fatty acids [27-30]. The broad

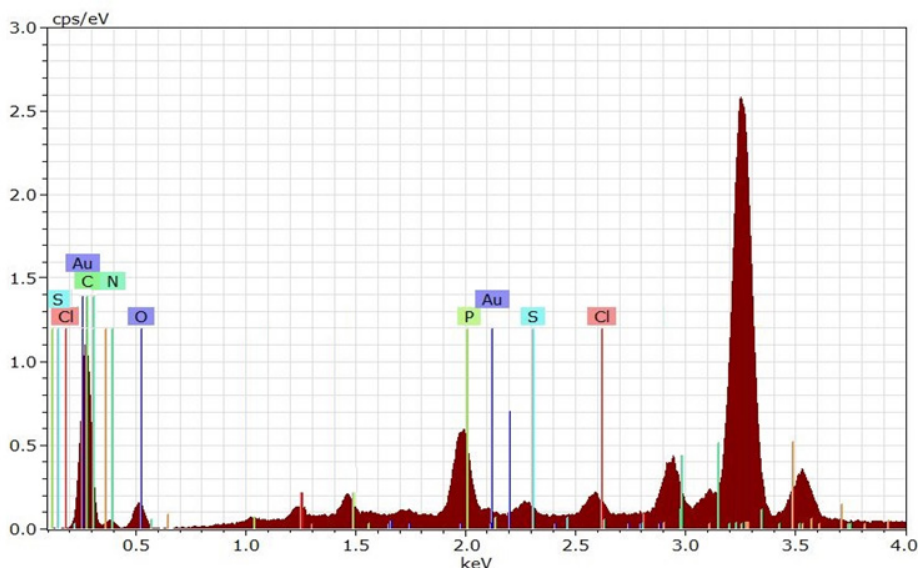
absorption band at  $3,256\text{ cm}^{-1}$  confirms the presence of a stretch vibration of the group -OH in the extract and thus confirms the finding of phenolic compounds [31], monosaccharide [18], and melanin pigment [32].

In **Figure 2B**, the FTIR spectrum of AuNPs is observed in the emergence of a new band that differs radically in its location at the site  $1,636\text{ cm}^{-1}$  compared with Figure 2A for just the mushroom extract. This band belongs to the carbonyl group (C=O), where it is believed the presence of compounds containing the amide group (NH-CO) as in peptides and proteins, as well as flavonoids, monosaccharide, and phenolic compounds [31], which covered element atoms and raised the surface area of metallic nanoparticles [24].



**Figure 2** FTIR of the mushroom extract (a) and mycosynthesized gold nanoparticles (b).

**Figure 3** represents the specific EDX analysis of the residue resulting from the interaction of the fungus extract with the gold salt. This indicates the presence of the gold component, which comes from AuNPs [11]. Generally, **Figure 3** also shows the presence of carbon, nitrogen and oxygen, due to the various organic compounds such as amino acids, fatty acids, and polyphenols present in the *Coprinus comatus* extract [18,30]. Also, the peak of chlorine may be a noise peak especially at 0 keV caused by the noise of the electronics of the detector of EDX device. However, sulfur is related with some amino acids types [33,34] and phosphorus is related to their high amounts in this mushroom [35,36]. The FTIR after 2 and 3 h did not show any significant alters in the peaks thus their images did not include here.



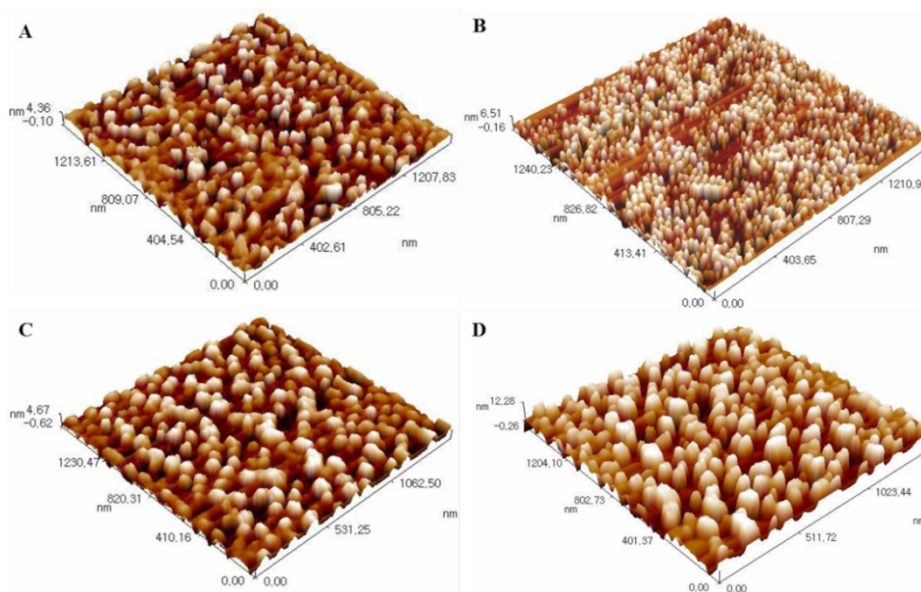
**Figure 3** EDX of the mycosynthesized gold nanoparticles using *Coprinus comatus*.

**Table 1** Amplitude, hybrid and functional parameters of gold nanoparticles with and without UV irradiation.

Parameters	Time of UV Irradiation			
	0 h	1 h	2 h	3 h
<b>Amplitude parameters</b>				
Roughness average (Sa)	0.997 nm	1.67 nm	1.25 nm	2.87 nm
Root mean square (Sq)	1.17 nm	1.92 nm	1.46 nm	3.34 nm
Ten-point height (Sz)	4.46 nm	6.60 nm	5.29 nm	6.76 nm
<b>Hybrid parameters</b>				
Root mean square slope (Sdq)	0.138 nm <sup>-1</sup>	0.34 nm <sup>-1</sup>	0.16 nm <sup>-1</sup>	0.331 nm <sup>-1</sup>
Mean summit curvature (Ssc)	-0.0155 nm <sup>-1</sup>	-0.0263 nm <sup>-1</sup>	-0.0214 nm <sup>-1</sup>	Non-detected
Surface area ratio (Sdr)	0.908	5.27	1.21	4.64
<b>Functional parameters</b>				
Reduced summit height (Spk)	0.346 nm	0.742 nm	0.491 nm	0.223 nm
Core roughness depth (Sk)	3.56 nm	5.76 nm	4.61 nm	11.20 nm
Reduced valley depth (Svk)	0.521 nm	0.15 nm	0.178	1.47 nm

**Figure 4** illustrates 4 images (A, B, C and D) of Atomic Force Microscope (AFM) including the lateral (2D) and **Figure 5** (A, B, C and D) shows 4 images of 3D (3-dimensional) that exhibit surface roughness of the mycosynthesized AuNPs at size images 1,618×1,610, 1,654×1,615, 1641×1594 and 1605×1535 nm<sup>2</sup> for AuNPs before UV irradiation (A) and AuNPs after 1, 2 and 3 h from UV irradiation (B, C and D), respectively. Surface roughness analysis exhibits various parameters like amplitude, hybrid and functional parameters, as in **Table 1**.

However, amplitude parameters had measured such as roughness average reach to 0.997 nm before UV irradiation and increased to 1.67, 1.25, and 2.87 nm after UV irradiation for times 1, 2, and 3 h, respectively. Hybrid parameters had measured, such as surface area ratio, which reached 0.908 before UV irradiation and increased to 5.27, 1.21, and 4.64 after UV irradiation for times 1, 2, and 3 h, respectively. Moreover, functional parameters had measured like core roughness depth recorded 3.56 nm before UV irradiation and increased to 5.76, 4.61, and 11.20 nm after UV irradiation for times 1, 2, and 3 h, respectively. This is an indicator to form gold nanoparticles in a small size with an average reached 49.68, 64.43, and 54.16 nm, respectively, but they stay less than 100 nm, as in **Figure 6**. These findings agree with Owaid *et al.* [24], especially for the Cumulation of AuNPs. After one h, the best results were observed about the effect of UV irradiation on sizes of AuNPs, which reached smaller nanoparticles compared with times 2 and 3 h, and this agreed with the results of Watcharaporn *et al.* [37]. In this study, only one h instead of more than three h was best to enhance small sizes of AuNPs but close of sizes of AuNPs without UV irradiation, as shown in **Figures 4, 5, and 6**. Furthermore, that leads to enhancing the properties of retention and dissolution of AuNPs to enhance gold ions release [38], and that may be exploited for reducing effects of UV on human cells by AuNPs [39]. **Figure 6** showed a range of granularity Cumulation Distribution ranged from 38 - 83 nm before UV irradiation and changed to 25 - 45, 38 - 142 and 43 - 73 nm after 1, 2, and 3 h from UV irradiation, respectively.



**Figure 4** AFM of AuNPs, 3-dimensional A: AuNPs before UV irradiation, B, C, D: AuNPs after 1, 2 and 3 h from UV irradiation, respectively.



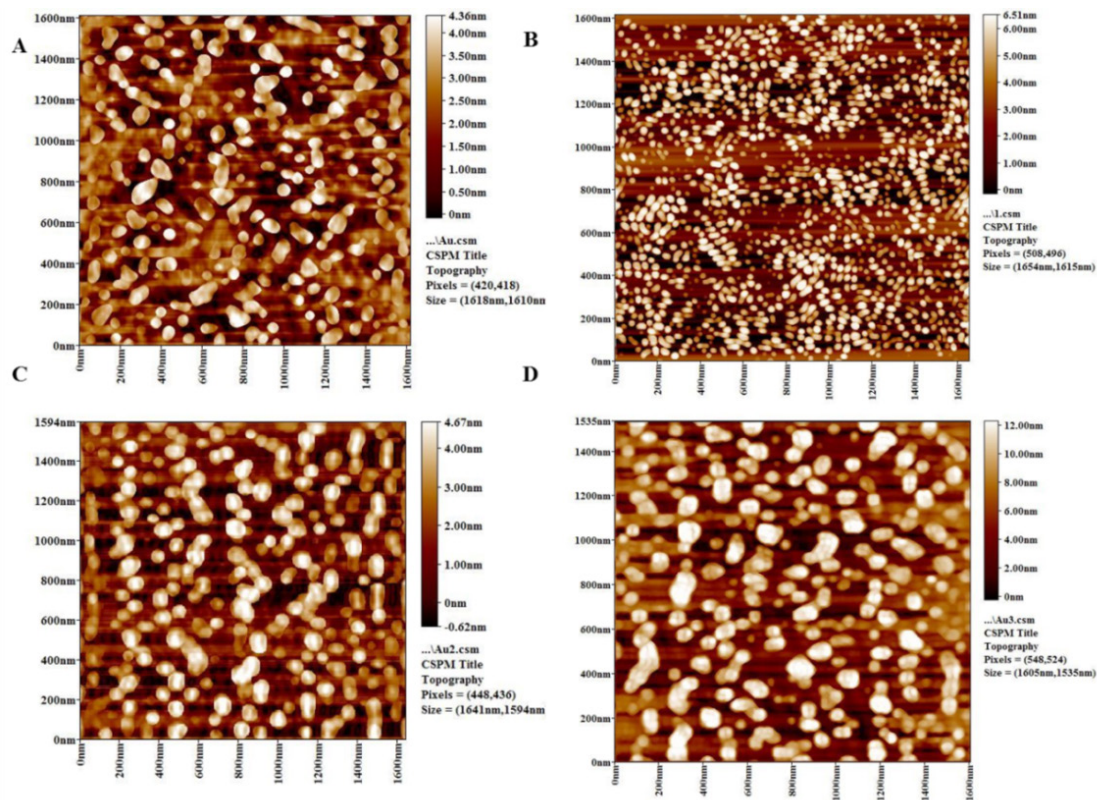


Figure 5 AFM of AuNPs, lateral dimensional.

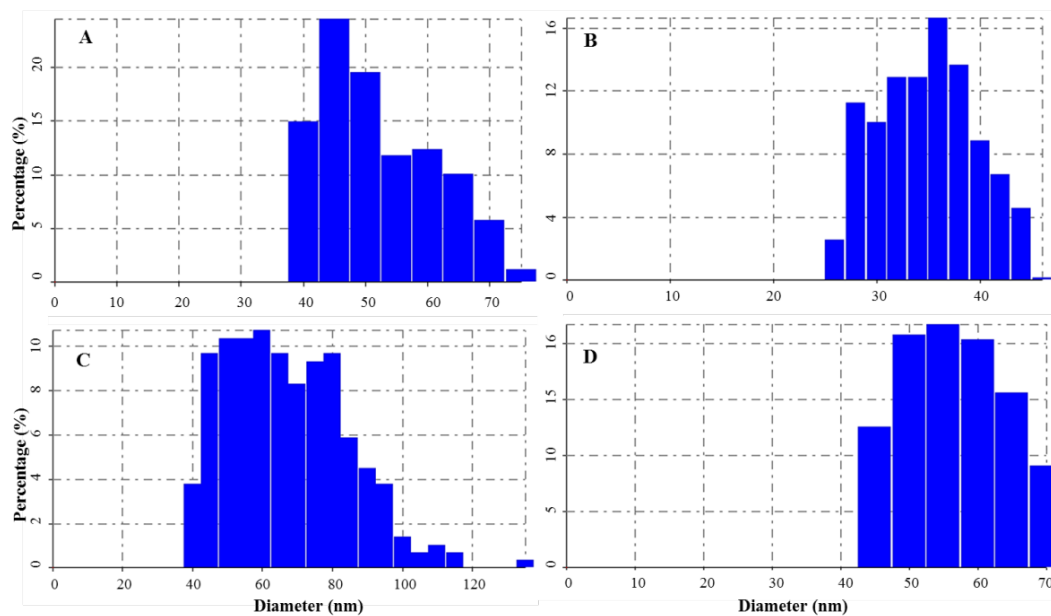
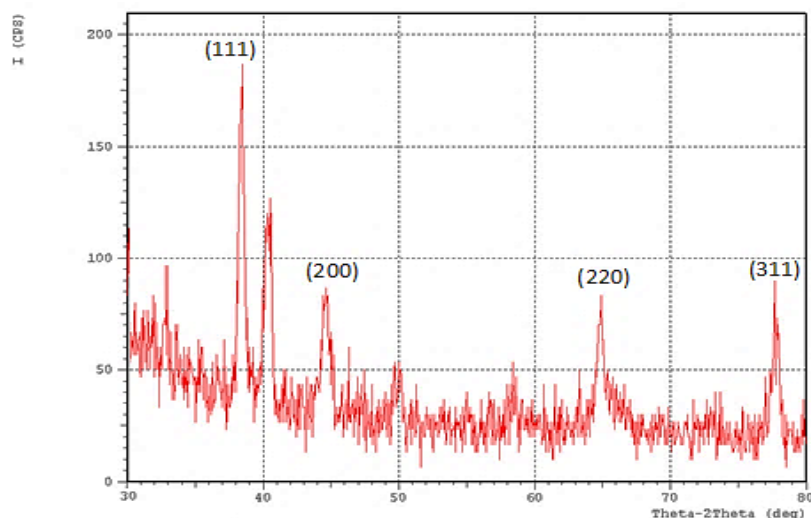


Figure 6 Histogram of granularity Cumulation Distribution of AuNPs.

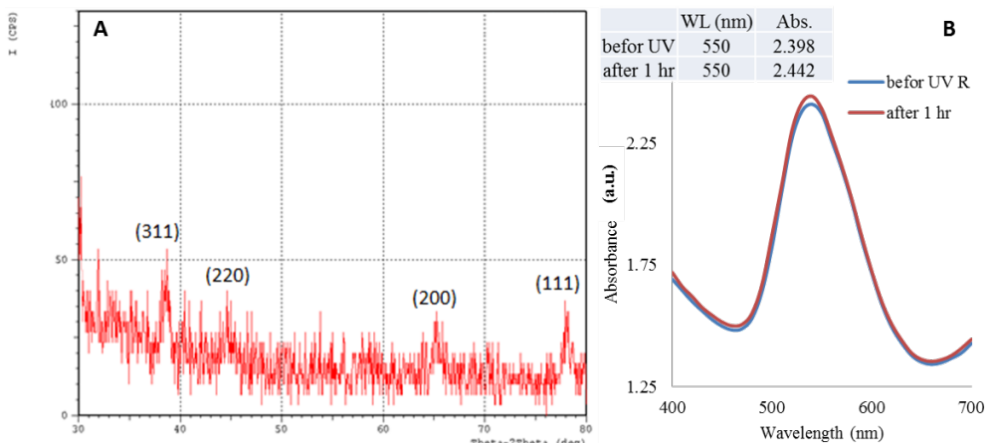
**Figure 7** shows the XRD pattern of nanoparticles before UV irradiation at  $2\theta$  values ranging from  $30^\circ$  to  $80^\circ$ ; it can be seen that 4 peaks (38.76, 44.91, 65.14 and 77.51). All these diffraction peaks correspond to the diffraction planes (111), (200), (220) and (311), respectively, were indexed to the gold metal with face-centered cubic (fcc) structure. This indicates that the precipitation consists of pure crystalline gold according to (International Center for Diffraction Data, ICDD No 4-0783). This is also consistent with previous studies [12]. The Lattice constant is  $a = 4.05 \text{ \AA}$  in good agreement with the standard diffraction pattern of cubic gold metal. From the XRD pattern, a powerful Bragg reflection of level (111) is observed, and the average particle size  $D$  is estimated using the Debye-Scherrer equation and the calculated  $D$  value of reflection is 111.39 nm.



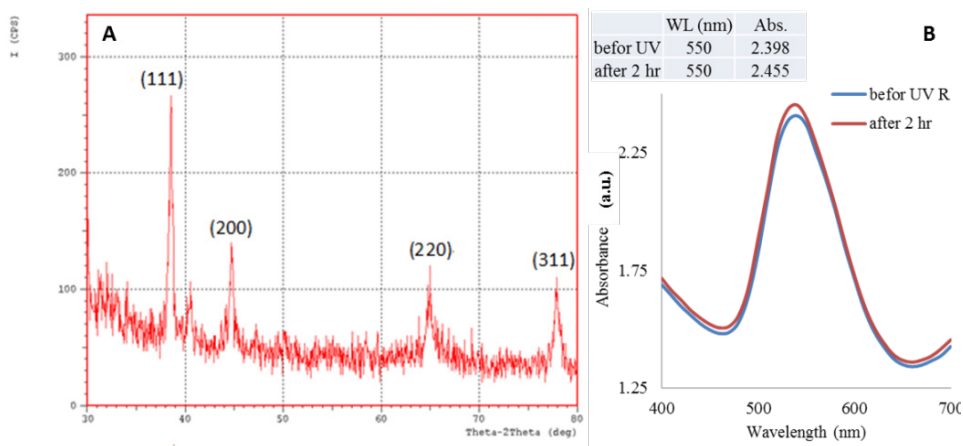
**Figure 7** The XRD pattern of nanoparticles before UV irradiation.

**Figures 8 - 10** represented UV-visible and XRD diffraction patterns of Au nanoparticles after UV irradiation for different times (3 & 2 h, one h). UV-visible spectra showed the highest peak was constant, which reached 550 nm, but the optical intensity of lambda max changed for 2.398 before UV irradiation to 2.442, 2.455, and 2.434 (a.u.) after 1, 2, and 3 h from UV irradiation and that agree with Gasperini *et al.* [40]. They reported that UV irradiation increased the optical density. The crystallite size  $D$  of reflection (111) for each figure increased to 58.16, 59.13, and 47.35 nm after 1, 2, and 3 h from UV irradiation. Also, it can be observed that the irradiation of nanoparticles by ultraviolet at different times (1, 2, and 3 h) reduces the height of X-ray intensity peaks from crystalline levels (111), (200), (220), and (311), except for the 2-hour radiation period (Figure 9), we again observe the peaks of the intensity of the reflection of crystalline levels due to the effect of UV radiation, like ionizing radiation, on the electron distribution in crystals. As a result of the response of electrons to the high frequencies of ultraviolet waves, the material's electronic structure is destroyed because electrons move from lower energy levels to higher energy levels [41]. Also, the intensity of absorbance showed an increase with UV irradiation compared without the irradiation, as in Figures 8, 9, and 10, and that agreed with Watcharaporn *et al.* [37].

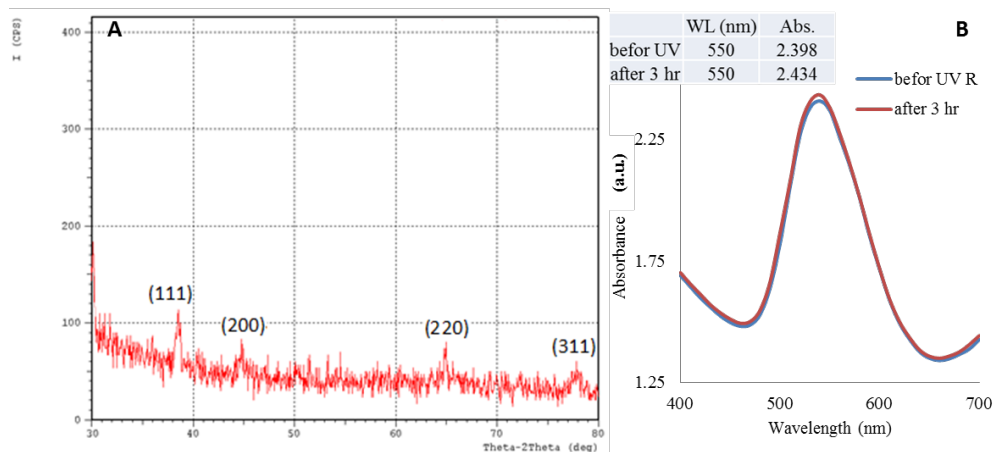




**Figure 8** XRD and UV-Vis analyses for AuNPs after UV irradiation for 1 h.



**Figure 9** XRD and UV-Vis analyses for AuNPs after UV irradiation for 2 h.



**Figure 10** XRD and UV-Vis analyses for AuNPs after UV irradiation for 3 h.

However, **Table 2** exhibits the crystallite size D of reflection (111) each time. The nanoparticles' average size increases when these particles are exposed to ultraviolet waves, but their size remains within the nanoscale range of fewer than 100 nanometers. The size of AuNPs was 17.39 nm before UV irradiation and increased to 58.16, 59.13, and 47.35 nm after 1, 2, and 3 h from UV irradiation. The surface plasmodium oscillation of surface gold nanoparticles, which becomes heterogeneous with the rest of the particles, makes it a nucleus to accumulate more gold nanoparticles. This increases the proportion of NPs in the colloidal solution and increases the particle size, which agrees with the results of parameters of roughness surface in AFM, as in **Table 1**.

**Table 2** The average particle size of D for AuNPs before and after exposure to UV.

Time of UV Irradiation	$2\theta$	$\text{Cos } \theta$	d	a (Å)	FWHM	D (nm)
0 h	38.4055	0.9443	2.34196	4.05	0.4839	17.39
1 h	38.8220	0.9431	3.17495	5.49	0.1449	58.16
2 h	38.5424	0.9439	3.17380	5.48	0.1424	59.13
3 h	38.5424	0.9440	2.33504	4.03	0.1778	47.35

### Conclusions

This research is considered the 1st attempt to mycosynthesize AuNPs from the extract of *Coprinus comatus* fruiting bodies, and it aims to screen the influence of ultraviolet irradiation at different times on characteristics of AuNPs, and their crystalline nature. The present research results showed that the color change of the mixture from light yellow to purple after 25 min and the lambda max of the absorbance reached 530 nm using the UV-Visible spectrum, which is evidence of the formation of AuNPs from the extract of *Coprinus comatus*. The FTIR spectra revealed active groups like carbonyl group (C=O) and a hydroxyl group (-OH), belonging to the peptides, proteins, flavonoids, monosaccharides, polysaccharides, and phenolic compounds. The EDX technique showed that the formed nanoparticles were AuNPs with C, H, N, and S elements due to the amino acid composition and the organic matter of the mushroom. XRD results showed that gold nanoparticles have a face-centered cubic (fcc) crystal and that the UV irradiation at different times increased the intensity of absorbance and sizes of NPs from 17.39 nm AuNPs before the irradiation and switched to 58.16, 59.13, and 47.35 nm after 1, 2, and 3 h, respectively, but their sizes remained within the nanoscale range (less than 100 nm). Finally, the best result was observed after one h about the effect of UV irradiation on sizes of AuNPs, which reached smaller nanoparticles compared with times 2 and 3 h.

### Acknowledgements

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